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# Effects of water impoundment and water-level manipulation on the bioaccumulation pattern, trophic transfer and health risk of heavy metals in the food web of Three Gorges Reservoir (China)

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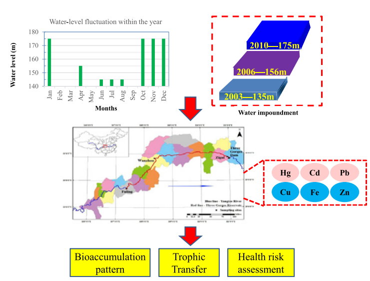
## HIGHLIGHTS

- We evaluated the effects of water impoundment and water-level manipulation on HMs.
- Cu, Fe, Zn and Hg in biota were higher whereas Cd and Pb were lower than before impoundment.
- Water-level manipulation shaped spatiotemporal pattern of HMs in fish and invertebrates.
- Only Hg, Cd and Pb in biota exhibited a declining trend towards the dam.
- Hg and Cd biomagnified during different hydrological periods, whereas other HMs showed weak power.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The Three Gorges Reservoir (TGR) of China, the largest hydropower project over the world, has attracted much attention to the water impoundment and water-level manipulation. In this study, we evaluated potential effects of water impoundment and seasonal water-level manipulation on the bioaccumulation, trophic transfer and health risk of HMs (Cu, Fe, Zn, Hg, Cd and Pb) in food web components (seston, aquatic invertebrate and fish) in TGR. Our results show that, after the impoundment for eight years (2003–2010), all of the six metal concentrations in aquatic biota fell within the criteria of safety quality guidelines. The concentrations of Cu, Fe, Zn and Hg in fish and aquatic invertebrates were higher than those before impoundment, whereas Cd and Pb were lower than those before impoundment. Nonetheless, Hg, Cd and Pb in aquatic consumers underwent an increasing trend during the entire impoundment, implying potential reservoir effect in the future. Only the concentrations of Hg, Cd and Pb in aquatic consumers exhibited a declining trend towards the dam, showing consistent with the background level at the three reaches. Seasonal variations in HM concentrations of fish and aquatic invertebrates were ascribed to the water-level manipulation associated with reservoir management. Our findings show that Hg or Cd biomagnified through aquatic food web during different hydrological

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periods, whereas Pb, Cu, Fe and Zn exhibited weak biomagnification power. Overall, Hg, Cd and Pb showed a higher risk than that of Cu, Fe and Zn.



## 1. Introduction

Heavy metals (HMs) from natural and anthropogenic sources are increasingly released to aquatic environments (Agah et al., 2009; Bravo et al., 2014), resulting in changing ecosystem biodiversity, eliminating sensitive species or reducing species abundance through reproductive impairment and increasing incidence of diseases (Kibria et al., 2012). Bioavailability of HMs and the accumulation in aquatic fauna depend on both abiotic (e.g., metal concentration, pH, dissolved organic carbon and temperature) and biotic (e.g., feeding habits, exchange surface and metabolic activity) factors (Goutte et al., 2015). Higher levels of metals could be also reflected in the sediments, water column and biological tissues (Chi et al., 2007; Monroy et al., 2014; Li et al., 2015). Specifically, high background level of nonessential HMs (e.g., Pb, Hg and Cd) tends to be associated with terrestrial inputs and anthropogenic (both domestic and industrial) sources. However, the distribution of essential HMs such as Cu, Fe and Zn is affected by primary production because these elements serve as nutrients in biological activities (Balkis et al., 2010). HMs can bio-accumulate up to three to six times higher concentrations in aquatic invertebrate and fish than those of the surrounding environment (Luoma and Rainbow, 2008), thereby they pose a potential health risk to predators (Liu et al., 2015) and humans (Hruba et al., 2012) through biomagnifications.

Water level fluctuation (WLF) regulates the biogeochemistry of aquatic ecosystems by altering the acid-base characteristics and redox potential of sediments and the response of microbial community, which can facilitate the release or sorption of HM contaminants (Jonge et al., 2012; Nedrich et al., 2017). The sediments, which have been exposed for months or years and then are rewetted, are crucial sources of HM inputs to aquatic food webs (Snodgrass et al., 2000; Sorensen et al., 2005; Selch et al., 2007). The impoundment leads to the increase of inundated land area. A noted “reservoir effect” could occur because HMs from the inundated soil releases into the water column. For example, Hg concentrations in fish from newly inundated reservoirs are elevated relative to those before the impoundment (Bodaly et al., 2007; Brinkmann and Rasmussen, 2010). After the impoundment of TGR in China, Gao et al. (2015a, b) show that the concentrations of HMs exhibit an increasing tendency in sediments compared with those before impoundment (e.g., Qi et al., 2002), but the HM contents in fish muscle do not notably increase. Li and Xie (2016) suggest that the concentrations of Hg in fish do not significantly increase after impoundment, while those of Pb, Cr, Cd, and As decrease. Although the water impoundment is reported to affect the HM level in TGR, the tendency seems to be totally different among sediments, water column and biological samples or for different metal categories. Moreover, little is known about whether manipulation-induced WLF can modify the bioaccumulation pattern and health risk of HMs in TGR.

The Three Gorges Dam (TGD), with 2309 m long and 181 m high, is situated at the main stem of the Yangtze River of China and serves as one of the biggest hydroelectric dams in the world (China Three Gorges Project Corporation, CTGPC, http://www.ctgpc.com.cn). Construction of TGD forms a giant, typical river-type reservoir (TGR) from Chongqing Province down to Yichang City (Hubei Province). The TGR has been impounded through three stages to elevations of 135 m, 156 m, and 175 m above sea level in 2003, 2006, and 2010, respectively (Bao et al., 2015). After the completion of the project, the water-level varies between 145 m and 175 m periodically all the year round. Meanwhile, the TGR has been operated by anti-season water-level manipulation: high water-level in winter (dry season) whereas low water-level in summer (wet season). Such hydrological changes will alter the original inputs or outputs of contaminants. Over the past decades, reports on the contamination of HMs in TGR are limited to the soil and sediments (Tang et al., 2014; Han et al., 2015), and little attention has been paid to the biotic community. It's well known that people in China rely, to a larger extent, on the requirement of proteins from freshwater fish. Thus, research on higher trophic level predators (e.g., fish and birds) can explicitly evaluate the environmental health risk of HMs (Yi et al., 2011; Yu et al., 2012). Stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) have been proved to be a valuable tool to address the trophic relationship and potential biomagnification of contaminants in aquatic food webs (Dehn et al., 2006). In particular, stable nitrogen isotopes ($\delta^{15}N$) can produce a constant enrichment (3–4‰) at each trophic transfer (Jarding et al., 2006; Vander Zanden and Fetzer, 2007), which allows $\delta^{15}N$ to be used as a reliable indicator of trophic positions in the food webs (Minagawa and Wada, 1984).

Although no reservoir effect of HMs has been shown since the impoundment (Li et al., 2015) reveal a significant temporal trend of Hg concentrations associated with regular water-level manipulation, suggesting the impact of WLF on HM level. After the inundation (175 m) in 2010, Hg proves to biomagnify along aquatic food chains at the main stem of TGR (Yu et al., 2013; Li et al., 2015), implying that nonessential HMs such as Hg, Cd and Pb may biomagnify along the aquatic food chain. However, essential HM concentrations (Cu, Fe and Zn) could be diluted by biological absorption due to the thriving of phytoplankton (especially cyanobacteria) (Zhou et al., 2011; Liu et al., 2012) and lentic fish assemblages (Gao et al., 2010). Therefore, we hypothesize that water-level manipulation within one year would make a difference to HMs in TGR but the water impoundment could be more crucial to nonessential HMs than to essential HMs. In the present study, we evaluated the spatiotemporal pattern of HM (Cu, Fe, Zn, Hg, Cd and Pb) concentrations in food web components (seston, aquatic invertebrate and fish). The main objectives of this study were to illuminate the bioaccumulation of HMs and trophic transfer in the food web of TGR, and potential health risk based on consumption of aquatic products. In addition, we focused on evaluating potential effects of the water impoundment and water-level manipulation on HM bioaccumulation. We attempted to provide the readers with valuable perspectives associated with reservoir management and anthropogenic activities, and a better understanding of HM pollution in large-scale river-type reservoirs.

