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Analyzing trophic transfer of heavy metals for food webs in the newly-formed wetlands of the Yellow River Delta, China

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The newly-formed wetlands show slight heavy metal contamination and weak biomagnification through the food webs in the Yellow River Delta.

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ABSTRACT

Nine heavy metals sampled from water, sediments, and aquatic organisms in the newly-formed wetlands of the Yellow River Delta (YRD) of China were analyzed to evaluate their concentrations and trophic transfer in food webs. The stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were used to investigate trophic interactions. Results show that most of heavy metals detected in water and sediments are lower than that in Yangtze River Delta and Pearl River Delta. The longest food web is approximately 4 with the highest trophic level of birds. The difference of heavy metal concentrations between endangered Saunders's Gull and other three kinds of protected birds is not obvious. Cd, Zn, and Hg were identified to have an increase with the trophic level (TL), while As, Cr, Cu, Mn, Ni and Pb show an opposite trend, however, the biomagnification of the selected nine heavy metals in the food webs is not significant.

1. Introduction

Heavy metal contamination has aroused wide concerns around the world. Most of heavy metals identified in wetlands are caused by runoff and point source contaminant discharge (Birch et al., 1996), atmospheric deposition (Berg and Steinnes, 1997), and erosion due to rainfall precipitation (Duman et al., 2007). Thus, organisms in wetlands might be exposed to a higher level of heavy metals. As a result, heavy metals are ingested and accumulated in biological bodies or transport to a higher trophic level through wetland food webs (Green et al., 2010; Xie et al., 2010; Yu and Wang, 2004). Adverse effects such as deformities, cancers, and death in aquatic animals together with their terrestrial predators may be induced (Bird et al., 2008; Cai et al., 2009; Coeurdassier et al., 2010; Volpe et al., 2009). Thus, aquatic invertebrate, fish, and birds are usually used as sentinel organisms in monitoring the fate and transport of heavy metals in wetland food webs (Azevedo et al., 2009).

Traditional analyses such as direct field observation and gut content analysis can be used to evaluate trophic relationships (Pinnegar and Polunin, 2000). The direct field observation better reflects the feeding activities, but higher costs (time, labors and material resources) are required. Usually, gut content analysis is used to describe the feeding habits for prey and predator in a short-term. Stable isotope analysis, which reflects the whole diet history of a species, is commonly used to quantify relative trophic positions for various species (Bond, 2010; Michener and Schell, 1994). Recently, naturally occurring tracers of stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are recommended to investigate the trophic relationships and the biomagnification of contaminants (Bucci et al., 2007). The $\delta^{15}N$ is effective in quantifying the trophic position because the enrichment of nitrogen isotope occurs incrementally across trophic levels with a constant rate (3–4‰). For $\delta^{13}C$, the enrichment of carbon isotope is not obvious (i.e., approx.1‰) amongst different trophic levels (Hobson and Welch, 1992). Thus, it is considered as a valuable biomarker for identifying different sources of primary production (Hobson et al., 2002; Hoekstra et al., 2003). Stable isotope analyses are widely used in ecotoxicological studies to elucidate contaminant behavior (e.g., bioconcentration and biomagnifications) through the whole trophic chains (Borga et al., 2001; McIntyre and Beauchamp, 2007).

In the last decades, with the rapid development of economy in Asian countries, environmental pollution caused by heavy metals was identified in several delta areas, e.g., the Mekong Delta (Cenci and Martin, 2004), the Pearl River Delta (Cheung et al., 2008), and the Yangtze River Delta (Zhao et al., 2007). The Yellow River Delta (YRD) is an over-wintering and breeding site for migrating birds in the Northeast Asian Inland and the Western Pacific Rim. However,

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few studies focused on heavy metal pollution in the newly-formed wetlands. Further investigation of the trophic positions and feeding organisms within wetland food webs at different contaminant levels is required in order to provide sound basis for habitat protection.

The objectives of this paper are to 1) determine the levels of heavy metals (i.e., Mn, Zn, Cu, Cd, Cr, Pb, Ni, As and Hg) in wetland food webs of the YRD; 2) evaluate and quantify biomagnification of heavy metals by calculating the bioaccumulation factor (BAF) and biomagnification factor (BMF).

2. Materials and methods

2.1. Study sites

The YRD is located in the northern Shandong province, China. It lies on the south side of the Bohai Sea, spanning from 118°07′E to 119°18′E, and from 36°55′N to 38°12′N, with an area of 6010 km^2 (Fig. 1). This region is characterized as a temperate, semi-humid continental monsoon climate. The YRD is one of the most active land–ocean interaction regions in the world. Substantial sands are carried by the Yellow River and deposit at the river mouth every year. New lands are then formed. As a result, an abundance of wetland vegetation and aquatic resources provide exceptional habitats for many species of birds for breeding, migrating, and wintering. This region has been an important stopover in the inland of northeast Asia and around the western Pacific Ocean for bird migration. In 1992, the YRD was designated as a national nature reserve in order to protect the newly-formed wetlands, and rare and endangered bird species, where 7 bird species are listed as the first priority for national conservation and 33 species are listed as the second priority. In the Convention on International Trade in Endangered Species (CITES) of wild fauna and flora, 7 bird species are listed in the annex I, 26 species in the annex II, and 7 species in the annex III.

2.2. Sampling

Samples of water and sediments were collected from nine sites (A, B, C, D, E, F, G, H, and I) with three paralleled samples for each site. Water milfoil, invertebrates and fish were obtained from five sites (A, B, C, D, and E) in August 2008 (Fig. 1). Bird samples were obtained from the Rescue Service Center of YRD and local residents.