## 2. Material and methods

### 2.1. Study area and sampling site

The Yangtze River, the longest river in Asia and the third longest river in the world, originates from Qinghai-Tibet Plateau and flows

6397 km eastward to the East China Sea (Shanghai Province). The reach from the headwater to Yichang city (Hubei Province) is defined as the upstream of the Yangtze River (Yang et al., 2014).

The TGR (Lat. 29°16′-31°25′ and Long. 106°−111°50′), is situated at the upstream of the Yangtze River, spanning a 600 km valley from Chongqing City to the Zigui County of Yichang and covering a total surface area of 1,080 $km^2$ (Fig. 1). The reservoir has a water capacity of 39.3 $km^3$ when coming into full use. Climate in this region belongs to southeast subtropic monsoon, with an annual mean temperature of 16.5 °C. The annual precipitation is approximately 1100 mm and 80% of them occur from April to October (Ye et al., 2013). The riverbeds of original reaches in TGR exhibit different elevations and the maximal water-level impoundment results in an actual mean water depth of 70 m and a maximal depth of 170 m. According to the geographical and hydrological characteristics of TGR, we chose three types of habitats in the main stem of TGR as study stations (Fig. 1). The Zigui (Hubei Province), Wanzhou and Fuling reaches (Chongqing City) are 1 km, 277 km and 484 km away from TGD, respectively. The geographic, hydrological and climatic characteristics of these three stations were provided in Supplementary Table S1. For each station, three study sites (including the main channel and estuary of tributaries) were established for sample collections of water, aquatic invertebrate and fish.

### 2.2. Sample collection and processing

Sampling was performed in July (flooding period) and November (post-flooding period) 2011, and May (pre-flooding period) and August (flooding period) 2012 at the three sampling stations (Fig. 1). Seston, aquatic invertebrate and fish were collected from different stations on each sampling date. Specifically, sampling of seston was executed by site, whereas sampling of aquatic invertebrate and fish was conducted by station according to the availability of biological samples.

Water samples were well mixed from the surface and bottom layers and then filtrated through acid-washed glass fiber filter (0.45 μm, Whatman GF/C) using vacuum. In particular, water samples for THg analysis were preserved through the addition of HCl (guarantee reagent), to a 0.5% HCL concentration in the water sample, and then refrigerated in dark bags until analysis (Sinclair et al., 2012). Particulate matter kept on the filter was analyzed as seston sample and filtered water was used for the determination of dissolved metal (Hg, Cd, Pb, Cu, Fe, Zn) concentration in the water column.

Surface sediment samples were taken using a core sampler (K-B type, Wildco, USA) near the middle of stream flow. The sediment cores were sliced at 5 cm thick intervals with slice equipments whose surface is coated with a Polytetrafluoroethene (Wei et al., 2016). The sediment samples were stored in plastic bags, sealed into clean polyethylene bags and treated immediately after returning to the laboratory. Samples were wet sieved through an acid-cleaned 63 μm mesh nylon sieve to obtain chemically active materials (Gao et al., 2015a), dried at 40 °C to a constant weight, and ground into homogenous particles using an agate mortar.

Benthic animals were collected partially through a Peterson modified grab (grab surface area = 0.0625 $m^2$), partially from trawl net in the WLF region of TGR, and then filtered through a filter sieve of mesh size (75 μm). Freshwater shrimp was obtained by shrimp trawl net and mostly from fishermen. Aqun. Invertebrate samples were stored in either acid cleaned plastic bags or acid cleaned Teflon vials (for smaller organisms) and frozen. After field collecting frozen samples were thawed, rinsed with ultra-clean water, weighed, and freeze-dried. Molluscs (e.g., snail) were removed from their shells prior to freeze-drying.

Fish were collected by local fishermen using gillnets with a mesh size ranging from 10 to 30 mm length. All individuals were identified to species level. Five to ten individuals per species were

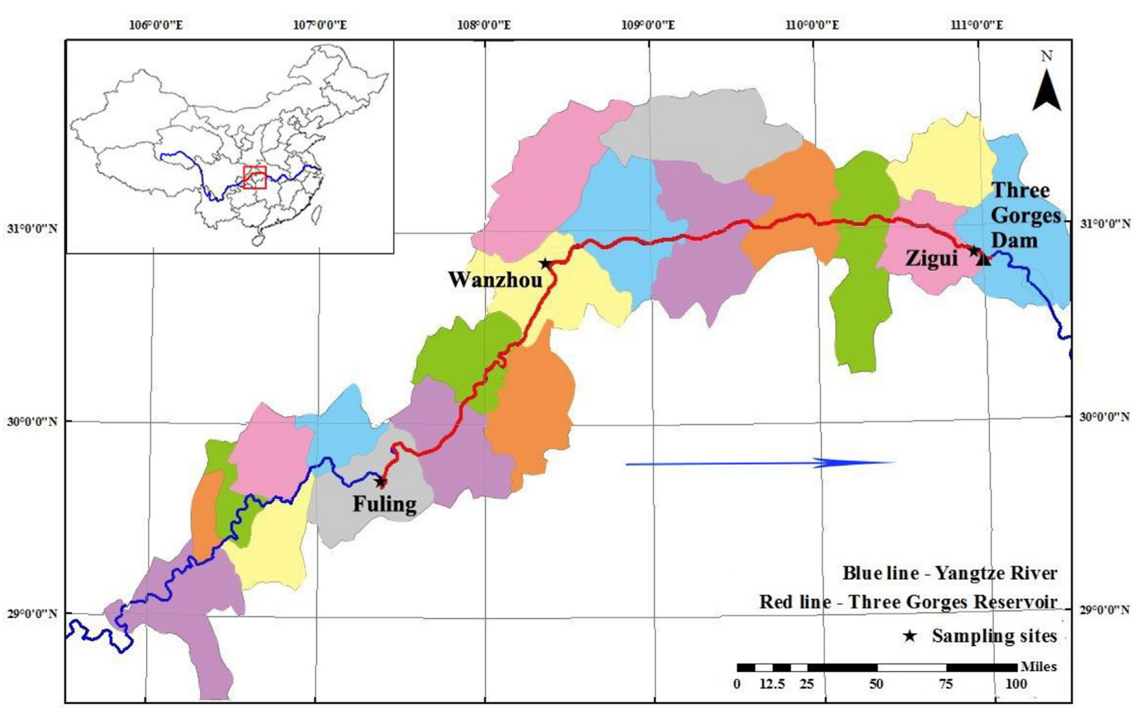


**Fig. 1.** Map of the Three Gorges Reservoir (TGR) showing the locations of water column and biological sampling. Bold lines (blue and red) in the middle of the map represent the main stem of the Yangtze River and small asterisks represent study stations. The polygons around the main stem represent the region of cities and counties. Blue arrow represents the direction of water flow in TGR.

used for metal analysis at each station and each sampling date. Each fish was measured for body weight and body length, whereas aquatic invertebrate was measured for body weight only (Supplementary Table S3). In general, a filet of 5–20 g of muscle tissue was cut from the dorsal area. For small fish and aquatic invertebrates (e.g., freshwater shrimp), muscle tissues of some individuals were usually incorporated into one sample to reach the requirement of minimum sample amount. Seston, fish muscle and aquatic invertebrate samples were immediately wrapped in acid-cleaned plastic bags and placed in an ice box, transported to the laboratory and kept in a freezer. All samples will be kept at −20 °C until analysis.

### 2.3. Heavy metal analysis and quality assurance/control (QA/QC)

The filtered water samples were digested with sulfuric acid, potassium permanganate and potassium persulfate for metal analysis. Biotic samples including seston, aquatic invertebrate and fish muscle were ground to a homogenized powder. Metals in the water column were measured with an ICP-MS after infiltration through a 0.45 mm membrane. For biotic samples, 0.2 g of dry powder was digested by a microwave system with $HNO_3$ in teflonlined vessels under the conditions of controlled pressure. Concentrations of Cu, Fe, Zn, Cd, and Pb were determined by Inductively Coupled Plasma Spectroscope analysis (ICP-JY Ultima, Jobin Yvon Co., France). The concentration of Hg was evaluated using the cold vapor atomic absorption spectroscopy (CVAAS) method described by McIntyre and Beauchamp (2007).

Procedural blanks, field blanks, equipment blanks, regent blanks and field replicates were routinely used with each batch of samples analyzed to evaluate and maintain the quality assurance/quality control (QA/QC). Blanks were tested every three samples and, two samples were spiked to calculate the recovery of the method. The HM concentrations in the blanks were less than 1% of the samples and all the relative standard deviations (RSD) of triplicate samples were less than 10%. The HM concentrations in aquatic invertebrate and fish are presented as mean ± standard deviance (SD) in ng $g^{-1}$on a wet weight (ww) basis, whereas that in the water was presented by ng $L^{-1}$. Specifically, reagent blanks that deduct the influences of sampling facilities (procedural blanks) were 0.1 μg $L^{-1}$, 0.05 μg $L^{-1}$, 0.5 μg $L^{-1}$, 3 μg $L^{-1}$, 5 μg $L^{-1}$ and 3 μg $L^{-1}$ for Hg, Cd, Pb, Cu, Fe and Zn, respectively. RSD of replicate samples were 3.5–6.8%, 4.3–7.3%, 4.5–7.5%, 2.7–5.6%, 2.1–4.8% and 2.5–5.4% for Hg, Cd, Pb, Cu, Fe and Zn, respectively.

### 2.4. Calculation of health risk index and biomagnification power

The Target hazard quotients (THQs) and hazard index (HI) have been widely utilized to assess the health risk induced by HMs (Yi et al., 2011; Ahmed et al., 2015). The THQ is a ratio of the determined dose of a pollutant to the reference dose level that provides an indication of the risk level associated with pollution exposure (Bortey-Sam et al., 2015; Gao et al., 2015b). If the ratio is less than 1, there is no obvious health risk for the exposed population. Higher THQ reflect a higher probability of experiencing long-term non-carcinogenic effects (Gu et al., 2016). The method for THQ determination is described in details by the U.S. EPA (USEPA, 2000). The THQ is given by the following equation:

$$THQ = \left(EF \times ED \times FIR \times C \times 10^{-3}\right) \Big/ (RfD \times W_{ab} \times AT) \quad (1)$$

where EF, ED, FIR, C, RfD, $W_{ab}$, AT represent the exposure frequency (365 days/year), exposure duration (70 years), food ingestion rate (g/d), mean HM concentration in fish muscular tissue (μg/g), oral reference dose (mg/kg/d), average body weight (60 kg), and average exposure time for non-carcinogens (365 days/year × exposure years, assuming 70 years in this study). Assuming that cooking has no effect on the toxicity of HMs in aquatic products (Chien et al., 2002), the hazard index (HI) can be expressed as the sum of the hazard quotients for all selected metals:

$$HI = THQ_1 + THQ_2 + \cdots\cdots + THQ_n \quad (2)$$

where $THQ_n$ is the target hazard quotient for n selected metals.

The ability of a contaminant to biomagnify can be expressed in terms of Trophic Magnification Factors (TMFs), and when TMF > 1 indicating biomagnifications (Hop et al., 2002; Jaeger et al., 2009). The TMF was based on the relationships between trophic level and contaminant concentrations using the following equations:

$$Log\ (\text{contaminant concentration}) = A + B{*}TP \quad (3)$$

$$\text{TMF} = 10^{\text{B}} \quad (4)$$

where A is the intercept; B is the slope; and the level of statistical significance of the regression was set at $p < 0.05$. The trophic magnification factor provides a mean rate of increase per trophic position in the food chain and assumes that uptake from the diet is the main exposure route (Hop et al., 2002).

### 2.5. Stable isotope analyses

Seston samples for stable isotope analysis were first acidified with 1 N HCl overnight to remove carbonate and roasted to a constant weight at 60 °C, and then ground to a fine powder using acidic clean mortar and pestle. Freeze-dried biota samples for $\delta^{13}C$ analysis were treated with 1 N HCl, followed by treatment with 1% dichloromethane in methanol to eliminate lipids (Sweeting et al., 2006). Samples for $\delta^{15}N$ analysis did not undergo any treatment after lyophilization. Homogenized biota samples were weighed in a tin foil cup, wrapped, and analyzed with a Flash EA-1112 HT elemental analyzer accompanied by a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., USA). Isotopic ratios were expressed relative to international standards (Pee Dee Belemnite for carbon and atmospheric $N_2$ for nitrogen). Delta values were defined as:

$$\Delta R = \left[\left(X_{\text{sample}} - X_{\text{standard}}\right) \Big/ X_{\text{standard}}\right] \times 10^3 (\%) \quad (5)$$

where $R = {}^{13}C$ or ${}^{15}N$ and X is the corresponding ratio ${}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The analytical precision was within 0.1‰ and 0.2‰ for carbon and nitrogen isotope values, respectively.

Trophic positions (TPs) were determined for all common species at three study stations of TGR and used to compare the $\delta^{15}N$ data across sites. Estimates of TP were calculated based on the $\delta^{15}N$ values in fish muscle and primary consumer (baseline of food web). Freshwater snail *Turbo fluctuosa* is a sedentary primary consumer species with long lifespan and was used as baseline indicator (TP = 2). The TPs of fish and aquatic invertebrates were assessed by an equation as follows:

$$TP_{\text{consumer}} = 2 + \left(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{baseline}}\right) \Big/ \Delta\delta^{15}N \quad (6)$$

where $\delta^{15}N_{\text{consumer}}$ and $\delta^{15}N_{\text{baseline}}$ were the nitrogen isotope values for aquatic consumers (fish and aquatic invertebrates) and baseline indicator, respectively. The discrimination factor ($\Delta\delta^{15}N$) at each trophic step was defined as 3.4‰ (Post, 2002; Jardine et al., 2006).

### 2.6. Statistical analysis

Statistical analyses were conducted through SPSS Ver 19.0 (Chicago, IL, USA). The interspecific difference, spatial and temporal trends in HM concentrations were evaluated using multi-factor analysis of variance (MANOVA), in which species, season and site were considered to be fixed factors, whereas metal concentrations (e.g., Hg, Cd and Pb) to be dependent variable. The ANOVA result was followed by a Tukey post hoc test. The P value less than 0.05 was defined as statistically significant. The ability of biomagnification for each metal element was evaluated through the regression slope of log-transformed metal elements vs. $\delta^{15}N$ (Jardine et al., 2006). The linear regression equation was expressed by $Log_{10}$ [metal element] $= (a \times \delta^{15}N + b)$ with a 0.95 confidence interval and the regression slope.

## 3. Results and discussion

### 3.1. Concentrations of HMs in food web components and health risk assessment

Great variations in HM concentrations of seston and aquatic fauna were shown in Table 1. The metal concentrations (ng $g^{-1}$ ww) were summarized as: Hg from 17.8 to 117.0, Cd from 3.2 to 145.8, Pb from 20.3 to 92.6, Cu from 258.6 to 35682.5, Fe from 6632.6 to 93306.2 and Zn 4210.4 to 64483.1. The lowest concentrations for the six metals were all found in seston samples, suggesting the bioaccumulation of all the six metals from primary producers to higher trophic level consumers. The highest concentrations for Cu, Fe and Zn were shown in invertebrates, while the highest concentrations of Hg and Pb were seen in carnivorous fish (Table 1). Snail *Turbo fluctuosa*, which lives in the benthic sediment, exhibited the highest concentration of Cd, suggesting that Cd was a primary pollutant in the TGR region (Xu et al., 2016). Of all fish species, the highest concentrations of Fe and Zn were found in omnivorous *Misgurus anguillicaudatus* and that of Cu was observed in omnivorous *Coreius heterodon*, whereas the lowest concentrations of Cu, Fe and Zn were found in carnivorous *Erythroculter ilishaeformis*. For nonessential HMs, the highest concentrations were observed in carnivorous *Silurus asotus* (Hg), *Siniperca chuatsi* (Cd) and *E. ilishaeformis* (Pb). The lowest concentrations were found in herbivorous *Ctenopharynodon idellus* (Hg), planktonic *Aristichthys nobilis* (Cd) and omnivorous *C. heterodon* (Pb). Therefore, the lowest level of Hg, Cd and Pb was not consistent with trophic guilds.

The maximum allowable concentrations of Hg, Cd, Pb, Cu, Fe, Zn in fish were also shown in Table 1, as specified by the Ministry of Agriculture, Fisheries and Food (MAFF, 2000) and United Nations Food and Agriculture Organization (Food and Agriculture Organization of the United Nations, 1983). In the present study, the concentrations of HMs in fish muscle were all below the safety standard, suggesting less or no human health risk at initial period of the largest water-level impoundment. Overall, aquatic invertebrates showed higher averaged concentrations of Cd, Cu, Fe and Zn, whereas fish exhibited higher Pb and Hg concentrations (Table 1). Sivaperumal et al. (2007) illustrated that invertebrates accumulated more HMs (Cu, Zn, Co, Mn) than fish in Indian fish markets. Yi et al. (2008) reported that the highest concentrations of Cd, Cu, Zn and Cr were found in crayfish *Eriocheir* in the Yangtze River (China). Therefore, our findings might imply that nonessential metals biomagnify while essential metals biodilute along aquatic food webs.