Sediment samples were collected at depths varying from 0 to 20 cm. These samples were then pooled and homogenized within polyethylene bags. After these samples were frozen for drying and milled with agate mortar, they were then sieved (0.15 mm) for further testing and analyzing. Water samples were gathered from surface water with 50 ml acid-washed polyethylene sample bottles, and then filtered using membrane with 0.45 μm. Nitric acid was put into water samples treated to the pH value which was controlled at 2. All water and sediment samples were stored at 4 °C before analysis.

Sample details for primary producers (water milfoil (*Myriophyllum spicatum*)), four invertebrate species (Chinese mitten crab (*Eriocheir sinensis*), crab (*Helice tridens tientsinensis*), Chinese shrimp (*Fenneropenaeus chinensis*), clam (*Mactra veneriformis*)), six fish species, i.e., weever (*Lateolabras japonicus*), catfish (*Chaeturichthys sitgmatias*), common carp (*Cyprinus carpio Linnaeus*), Silver carp (*Hypophthalmichthys molitrix*), Redeye mullet (*Liza haematocheila*), and javelin goby (*Acanthogobius hasta*)), and four water bird species (Saunders's Gull (*Larus saundersi*), Purple Heron (*Ardea purpurea*), Little Egret (*Egretta garzetta*), and Spot-billed Duck (*Anas poecilorhyncha*) are shown in Table 1. All organism samples were stored at −20 °C before analysis.

2.3. Analysis for heavy metals

The filtered samples of water were digested with sulfuric acid and potassium bromate- potassium bromide for Hg analysis, and were reduced with ascorbic acid-thiourea for As (PRC EPM, 2002). The samples of sediments and organisms were pooled and homogenized, respectively. For analysis of heavy metals, 0.2 g of powdered dry sample was digested by a microwave system with HNO_3 in teflon-lined vessels under the conditions of controlled pressure. Concentrations of Mn, Zn, Cu, Cd, Cr, Pb, and Ni were determined by Inductively Coupled Plasma Spectroscope analysis (ICP-JY Ultima, Jobin Yvon Co., France). The concentrations of Hg and As were evaluated using Atomic Fluorescence Spectrometry (AFS-930, Beijing Vital Co., Beijing, China). For quality assurance and quality control, standard reference materials (GBW07401 and homogenate muscle sample, IAEA-407, from the International Laboratory of Marine Radioactivity, IAEA, Monaco) and process blanks were digested and analyzed with each batch of samples. The recovery rates of metals in the standard reference materials are about 90–110%. In this study, heavy metal concentrations in the blanks are less than 1% of the samples and all of the relative standard deviations of the replicate samples are less than 10%. The detection limits of detectors for each metal are: As 0.00005 μg ml^{-1}, Cd 0.0001 μg ml^{-1}, Cr 0.0003 μg ml^{-1}, Cu 0.0005 μg ml^{-1}, Ni 0.0003 μg ml^{-1}, Pb 0.001 μg ml^{-1}, Zn 0.0001 μg ml^{-1}, Hg 0.000005 μg ml^{-1}, and Mn 0.00005 μg ml^{-1}.

2.4. Stable isotopes

To analyze the stable isotopes for nitrogen and carbon, samples of water milfoil, Chinese shrimp, crabs, fish, and water birds (Table 1) were first homogenized respectively by an analytical mill and then were freeze-dried. Lipids were removed from all samples by methanol extraction within 12 h to reduce the variability caused by isotopically lighter lipids. Before implementation of isotope analysis, the samples were further dried at 80 °C for 4 h and then 0.5 mg samples were set in 8 × 5 mm Sn capsules and combusted at 1000–1050 °C. Nitrogen and carbon were transported through the interface (ConFlo III, Finnigan MAT) and were analyzed using a mass spectrometer (THERMO Delta plus, Finnigan MAT).

The stable isotope abundance (δ) is given by

$$\delta X = \left(\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right) \times 1000(‰) \quad (1)$$

where X is ^{13}C or ^{15}N and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The values of R_{standard} can be determined based on the PeeDee Belemnite (PDB) for ^{13}C and atmospheric N_2 (AIR) for ^{15}N. Replicate measurements of internal laboratory standards (albumen) show that the measurement errors are ±0.3‰ and ±0.2‰ for stable carbon and stable nitrogen isotope measurements, respectively.

Trophic level (TL) for each aquatic organism is given as

$$TL_{\text{consumer}} = 2 + \left(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{clam}}\right)/3.8 \quad (2)$$

where TL_{consumer} is the trophic level and the trophic level of clam is assumed to be 2. The calculation of trophic level for birds was given by