According to USEPA (2009), oral reference doses were 0.0005, 0.001, 0.004, 0.04, 0.7, 0.3 mg/kg/d for Hg, Cd, Pb, Cu, Fe and Zn, respectively. In this study, the dietary intake of resident area was 105 g/d according to the reference value of Gao et al. (2015a, b). The THQ for six metals and the HI from fish consumption were estimated by the general population of the TGR region. The health risk of intake for any metal was low because all the values were less than 1.0 (Table 2). Specifically, Hg (0.0622–0.4094) showed a higher THQ value than other HMs. The THQ of Pb was low, with the highest value of 0.0405. The THQ of Cd ranged from 0.0056 to 0.0728. For essential HMs, the THQ values of Cu, Fe and Zn ranged from 0.0113 to 0.0997, 0.0166–0.0459 and 0.0246–0.1762, respectively. Due to possible synergic effects of metals (Iwegbue, 2015), individual THQ values of all selected metals were summed up to form the hazard index (HI). The HI of carnivorous *S. asotus* (SAS) was the highest (0.6421) because of the highest Hg and the second highest Pb of all THQ values. The herbivorous *C. idellus* (CI) (0.1829) showed the lowest HI value due to the lowest THQ of Hg. As a major risk contributor, nonessential HMs (Hg, Cd, Pb) accounted for more than 50% (58.5%–86.7%) of the HI for most species, except omnivorous *M. anguillicaudatus* (OW) (Supplementary Fig. S2). In contrast, essential HMs (Cu, Fe, Zn) contributed 50.5% to the HI of omnivorous *M. anguillicaudatus* (OW) due to the highest concentration of Fe and Zn. Although the HI values of all fish species were less than 1.0, only fish consumption was considered for the health risk assessment induced by HMs. We didn't take into account the synergic effect of other aquatic products, drinking water, vegetable, rice and poultry that possibly accumulate HMs (Yu et al., 2012). Furthermore, the concentrations of Hg, Cu, Fe and Zn in our study were higher than those before impoundment (Supplementary Table S9). This is an important point for the food-chain as Hg increases through the upper level, ending up in the diet of humans (Canli and Atli., 2003). With the rapid economic development and increasing human activities, much higher HM level could be expected in TGR. Therefore, the actual health risk through dietary intake of HMs for local people can be higher than that of present study.

### 3.2. Spatial pattern of HM concentrations in aquatic biota

Overall, the concentrations of Hg and Cd in fish and invertebrate at three sampling stations ranked in the following order: upper reach > middle reach > lower reach (Table 1, Supplementary Table S7). Significant spatial differences were also found across the three stations (MANOVA, Hg: $F = 1.332$, $p < 0.05$; Cd: $F = 5.760$, $p < 0.01$, Supplementary Table S4). However, there was no significant difference for Pb across stations (MANOVA, $F = 1.069$, $p = 0.347$, Supplementary Table S4). For Cu, Fe and Zn, there were no significant difference at the three stations, even if the lowest concentrations of them were all observed at the lower reach (carnivorous *E. ilishaeformis*). Accumulation of HMs in fish mainly results from the surface contact with water, and by breathing and via food chain biomagnification. The uptake through these three routes depends on the background level of HMs in the habitats (Yi et al., 2011). Gao et al. (2016) investigated spatial patterns of HM concentrations in TGR and illuminated that higher level of metals (Cu, Hg, Cd, As, Pb and Zn) in the water column were observed at the location farthest from the dam. The upper reach of the TGR is close to urban (Chongqing, Changshou, Fuling) and industrial areas, where there are poor water quality and high background level of HMs. Huge metropolitan centers (Chongqing, Changshou, Fuling and Wanzhou) become the main pollution sources in the TGR and, have yielded 220 million tons of industrial effluent and 486 million tons of urban sewage in 2010 (CNEMC, 2011; Deyerling et al., 2014). Meanwhile, anthropogenic activities such as agricultural and domestic run-off make great contribution to HM contamination (Ma et al., 2016). Besides, the upper reach shows the largest flooded area and the highest flow flux among the three reaches (Supplementary Table S1) and, receives a large amount of waste

**Table 1**
Averaged HM concentrations (ng $g^{-1}$ wet weight), $\delta^{15}N$ signatures of food web components (seston, aquatic invertebrate and fish) and trophic positions of aquatic consumers in TGR. The species code was in accordance to Supplementary Table S2. M ± SD represents Mean ± SD. TP: trophic position. N: number of samples.

| Catergery | Hg | Cd | Pb | Cu | Fe | Zn | $\delta^{15}N$ | N | TP |
|---|---|---|---|---|---|---|---|---|---|
| | M ± SD | M ± SD | M ± SD | M ± SD | M ± SD | M ± SD | M ± SD | | |
| **Seston** | 5.2 ± 4.5 | 2.1 ± 0.3 | 2.2 ± 0.4 | 22.7 ± 3.3 | 367.4 ± 14.7 | 421.5 ± 24.7 | 8.2 ± 1.1 | 27 | |
| **Invertebrate** | | | | | | | | | |
| Snail *Turbo fluctuosa* | 22.6 ± 3.8 | 145.8 ± 9.4 | 57.2 ± 14.5 | 35682.5 ± 361.2 | 93306.2 ± 143.2 | 64483.1 ± 159.4 | 8.6 ± 3.6 | 25 | 2.00 |
| Shrimp *Macrobrachium nipponense* | 37.8 ± 9.7 | 20.3 ± 7.0 | 42.5 ± 14.5 | 5205.5 ± 92.5 | 6593.5 ± 125.8 | 12080.7 ± 70.0 | 9.9 ± 1.7 | 25 | 2.36 |
| Shrimp *Exopalaemon modestus* | 41.5 ± 13.0 | 19.5 ± 5.9 | 47.4 ± 9.9 | 3611.8 ± 61.7 | 9176.2 ± 136.7 | 13829.1 ± 64.4 | 13.0 ± 2.3 | 26 | 3.28 |
| **Fish** | | | | | | | | | |
| **Herbivore** | | | | | | | | | |
| *Ctenopharynodon idellus* | 17.8 ± 7.8 | 18.8 ± 10.5 | 27.3 ± 6.9 | 515.0 ± 12.1 | 6969.7 ± 46.4 | 6163.0 ± 38.5 | 6.2 ± 1.9 | 25 | 1.29 |
| **Planktivore** | | | | | | | | | |
| *Hypophthalmichthys molitrix* | 35.4 ± 7.0 | 12.8 ± 3.9 | 44.7 ± 7.0 | 887.1 ± 16.9 | 9284.7 ± 65.9 | 7969.0 ± 39.0 | 8.2 ± 1.5 | 16 | 1.87 |
| *Aristichthys nobilis* | 46.4 ± 14.3 | 3.2 ± 0.8 | 53.2 ± 13.4 | 1038.5 ± 22.1 | 7306.6 ± 29.1 | 6416.7 ± 32.4 | 9.4 ± 1.6 | 19 | 2.22 |
| **Omnivore** | | | | | | | | | |
| *Hemiculter leucisculus* | 57.5 ± 19.9 | 7.5 ± 2.0 | 32.3 ± 8.4 | 756.2 ± 11.7 | 17011.5 ± 156.5 | 11044.2 ± 37.1 | 8.7 ± 1.8 | 18 | 2.03 |
| *Hemiculter bleekeri warpachowsky* | 78.2 ± 17.6 | 17.0 ± 3.3 | 55.0 ± 5.5 | 1345.6 ± 12.5 | 14042.8 ± 150.5 | 9924.9 ± 75.9 | 9.6 ± 0.1 | 17 | 2.27 |
| *Cyprinus carpio* | 80.4 ± 16.8 | 12.1 ± 6.8 | 34.6 ± 9.6 | 558.7 ± 18.2 | 8722.1 ± 35.7 | 7842.9 ± 26.7 | 8.5 ± 2.3 | 26 | 1.95 |
| *Carassius auratus* | 54.9 ± 12.2 | 17.1 ± 7.1 | 50.1 ± 14.4 | 792.4 ± 10.5 | 10395.2 ± 69.4 | 12022.2 ± 59.3 | 8.9 ± 1.7 | 33 | 2.09 |
| *Coreius heterodon* | 55.8 ± 11.9 | 29.5 ± 1.0 | 92.6 ± 7.4 | 2278.3 ± 12.6 | 6842.3 ± 29.9 | 5178.8 ± 45.3 | 10.8 ± 0.6 | 23 | 2.63 |
| *Coreius guichenoti* | 70.4 ± 4.6 | 10.3 ± 6.1 | 52.5 ± 9.0 | 729.3 ± 6.3 | 11934.0 ± 45.3 | 8171.0 ± 58.2 | 10.0 ± 1.0 | 19 | 2.40 |
| *Saurogobio dabryi* | 64.7 ± 15.6 | 13.1 ± 2.1 | 52.5 ± 13.6 | 863.1 ± 12.4 | 9638.6 ± 93.7 | 8771.6 ± 61.0 | 10.6 ± 1.6 | 29 | 2.58 |
| *Squalidus argentatus* | 50.4 ± 7.9 | 25.5 ± 7.1 | 36.6 ± 12.7 | 538.2 ± 7.9 | 7632.7 ± 64.2 | 6826.8 ± 79.5 | 10.5 ± 1.7 | 21 | 2.56 |
| *Pelteobagrus vachelli* | 61.9 ± 12.8 | 14.0 ± 1.6 | 34.9 ± 11.0 | 562.4 ± 13.4 | 7905.3 ± 48.3 | 4955.6 ± 27.0 | 10.1 ± 1.5 | 24 | 2.44 |
| *Misgurnus anguillicaudatus* | 71.4 ± 14.2 | 12.3 ± 4.8 | 34.8 ± 12.3 | 1501.4 ± 14.4 | 18354.7 ± 157.9 | 30211.7 ± 75.0 | 11.8 ± 1.9 | 16 | 2.94 |
| **Carnivore** | | | | | | | | | |
| *Erythroculter dabryi* | 62.4 ± 9.9 | 29.7 ± 8.7 | 62.7 ± 13.8 | 647.7 ± 7.4 | 6980.8 ± 75.3 | 4658.7 ± 83.4 | 13.3 ± 0.8 | 19 | 3.37 |
| *Erythroculter ilishaeformis* | 80.9 ± 4.7 | 10.8 ± 2.1 | 92.6 ± 9.2 | 258.6 ± 6.3 | 6632.6 ± 57.2 | 4210.4 ± 23.8 | 11.9 ± 1.9 | 19 | 2.96 |
| *Silurus asotus* | 117.0 ± 10.1 | 21.9 ± 2.7 | 84.3 ± 21.9 | 1025.9 ± 13.4 | 16241.1 ± 141.6 | 12340.8 ± 42.9 | 13.2 ± 1.1 | 23 | 3.33 |
| *Siniperca chuatsi* | 112.7 ± 23.5 | 41.6 ± 12.8 | 75.8 ± 14.7 | 629.5 ± 11.5 | 9624.7 ± 26.4 | 7627.1 ± 48.6 | 13.9 ± 1.7 | 15 | 3.55 |
| FAO | – | 500 | 500 | 30000 | – | 50000 | | | |
| MAFF | 300 | 200 | 500 | 20000 | 50000 | 50000 | | | |

**Table 2**
Estimated target hazard quotient (THQ) and hazard index (HI) of HMs in fish.

| Species | THQ | | | | | | HI | %Nonessential to total HI |
|---|---|---|---|---|---|---|---|---|
| | Nonessential HMs | | | Essential HMs | | | | |
| | Hg | Cd | Pb | Cu | Fe | Zn | | |
| *Ctenopharynodon idellus* | 0.06 | 0.03 | 0.01 | 0.02 | 0.02 | 0.04 | 0.18 | 58.5 |
| *Hypophthalmichthys molitrix* | 0.12 | 0.02 | 0.02 | 0.04 | 0.02 | 0.05 | 0.27 | 60.5 |
| *Aristichthys nobilis* | 0.16 | 0.01 | 0.02 | 0.05 | 0.02 | 0.04 | 0.29 | 65.4 |
| *Hemiculter leucisculus* | 0.20 | 0.01 | 0.01 | 0.03 | 0.04 | 0.06 | 0.37 | 62.8 |
| *Hemiculter bleekeri warpachowsky* | 0.27 | 0.03 | 0.02 | 0.06 | 0.04 | 0.06 | 0.48 | 68.3 |
| *Cyprinuscarpio* | 0.28 | 0.02 | 0.02 | 0.02 | 0.02 | 0.05 | 0.41 | 77.5 |
| *Carassius auratus* | 0.19 | 0.03 | 0.02 | 0.03 | 0.03 | 0.07 | 0.37 | 65.1 |
| *Coreius heterodon* | 0.20 | 0.01 | 0.01 | 0.10 | 0.02 | 0.03 | 0.36 | 59.5 |
| *Coreius guichenoti* | 0.25 | 0.02 | 0.02 | 0.03 | 0.03 | 0.05 | 0.40 | 72.4 |
| *Saurogobio dabryi* | 0.23 | 0.02 | 0.02 | 0.04 | 0.02 | 0.05 | 0.39 | 70.7 |
| *Squalidus argentatus* | 0.18 | 0.04 | 0.02 | 0.02 | 0.02 | 0.04 | 0.32 | 74.2 |
| *Pelteobagrus vachelli* | 0.22 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.33 | 77.8 |
| *Misgurnus anguillicaudatus* | 0.25 | 0.02 | 0.02 | 0.07 | 0.05 | 0.18 | 0.57 | 49.9 |
| *Erythroculter dabryi* | 0.22 | 0.05 | 0.03 | 0.03 | 0.02 | 0.03 | 0.37 | 80.3 |
| *Erythroculter ilishaeformis* | 0.28 | 0.02 | 0.04 | 0.01 | 0.02 | 0.02 | 0.40 | 86.7 |
| *Silurus asotus* | 0.41 | 0.04 | 0.04 | 0.04 | 0.04 | 0.07 | 0.64 | 75.5 |
| *Siniperca chuatsi* | 0.39 | 0.07 | 0.03 | 0.03 | 0.02 | 0.04 | 0.60 | 83.9 |

water from shipping, tidal pumping and trapping (Bing et al., 2016). Furthermore, the impoundment has turned the natural river flow of the dammed reaches into a man-made lacustrine regime (Xu et al., 2011; Wei et al., 2016). After the impoundment of TGR, the decreasing flow velocity and long residence time of water accelerate the sinking process of particular matters as well as attached metals (Müller et al., 2008; Bao et al., 2015; Tang et al., 2016). Therefore, the decrease of Hg and Cd towards the dam can be explained by different background levels of HMs at the three reaches. In contrast, Cu, Fe and Zn serve as nutrients in biological activities and are affected by primary production (Balkis et al., 2010). Previous studies have reported the thriving of phytoplankton (especially cyanobacteria) (Zhou et al., 2011; Liu et al., 2012) since the inundation of TGR. Gao et al. (2016) demonstrated that the concentrations of nonessential HMs in the water column decreased from upstream to downstream, while essential HMs (Cu, Fe, Zn) in aquatic fauna did not have such trend. These results probably support the self-purification of Cu, Fe and Zn due to biological absorption of plankton.