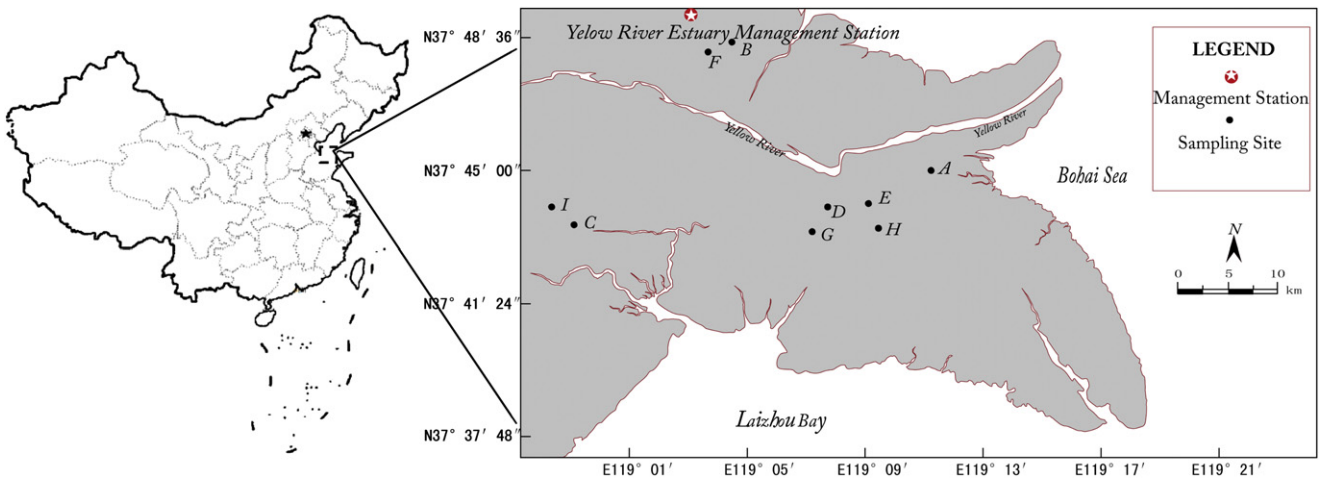

Fig. 1. Study area with the designed sites (A, B, C, D, E, F, G, H, and I) for sample collection in the Yellow River Delta.

Table 1
Sample details of organisms from the YRD wetlands.

Names	$\delta^{13}C$	$\delta^{15}N$	Nd[a]	Na[b]	Body weight (g)		Body length (cm)		TL[c]
	Mean ± SD	Mean ± SD			Min–Max	Mean ± SD	Min–Max	Mean ± SD	
Primary producer									
Water milfoil	−10.74 ± 0.67	4.47 ± 0.28	3	3					1.50
Invertebrates									
Clam	−21.04 ± 0.47	6.40 ± 0.45	5	3					2.00
Crap	−19.98 ± 0.59	6.85 ± 0.14	5	3					2.11
Chinese shrimp	−21.18 ± 0.58	6.65 ± 0.32	5	3					2.08
Chinese mitten crab	−25.34 ± 0.71	5.83 ± 0.26	5	3					1.88
Fish									
Catfish	−20.11 ± 0.40	9.57 ± 0.36	6	3	250–400	330 ± 75.49	33–42	37 ± 4.72	2.85
Common carp	−23.15 ± 0.52	8.93 ± 0.66	6	3	99–2545	911 ± 1117.55	20–47	33 ± 11.22	2.65
Javelin goby	−13.98 ± 0.21	12.65 ± 0.05	4	3	200–400	305 ± 82.25	42.5–49	46 ± 2.86	3.65
Redeye mullet	−21.27 ± 0.50	7.06 ± 1.00	6	3	91–1850	478 ± 685.77	24.2–57	33 ± 12.51	2.18
Silver carp	−24.82 ± 0.37	7.71 ± 1.01	5	3	115–850	443 ± 373.48	23–41	30 ± 8.79	2.29
Weever	−21.18 ± 0.58	9.23 ± 0.27	4	3	125–400	289 ± 116.35	27–30	29 ± 1.31	2.74
Water Birds									
Spot-billed Duck	−20.38 ± 3.06	9.69 ± 1.09	2	3	1310–1500	1405 ± 134.35	53–62	58 ± 6.36	3.28
Purple Heron	−24.69 ± 0.34	8.34 ± 0.27	3	3	804–1120	983 ± 162.13	92–109	102.33 ± 9.07	2.85
Saunders's Gull	−20.00 ± 0.89	11.95 ± 0.69	2	3	173–198	186 ± 17.67	33–36	35 ± 2.12	3.80
Little Egret	−21.43 ± 1.20	9.38 ± 0.31	3	3	356–403	377 ± 23.79	64–70	67 ± 3.65	3.11

[a] Nd: the number of organisms that were used to determine the concentrations of heavy metals.
[b] Na: the number of organisms that were used in stable isotopes analysis.
[c] TL: trophic level.

$$TL_{bird} = 3 + \left(\delta^{15}N_{bird} - 2.4 - \delta^{15}N_{clam}\right)/3.8 \quad (3)$$

where the diet-tissue isotope fractionation factor is +2.4‰, TL_{bird} is the trophic level of birds, and $\delta^{15}N_{bird}$ is the stable nitrogen isotope value of birds (Fisk et al., 2001).

The food web was then determined according to trophic level obtained from $\delta^{15}N$. Food sources were determined by $\delta^{13}C$ and the prey–predatory relation was described by literature (Gounter and Furness, 1997; Montesinos et al., 2008).

2.5. Calculations for trophic magnification and biomagnification factors

The trophic magnification factor (TMF) represents the average increase rate per TL rather than specific predator–prey relationship. Thus, it is employed to measure the biomagnification of heavy metals in food webs. The continuous integrative measures of trophic position were obtained according to the stable nitrogen isotope ratios presented by Fisk et al. (2001) and Johnson and Schindler (2009). The TMF is determined based on a single linear regress relationship between trophic level and heavy metal concentration (HMC) (Hu et al., 2005) given by

$$\mathrm{Log(HMC)} = a + b \times \mathrm{TL} \quad (4)$$

where a and b are constants. The coefficient b was used to evaluate food web magnification factor (FWMF) (Dehn et al., 2006) and TMF (Hu et al., 2005) given by

$$\mathrm{FWMF} = b \quad (5)$$

and

$$\mathrm{TMF} = 10^{b} \quad (6)$$

Biomagnification factor (BMF) was evaluated by

$$\mathrm{BMF} = \left(\mathrm{Metal_{Predator}}/\mathrm{Metal_{Prey}}\right)/\left(\mathrm{TL_{Predator}}/\mathrm{TL_{Prey}}\right) \quad (7)$$

where $\mathrm{Metal_{Predator}}$ and $\mathrm{Metal_{Prey}}$ are metal concentrations of predator and prey species, respectively (Hoekstra et al., 2003).

Bioconcentration factor (BCF) is defined as (McGeer et al., 2003):

$$\mathrm{BCF} = Css/Cw \quad (8)$$

where Css is the heavy metal concentration in organisms at steady state (μg g^{-1}dry weight), and Cw is the heavy metal concentration in water (μg ml^{-1}).

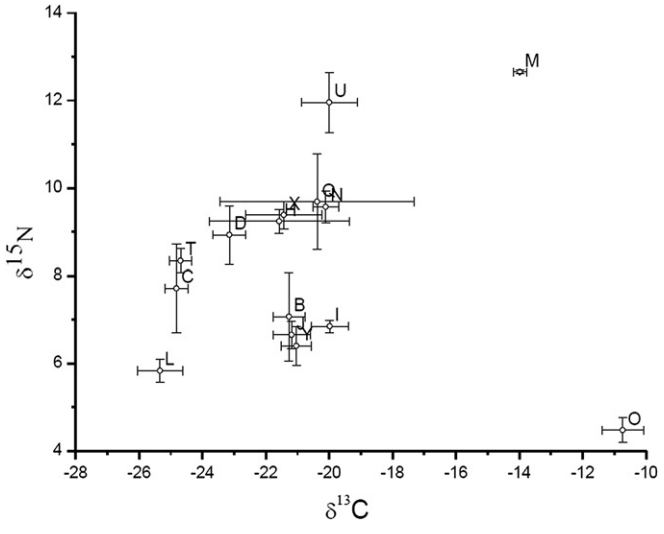

Fig. 2. Stable isotope diagram of members of wetland food web in the YRD: $\delta^{15}N$ ‰ (mean ± SD) versus $\delta^{13}C$ ‰ (mean ± SD).

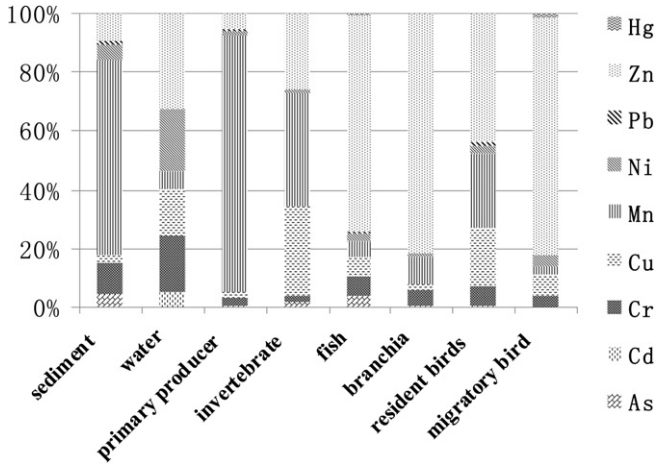

Fig. 3. Composition of nine heavy metals in different samples.

Table 2
Heavy metal concentrations in water (μg ml^{-1}) and sediment (μg g^{-1} DW) of the YRD.

Names	Site	N	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
Water	A	3	ND	0.003	0.009	0.007	0.002	0.008	ND	0.013	ND
	B	3	ND	0.002	0.007	0.007	0.002	0.009	ND	0.013	ND
	C	3	ND	0.002	0.005	0.007	0.002	0.012	ND	0.016	ND
	D	3	ND	0.001	0.005	0.006	0.005	0.006	ND	0.014	ND
	E	3	ND	0.001	0.006	0.006	0.001	0.009	ND	0.013	ND
	F	3	ND	0.001	0.007	0.006	0.001	0.006	ND	0.016	ND
	G	3	ND	ND	ND	0.003	ND	ND	ND	0.005	ND
	H	3	ND	0.001	ND	0.003	ND	ND	ND	0.004	ND
	I	3	ND	ND	ND	0.004	0.001	0.003	ND	0.004	ND
	mean		ND	0.002	0.007	0.005	0.002	0.007	ND	0.011	ND
Sediment	A	3	27.18	0.12	62.62	14.69	336.36	28.51	9.01	55.04	0.002
	B	3	25.88	0.08	66.56	13.87	378.60	26.60	8.40	48.10	0.004
	C	3	26.17	0.01	56.54	11.23	314.91	21.25	6.52	37.89	0.004
	D	3	25.54	0.10	56.06	13.00	348.94	27.30	9.77	54.30	0.006
	E	3	20.88	0.07	57.82	12.18	305.36	23.87	7.30	47.23	0.004
	F	3	26.81	0.08	50.86	18.15	314.01	29.47	8.61	53.90	0.011
	G	3	29.28	0.09	58.65	19.85	474.67	33.66	12.68	57.11	0.008
	H	3	27.97	0.10	63.06	19.58	468.90	31.27	9.87	53.77	0.145
	I	3	34.09	0.19	61.06	20.15	496.35	33.27	11.44	65.97	0.018
	mean		27.09	0.09	59.25	15.86	382.01	28.36	9.29	52.59	0.022

ND: not detected, N: sample quantity.

2.6. Statistical analysis

Statistical analysis was implemented using SPSS for Windows (Ver 17.0; SPSS, Chicago, IL, USA). Correlation between heavy metal concentration and trophic level was examined using Pearson's rank correlation test. When the p value is less than 0.05, the linear regression between the heavy metal concentration and the trophic level is considered as significant. The profile patterns of heavy metals were evaluated by principal component analysis (PCA) for all samples. This analysis was carried out on log-transformed data. In a case of missing values (e.g., below the detection limit), the concentrations were treated as half of the detection limit.