### 3.3. Effects of water-level manipulation and water impoundment on HM bioaccumulation pattern

Operated water-levels at different sampling seasons were shown in Supplement Fig. S1. Hg and Cd concentrations of aquatic fauna exhibited significant difference during different sampling seasons (MANOVA, Hg: $F = 11.93$, $p < 0.01$; Cd: $F = 7.138$, $p < 0.01$, Supplementary Table S4 and S8). Moreover, the concentrations of Cu and Zn showed significant temporal differences (MANOVA, Cu: $F = 5.99$, $p < 0.01$; Zn: $F = 2.616$, $p < 0.05$, Supplementary Table S4 and S8). The tendency in concentrations of four HMs (Hg, Cd, Cu and Zn) was in the sequence as: post-flooding (Nov. 2011)>pre-flooding (May 2012)>flooding (Jul. 2011)>flooding (Aug. 2012). Based on the characteristics of hydrology, environment and climate, the trend of HM concentrations may be related to such factors as the rainy season, frequency of inundation (Ye et al., 2011) and change in water quality parameters (Malik et al., 2010). According to the operation rhythm of water-level within one year, TGR formed a 30 m WLF zone (145–175 m) that varies seasonally (Ye et al., 2011; Gao et al., 2016). After flooding period, high water-level inundates the alluvial soil in the WLF zone, thereby puts high HM loads into the reservoir as revealed by previous studies (St-Louis et al., 2004; Sorensen et al., 2005). Besides, due to the decrease in the rainfall, both the water discharge and sand transport rate from the upstream degrade (Han et al., 2018). Furthermore, the low velocity and prolonged residence time of water forms a stable hydrodynamic condition for particle deposition. Thus, suspended particles in TGR sink greatly during high water-level (Yuan et al., 2013; Sun et al., 2018). Fine particles prove to notably increase HM accumulation in the sediments (Yao et al., 2016). Accordingly, HMs can be greatly accumulated in aquatic biota in November. Water-level manipulation can change the disturbance in physical environment and water chemistry, thereby alter the phytoplankton and cyanobacteria assemblages (Haldna et al., 2008; Solis et al., 2016). Naselli-Flores et al. (2007) reported that *Microcystis* spp. thrives when sudden decrease of water-level occurs in deep artificial reservoirs. In spring, the increase of nutrients in water bodies may trigger algae blooms accompanied with suitable sunshine, temperature and other logical factors (Ma et al., 2011; Gao et al., 2016; Shi et al., 2018). Algae blooms occurred ten more times in the mainstream and tributaries of TGR since the impoundment in 2003 (Ma et al., 2011). Accordingly, biological absorption of algae blooms should be responsible for low HMs during pre-flooding period (May). In rainy season (low water-level), frequent storming in this region transport coarse particles from upstream to the WLF zone (Wu et al., 2016; Han et al., 2018). However, fast water velocity and short residence time can not facilitate the deposition of particles. Consequently, coarse sediments with less HMs preferentially deposite on the upper reaches of TGR, whereas fine particles with much more HMs tend to be transported downward (Shi et al., 2018). This explains why the lowest HM level in fish occurred during flooding period. Accordingly, seasonal patterns in HM concentrations of fish and aquatic invertebrates were ascribed to the water-level fluctuation (WLF) associated with reservoir management in TGR.

To understand potential effect of the impoundment on the HM level of the TGR, HM concentrations of carps (common carp) in TGR were compared with those before impoundment and in other domestic or international ecosystems (Table 3). The concentrations of Hg (80.4 ng $g^{-1}$ ww), Cu (559 ng $g^{-1}$ ww) and Zn (7843 ng $g^{-1}$ ww) for carps in TGR were higher than those before impoundment (Hg: 65 ng $g^{-1}$ ww; Cu: 227 ng $g^{-1}$ ww; Zn: 2429 ng $g^{-1}$ ww) (Qi et al., 2002), whereas those of Cd (12.1 ng $g^{-1}$ ww) and Pb (34.6 ng $g^{-1}$ ww) were lower than those before impoundment (Cd: 34 ng $g^{-1}$ ww; Pb: 37 ng $g^{-1}$ ww). In contrast with other reaches in the Yangtze River, our study showed that carps (common carp) in TGR was severely contaminated by Hg, moderately contaminated by Cu and Zn, and slightly contaminated by Cd and Pb. As revealed by Table 3, carps in TGR showed slightly higher concentrations of Hg, Cd, Cu and Fe whereas similar levels of Pb and Zn compared with those in other Chinese lakes. The concentrations of Cu, Fe and Zn for fish in TGR were comparable to those from other international water bodies, whereas those of Hg, Cd and Pb in TGR were in the medium range among international water bodies.

TGR underwent three important impoundments in 2003, 2006 and 2010 by raising the storage water-level up to 135, 156 and 175 m, respectively (Wang et al., 2013). For the sake of data analysis, we divided the impoundment time into four stages: before June 2003 (initial stage), June 2003 to November 2006 (second stage), November 2006 to December 2010 (third stage) and after December 2010 (last stage). We collected previous monitoring data of HM concentrations (exclusive of Fe, insufficient data) in fish, water column and sediment during different impoundment stages (Supplementary Table S9). According to averaged HM concentrations during different periods (Fig. 2a), Hg, Cd and Pb in fish decreased firstly and then started to rise, but the values at last stage were lower than that at initial stage. In contrast, Cu, Fe and Zn increased firstly and then declined, but the values at last stage were still higher than that at initial stage (Fig. 2a). Despite the dilution effect at initial impoundment, the concentrations of Hg, Cd and Pb showed an increasing trend during the entire impoundment. Our findings revealed the consistence of nonessential HM concentrations in fish with the background level. After the impoundment, the dam limits the natural exchange of water column, slows down the water velocity and increases the water depth, thereby prevents the water column from mixing in the vertical direction and ultimately causes the accumulation of HMs in the sediments (Bing et al., 2016; Gao et al., 2016). The six metals in the sediments decreased firstly and then started to rise, and the valus at last stage were higher than that at initial stage (Fig. 2b), suggesting an increasing trend of background HM level in TGR. Hence, higher concentrations of Hg, Cd and Pb in fish can be expected after the present survey (2011–2012). Overall, Cu, Fe and Zn showed a declining trend after the impoundment. This can be explained by biological absorption of phytoplankton due to growing algae blooms. Further studies should be conducted to track potential reservoir effect induced by HMs in that Hg, Cd and Pb exhibited an increasing trend after the impoundment of TGR.

### 3.4. Effect of water-level manipulation on trophic transfer of HMs along the aquatic food chain

The potentials of HM trophic transfer were estimated by the relationships between the HM concentration and trophic position of fish. Generally, a significantly positive correlation suggests that a pollutant biomagnifies through the food chain, whereas a negative correlation indicates the biodilution of pollutants (Jaeger et al., 2009). In the present study, the correlations between HM concentrations and trophic position during post-flooding period were described by the regression equations as: log-Hg $= 0.20X+1.36$ ($r^2 = 0.5601$, $F = 12.46$, $p = 0.008$), log-Cd $= 0.09X+1.14$ ($r^2 = 0.0775$, $F = 1.76$, $p = 0.222$) log-Pb $= -0.19X+1.46$ ($r^2 = 0.0785$, $F = 1.77$, $p = 0.220$), log-Cu $= 0.06X+2.85$ ($r^2 = 0.1089$, $F = 0.12$, $p = 0.742$), log-Fe $= 0.01X+4.03$ ($r^2 = 0.1245$, $F = 0.01$, $p = 0.954$) and log-Zn $= 0.03X+3.88$ ($r^2 = 0.1132$, $F = 0.08$, $p = 0.778$). The trophic magnification factors (TMFs) for Hg, Cd, Pb, Cu, Fe and Zn were 1.58, 1.23, 0.66, 1.15, 1.02 and 1.07, respectively. Lower $r^2$ values of regressions indicate that the underlying relationships between the logarithm of HM concentration and trophic position are non-linear (Jaeger et al., 2009). Thus, only the regression for THg showed a statistically significant increase ($p < 0.001$) with increasing TP during post-flooding period (Fig. 3). Likewise, pre-flooding period showed a statistically significant increase with increasing TP for Hg ($p < 0.001$). The regression

**Table 3**
Comparisons of HMs (ng $g^{-1}$, wet weight) in carps (common carp: *Cyprinus carpio*) from selected studies.

| Location | Cu | Fe | Zn | Hg | Cd | Pb | Reference |
|---|---|---|---|---|---|---|---|
| Thane Creek, Mumbai, India | 310 | na | 8360 | 15 | 20 | 80 | Mishra et al. (2007) |
| Topolnitsa Reservoir, Bulgaria | 500 | na | 8000 | na | 1 | 300 | Yancheva et al. (2014) |
| Sidi Salem Reservoir, Tunisia | na | 5139 | na | 34 | 20 | na | Khemis et al. (2017) |
| Danube, Zemun, Serbia | 688 | 9380 | 6160 | 393 | 59 | 59 | Jovanović et al. (2017) |
| Taihu Lake, China | na | na | 5000 | na | 4 | 35 | Chi et al. (2007) |
| Hanjiang River, China | 300 | 7120 | 6140 | 8 | 2 | 13 | Xu et al. (1998) |
| Wujiang River, China | 300 | na | 5540 | na | 5 | 10 | Xu et al. (1998) |
| Poyang Lake, China | 476 | na | 14500 | 36 | 5 | 26 | Wei et al. (2014) |
| The upstream of the Yangtze River | 390 | na | 29830 | na | 15 | 120 | Cai et al. (2011) |
| The downstream of the Yangtze River | 1040 | Na | 7390 | 20 | 120 | 510 | Yi et al. (2011) |
| TGR (before impoundment) | 227 | na | 2429 | 65 | 34 | 37 | Qi et al. (2002) |
| TGR (initial imoundment) | 240 | na | 5880 | 66.2 | 3 | 17 | Yu et al. (2013) |
| TGR (after impoundment) | 559 | 8722 | 7843 | 80.4 | 12.1 | 34.6 | Present study |

na: No data available.