3. Results

3.1. Trophic levels of organisms in wetland food web

The values of $\delta^{13}C$ and $\delta^{15}N$ respectively varying from −25.73‰ to −10.74‰ and from 4.48‰ to 12.78‰ were detected in food webs based on the water milfoil (Fig. 2). Significant differences of $\delta^{13}C$ and $\delta^{15}N$ amongst different aquatic species were also observed

Table 3
Heavy metal concentrations (μg g^{-1} DW) in organisms of the YRD wetlands.

	As Mean ± SD	Cd Mean ± SD	Cr Mean ± SD	Cu Mean ± SD	Mn Mean ± SD	Ni Mean ± SD	Pb Mean ± SD	Zn Mean ± SD	Hg Mean ± SD
Primary producer									
Water milfoil	2.14 ± 0.12	0.23 ± 0.05	15.30 ± 0.13	9.23 ± 2.32	466.40 ± 48.90	8.73 ± 1.22	3.32 ± 2.05	28.32 ± 5.12	0.05 ± 0.01
Invertebrate									
Crap	4.32 ± 0.23	0.44 ± 0.05	5.87 ± 0.77	112.86 ± 35.75	170.20 ± 32.32	3.92 ± 0.87	0.80 ± 0.53	63.45 ± 19.63	0.12 ± 0.01
Chinese shrimp	7.50 ± 0.39	ND	3.62 ± 0.43	80.59 ± 23.44	2.77 ± 0.26	2.17 ± 0.68	0.39 ± 0.12	58.00 ± 17.83	0.10 ± 0.02
Clam	4.52 ± 0.31	0.45 ± 0.07	3.34 ± 0.33	4.00 ± 1.27	22.68 ± 4.43	4.38 ± 1.04	0.14 ± 0.03	70.92 ± 24.65	ND
Chinese mitten crab	4.77 ± 0.27	0.29 ± 0.02	5.31 ± 0.82	76.88 ± 21.07	82.51 ± 17.62	3.70 ± 1.31	ND	92.79 ± 19.37	0.21 ± 0.01
Mean	5.28	0.39	4.54	68.58	69.54	3.54	0.44	71.29	0.14
Fish									
Redeye mullet	0.97 ± 0.32	ND	3.60 ± 1.00	3.72 ± 1.09	2.58 ± 0.81	1.58 ± 0.94	ND	21.60 ± 4.95	0.10 ± 1.22
S[a]	0.55 ± 0.02	0.03 ± 0.01	5.65 ± 0.35	12.40 ± 9.35	15.06 ± 0.26	2.21 ± 0.39	3.60 ± 2.12	55.26 ± 1.83	0.03 ± 1.16
Silver carp	1.46 ± 0.84	ND	3.97 ± 0.49	5.07 ± 1.85	3.40 ± 0.92	2.27 ± 1.22	0.50 ± 0.02	46.86 ± 21.97	0.59 ± 1.47
S[a]	0.81 ± 0.37	0.03 ± 0.00	5.76 ± 0.78	8.08 ± 2.28	43.29 ± 14.15	1.70 ± 0.14	0.58 ± 0.24	118.81 ± 2.76	0.11 ± 1.48
Common carp	0.23 ± 0.02	ND	4.84 ± 0.94	4.39 ± 2.23	2.68 ± 0.47	1.35 ± 0.83	0.91 ± 0.04	61.41 ± 16.03	0.46 ± 0.25
S[a]	ND	ND	49.15 ± 45.91	4.37 ± 1.26	20.24 ± 3.19	1.35 ± 0.66	0.41 ± 0.05	713.13 ± 103.86	0.11 ± 0.01
Weever	2.75 ± 0.32	ND	3.79 ± 0.10	2.32 ± 1.20	2.41 ± 1.07	1.66 ± 0.16	0.86 ± 0.14	29.24 ± 3.38	0.53 ± 0.01
S[a]	1.46 ± 0.24	ND	4.51 ± 1.29	4.43 ± 1.23	13.87 ± 9.40	1.31 ± 0.34	0.53 ± 0.07	104.42 ± 5.91	0.13 ± 0.18
Javelin goby	8.97 ± 2.25	ND	3.96 ± 0.24	11.20 ± 10.89	4.07 ± 1.52	1.79 ± 0.77	0.55 ± 0.37	79.36 ± 22.16	0.12 ± 0.09
S[a]	4.79 ± 0.67	0.05 ± 0.01	7.42 ± 3.31	3.64 ± 0.24	23.51 ± 6.29	2.56 ± 1.78	0.74 ± 0.02	160.42 ± 6.48	0.11 ± 0.02
Catfish	1.83 ± 0.58	ND	5.13 ± 0.33	2.62 ± 0.39	4.29 ± 0.75	3.52 ± 3.05	0.32 ± 0.04	60.35 ± 15.02	1.26 ± 1.25
S[a]	1.72 ± 1.54	0.05 ± 0.02	5.63 ± 1.63	3.02 ± 0.15	16.33 ± 2.03	1.93 ± 0.45	0.78 ± 0.22	107.40 ± 8.37	0.15 ± 0.02
Fish[b]	2.50	ND	5.09	14.63	4.00	2.39	1.43	60.91	0.51[d]
Branchia[c]	1.61	0.04	11.72	5.98	24.19	1.78	1.04	218.56	0.11[d]
Water bird									
Spot-billed Duck	0.24 ± 0.04	ND	5.00 ± 1.44	13.81 ± 2.18	18.06 ± 15.38	1.89 ± 1.04	0.85 ± 0.29	30.84 ± 1.91	0.20 ± 0.09
Purple Heron	0.21 ± 0.03	ND	16.29 ± 3.22	22.31 ± 2.17	10.63 ± 3.62	19.75 ± 5.33	0.20 ± 0.05	218.56 ± 21.38	3.42 ± 0.54
Saunders's Gull	0.36 ± 0.04	ND	6.46 ± 1.98	19.02 ± 3.13	3.95 ± 1.43	4.00 ± 1.31	0.45 ± 0.08	81.46 ± 9.73	3.70 ± 0.73
Little Egret	0.19 ± 0.02	ND	3.47 ± 1.83	10.83 ± 1.89	6.70 ± 2.46	1.08 ± 0.27	1.02 ± 0.42	272.35 ± 19.27	0.03 ± 0.01
Mean	0.25	ND	7.81	16.49	9.84	6.68	0.63	150.80	1.84

[a] S: the concentrations of heavy metals in the branchia for different fish (B,C,D,H,M and N).
[b] Mean concentration of heavy metals in all fish tissues.
[c] Mean concentration of heavy metals in fish branchia.
[d] The concentrations of Hg in fish were displayed as geomean ± GSD due to the high discrete data.

($p < 0.001$). From Fig. 2, the data points of $\delta^{13}C$ and $\delta^{15}N$ tend to intensive distribution, indicating that there are several primary food sources for those organisms at higher trophic levels. Assuming the $\delta^{15}N$ is the lowest level (4.48‰) in water milfoil, the difference of $\delta^{15}N$ values between the water milfoil and the Saunders's Gull, which is the highest trophic level, was estimated to be 7.47‰. According to the $\delta^{15}N$, all samples can be divided into four groups, i.e., water milfoil, invertebrates including crab, Chinese shrimp and clam, fish, and birds. Moreover, the $\delta^{15}N$ values were converted to trophic levels, which vary from 1.50 to 3.80 in organisms (Table 1).

3.2. Heavy metal concentrations

There were some significant variations of metals in water, sediments, and organisms (calculated as percentage of total heavy metals) (Fig. 3). In water, the concentration of Zn was the highest, followed by Cr, Ni, Cu, Mn and Cd. As, Hg and Pb were all less than 0.001 μg ml^{-1}. Mn was the highest in sediments, followed by Cr, Zn, Ni, As, Cu, Pb, Cd, and Hg (Table 2). Moreover, one can observe that the heavy metal concentrations in various aquatic organisms are different (Table 3). The concentrations of Mn, Ni, and Cr are the highest in water milfoil. Zn in branchia, Hg in water birds, and As, Cu and Cd in invertebrates have the highest concentrations amongst those selected organisms. The highest concentration of Hg was observed in Purple Heron (3.42 μg g^{-1} DW) and Saunders's Gull (3.70 μg g^{-1} DW). Cr, Ni, Zn, Hg in Purple Heron, As, Ni, Zn, and Hg in Saunder's Gull, and Zn in Little Egret were higher than that in migratory birds (Table 4).

3.3. Profile patterns of heavy metals

It was found that each species had a specific profile (Fig. 4). Four principal components (PC1, PC2, PC3, and PC4) were identified after varimax rotation and accounted for 32%, 28%, 17%, and 8% of the total variance, respectively. PC1 is mainly associated with Cr, Ni, Hg, and As, PC2 is dominated by Mn, Cd, and Pb, PC3 is characterized as high Cu load, and PC4 is correlated with Zn. All species can be classified into four groups according to each individual species, i.e., primary producer, invertebrates, fish, and water birds. These samples can also be classified into three groups based on trophic levels: the first group including water milfoil, Chinese shrimp, and Chinese mitten crab (TL: 1.50–2.08); the second group including Spot-billed Duck and Purple Heron (TL: 2.85–3.28); and the third one including fiddler crab, Chinese shrimp, catfish, common carp, javelin goby, redeye mullet, silver carp, weever, Saunders's Gull, and Little Egret (TL: 2.00–3.80).

3.4. Biomagnification of heavy metals

The relationships between trophic levels and heavy metal concentrations show a trophic level-dependent accumulation in food webs (Fig. 5 and Table 5). Cd, Zn and Hg were identified to increase with the TL, while As, Cr, Cu, Mn, Ni and Pb show an opposite trend (Fig. 5). From Table 5, one can find that the FWMF and the TMF vary from −0.58 (for Mn) to 0.45 (for Hg) and from 0.26 (for Mn) to 2.82 (for Hg), respectively. A slope greater than zero indicates

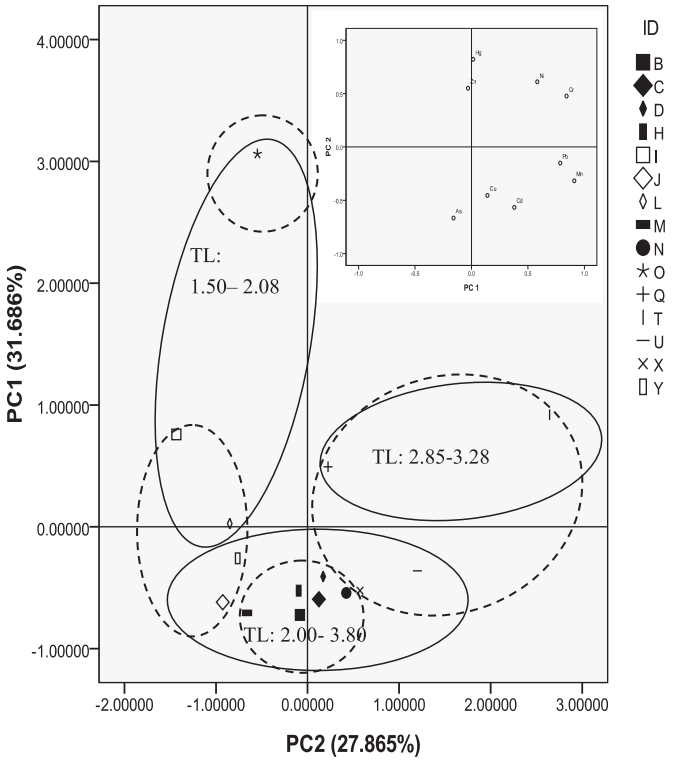

Fig. 4. Principal component (PC) 2 versus 1 from standardized data for the YRD. The variables used were As, Cd, Cr, Mn, Ni, Pb, Cu, Zn, and Hg. The graph in the upper right shows the contribution of variables associated with the principle components in a loading plot.

that metals are accumulated in food webs, while the slope less than zero suggests the elimination of the heavy metals from the food webs or an interrupted trophic transfer. Cd was found in a food web component, i.e., water milfoil to invertebrate (0.49 for FWMF and 3.12 for TMF). However, it cannot be detected in fish and water birds.