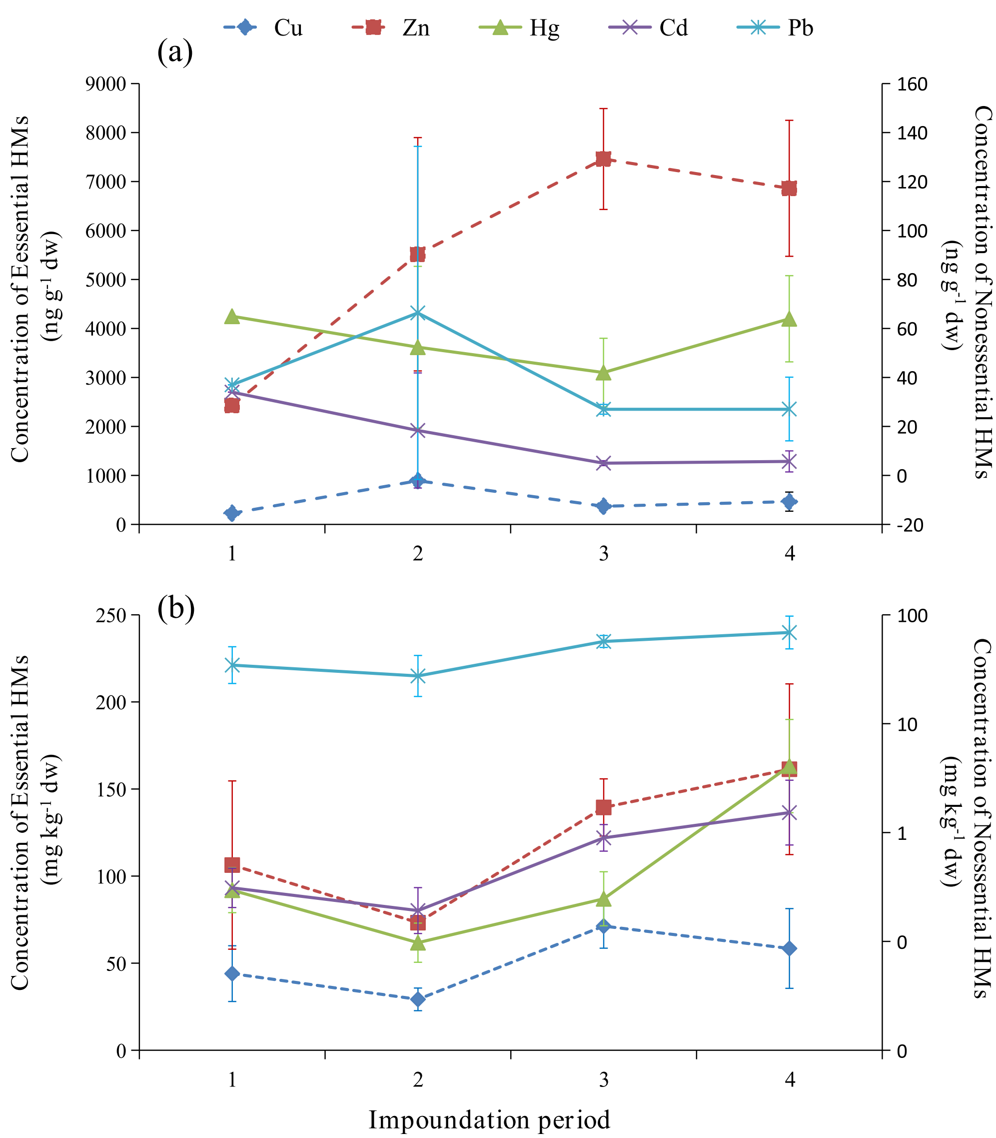


**Fig. 2.** Mean concentrations of HMs (Hg, Cd, Pb, Cu and Zn) in fish (a, *Cyprinus carpio*) and sediments (b) during four impoundment stages. 1-before June 2003; 2-June 2003 to November 2006; 3-November 2006 to December 2010; 4-after December 2010.

equation for Hg was described as: log-Hg = 0.26X+1.12 ($r^2$ = 0.4597, F = 15.46, p = 0.001). In contrast, during flooding period, only the regression for Cd indicated a statistically significant increase ($p < 0.001$) with increasing TP. The regression equation for Cd was log-Cd = 0.19X+0.63 ($r^2$ = 0.3125, F = 9.64, p = 0.006). Accordingly, our study indicated the biomagnifications of Hg and

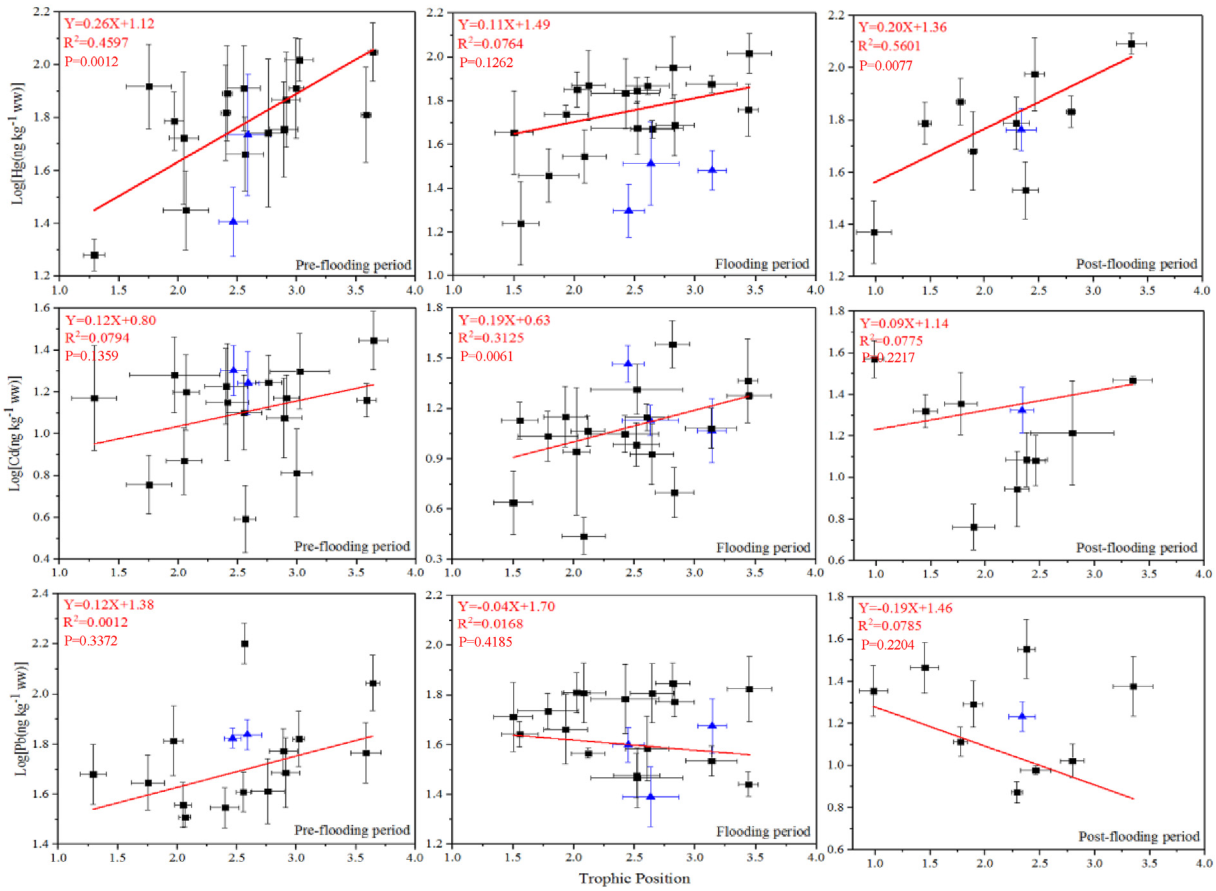


**Fig. 3.** The biplots of Log-transferred metal concentration vs. trophic position of fish for Hg, Cd and Pb during the three periods. Pre-flooding period: May 2012, Flooding period: averaged on July 2011 and August 2012, Post-flooding period: November 2011.