The BCF of heavy metal is different in various organisms. The highest BCFs of Cr, Mn, and Ni were found in primary producer, while the highest BCFs of Cd and Cu were identified in invertebrates (Table 6). The BMF varies from 0.02 (e.g., for As from Chinese shrimp to Little Egret) to 64.17 (for Hg from clam to Saunders's Gull) (Table 7). Although the concentrations of Hg, Cu, and Pb (corrected for TL) increase substantially, the biomagnification effects of As, Cd, Cr, Mn, Ni, Zn on species varying from clam to Saunders's Gull are not significant. Similarly, the metals of As, Cu, Pb were not transferred from Chinese shrimp to Purple Heron, while Cr, Mn, Ni, Zn, and Hg increased significantly.

4. Discussion

4.1. Heavy metal concentration in environmental media

No heavy metal concentrations in water exceed the Level II of China National Environmental Quality Standards for Surface Water (Level II for habitat protection of aquatic organism, spawning

Table 4
The concentration levels of heavy metals in resident birds (Spot-billed Duck) and migratory birds.

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
Spot-billed Duck	0.24	ND	5.00	13.81	18.06	1.89	0.85	30.84	0.19
Purple Heron	0.21\0.88	ND\–	16.29\3.26	22.31\1.62	10.63\0.59	19.75\10.44	0.20\0.23	218.56\7.08	3.42\17.10
Saunders's Gull	0.36\1.50	ND\–	6.46\1.29	19.02\1.38	3.95\0.22	4.00\2.12	0.45\0.53	81.46\2.64	3.70\18.50
Little Egret	0.19\0.79	ND\–	3.47\0.69	10.83\0.78	6.70\0.37	1.08\0.57	1.02\1.20	272.35\8.83	0.03\0.15

The left part of "\" was the heavy metal concentration, the right part of "\" was the ratio of trace metal concentration for migratory birds to resident birds.