Cd in the food web of TGR (Fig. 3), whereas Pb, Cu, Fe and Zn exhibited weak biomagnification power (Fig. 4), suggesting different bioaccumulation patterns for the six metals in fish.

The biomagnifications of HMs in aquatic fauna mainly rely on the water quality, feeding habit and assimilation properties of HMs (Blum et al., 2013). Previous studies reported the biomagnifications of Hg, Pb and Cs (Gray, 2002), and Cd and Zn (Quinn et al., 2003) along aquatic food webs. Our Hg TMFs (1.58–1.82) were comparable with those reported in different food webs. For instance, 1.15–2.19 (Coelho et al., 2013), 1.44–1.95 (Monferrán et al., 2016) and 1.23 (Signa et al., 2017). In recent decades, many studies have revealed significant, positive correlations between the Hg level and aqueous concentrations of organic matter (total or dissolved organic carbon, TOC/DOC) in water and aquatic biota (Chasar et al., 2009; Braaten et al., 2014). Jiang et al. (2018) investigated seasonal dynamics of DOC characteristics in Changshou Lake, a typical inland lake in the TGR area, and suggested that hydrological processes (e.g., terrestrial inputs resulting from runoff and humic-like component residences) and biological activities (e.g., microbial and algae growth) were the two main principal components controlling seasonal dynamics of DOC. TGR is a newly-built reservoir in the main stem of the Yangtze River. Currently, it suffers from the influences of allochthonous organic matter and cyanobacterial blooms that can yield large amounts of dissolved or particulate carbon. During pre-flooding period (in May), the water level drops to 155 m in TGR, riparian plants begin to grow as the temperature rises. With the increase of precipitation, the soil erosion makes more happen and the DOC content in water column increases with surface runoff (Supplementary Table S10). This probably explain why the Hg content during pre-flooding period was high. During post-flooding period (in November), the rainfall decreases and the input of allochthonous DOC to the water is greatly reduced. High water-level during post-flooding period makes the sediments in the WLF zone rewetted and thus pushes the recycling of sulfide in the sediments by turning sulfide into sulfate, thereby can enhance microbial methylation (Evers et al., 2007; Eckley et al., 2015, 2017). Although high contents of DOC and sulfate facilitated Hg biomagnification during pre- and post-flooding periods, low temperature during post-flooding period limited the methylation rate, and thus TMFs of Hg during post-flooding period was lower than that during pre-flooding. Cd biomagnifications have been recorded in marine and freshwater systems, from subtropical to Arctic food webs (Dietz et al., 2000; Ruelas-Inzunza et al., 2008). During post-flooding period (in November, Supplementary Fig. S1), the soil in the WLF zone is inundated and the pH and Eh in water column decline. The soil switches from oxidizing to reducing environment. The Cd concentrations tend to be low in that Cd converts from highly active exchangeable Cd to lowly active carbonate-bound Cd, oxide-bound Cd and sulfide-bound Cd (Kashem and Singh, 2004; Ji et al., 2012). During flooding period, the water level drops to the lowest (145 m), Cd in water column returns to highly active exchangeable Cd and is more accessible to aquatic food web in TGR.

## 4. Conclusions

After the complete operation (the largest water-level of 175 m) of the Three Gorges Reservoir, the concentrations of Cu, Fe, Zn and Hg in fish were higher than those before impoundment, whereas

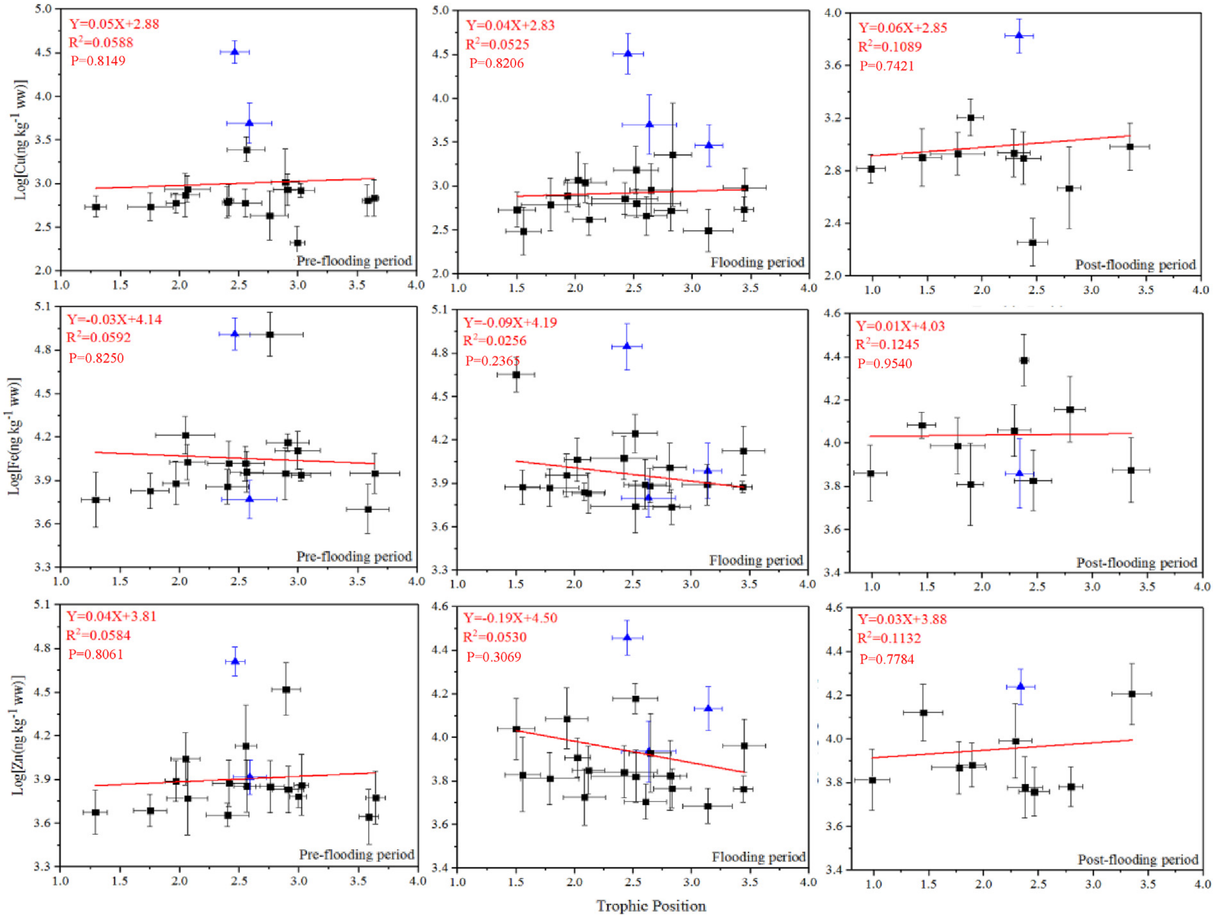


**Fig. 4.** The biplots of Log-transferred metal concentration vs. trophic position of fish for Cu, Fe and Zn during the three periods. Pre-flooding period: May 2012, Flooding period: averaged on July 2011 and August 2012, Post-flooding period: November 2011.

Cd and Pb were lower than those before impoundment. Nonetheless, the concentrations of Hg, Cd and Pb in fish and invertebrates showed an increasing trend during the entire impoundment (2003–2010), implying potential reservoir effect in the future. The decrease of Hg and Cd towards the Three Gorges Dam can be explained by different background levels of HMs at the three reaches. Seasonal water-level manipulation associated with reservoir management shaped spatiotemporal patterns in HM concentrations of aquatic consumers in TGR. Elements Hg and Cd biomagnify through aquatic food web during the three hydrological periods within the year, whereas Pb, Cu, Fe and Zn exhibited weak biomagnification power. Overall, Hg, Cd and Pb showed a higher risk than those of Cu, Fe and Zn.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.04.216.

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