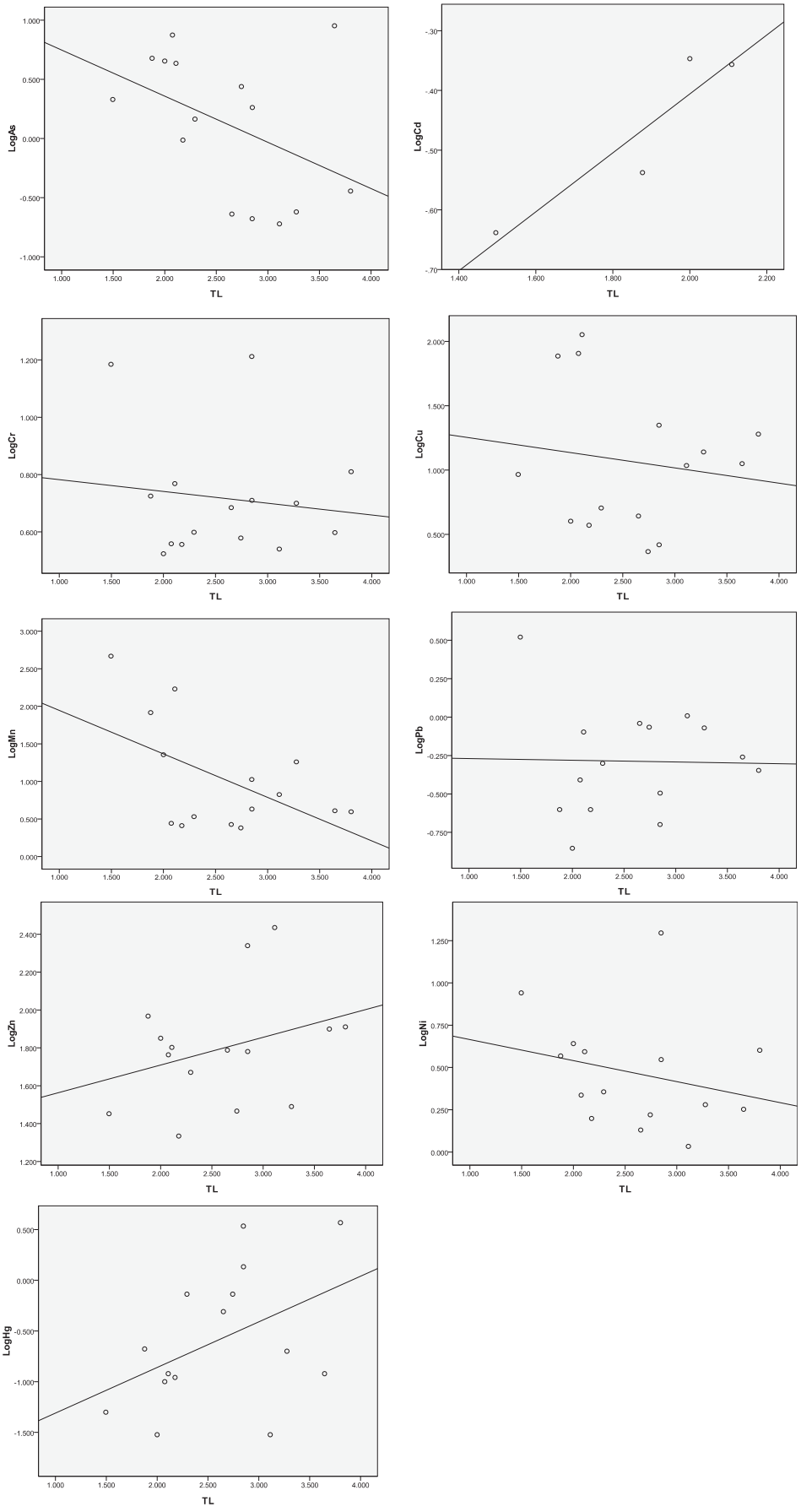

Fig. 5. The relationship between trophic levels and concentrations of heavy metals in biota.

Table 5
Regression analysis between logarithm of concentrations and trophic levels (slope, *p*-value of slope) and TMFs for heavy metals.

	As	Cd[c]	Cr	Cu	Mn	Ni	Pb	Zn	Hg
FWMF[a] (slope)	−0.39	0.49	−0.04	−0.12	−0.58	−0.13	−0.01	0.15	0.45
Intercept	1.14	−1.39	0.82	1.37	2.52	0.79	−0.26	1.42	−1.76
R	0.43	0.93	0.13	0.15	0.53	0.25	0.02	0.32	0.45
TMF[b]	0.41	3.12	0.91	0.76	0.26	0.75	0.98	1.40	2.82
p-value	0.11	0.08	0.65	0.60	0.04	0.37	0.94	0.24	0.10

[a] Food web magnification factor (slope of linear regression). R = coefficient of determination of linear regression. p-value of linear regression.
[b] Trophic magnification factors TMF = $10^{\text{slope of linear regression}}$.
[c] Cd is found in food web of water milfoil - invertebrate, however, the concentration of Cd was below the determination limit in fish and water bird.

ground of fish and shrimp, and food ground of juvenile fish) (PRC EPM and PRC AQSIQ, 2002), and the Standard of Aquatic Life Protection Chronic of USEPA (USEPA, 2009). Concentrations of As, Mn, Cu, Cr, and Pb in water are lower than that in Samborombon Bay wetlands, Argentina (Schenone et al., 2007). Cd, Cr, Cu, Pb, Zn are lower than that in Yangtze River Delta (Huang et al., 2006) and Pearl River Delta (Cheung et al., 2003). The concentrations of Zn, Pb, Cd, Cr, Cu, and Hg in sediments are in compliance with the Level I of China National Quality Standards for Soil (The Level I for protecting natural ecosystems and maintaining the soil baseline), while As is in compliance with the Level II of China National Quality Standards for Soil (Level II for agricultural production and human health) (PRC EPM and PRC AQSIQ, 1995). Compared with the quality baseline in sediment of Canada such as Threshold Effect Level (TEL) and the Probable Effect Level (PEL), the concentration order is Pb, Zn, Cd, Hg, and Cu < TEL < Cr < PEL< As (CCME, 1995). It is possible that pesticide used in farmlands around wetlands leads to higher As concentration. Moreover, all heavy metal concentrations in sediments are lower than that in Yangtze River Delta (Yi et al., 2008), and Cd, Cr, Cu, Ni, Pb, Zn are lower than that in Pearl River Delta (Cheung et al., 2003). This suggests lower external sources of heavy metals in the Yellow River Delta.

4.2. Heavy metal concentration in fish and invertebrates

The heavy metal concentrations in fish and invertebrates are similar to or lower than that reported by Anan et al. (2005) and Kojadinovic et al. (2007). However, Hg, Pb, Cu and Zn in fish and invertebrates (except common carp) are lower, and Cd in invertebrates is higher than that presented in MAFF (2000). As shown in Table 8, in invertebrates of the YRD, the levels of Mn, Zn, Pb, and Cd are lower or similar to, but Cr, Cu, As, and Hg are higher than that in Mekong Delta (Ikemoto et al., 2008). However, Cd, Cu, Zn, Pb, Ni, Cr are lower than that in the Pearl River Delta (Cheung et al., 2008). Mn in fish is lower, while Zn, Cu, Cr, and Hg are higher than that in Sagar Island, Sunderban (Saha et al., 2006). Cd in common carp is lower, and Cu, Pb, Zn, Hg, Cr, and As are higher, Cu and Cd in weever are lower, and Pb, Zn, Hg, Cr, and As are higher than that in the Yangtze River (Yi et al., 2008). This may be caused by different habitats, species or different growth rates (Farag et al., 2007; Marin-Guirao et al., 2008).

Table 6
The values of bioconcentration factor (BCF) of heavy metals in wetlands of YRD.

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
Primary producer	–	117	2185	1846	233 199	1247	–	2574	–
Invertebrate	–	171	711	16 669	51 547	524	–	6243	–
Fish	–	–	605	1018	1667	300	–	4688	–
Resident birds	–	–	715	2762	9030	269	–	2803	–
Migratory birds	–	–	936	2608	2659	886	–	13 008	–

"–" respects no detection.

Table 7
Predator/Prey biomagnification factor (BMF).

Predator	Prey	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
Purple Heron	Shrimp	0.02	ND	3.27	0.20	2.80	6.63	0.38	2.75	24.22
	Silver carp	0.12	ND	3.31	3.54	2.52	6.99	0.32	3.76	3.80
Saunders's Gull	Shrimp	0.03	ND	0.97	0.13	0.78	1.01	0.64	0.77	19.64
	Clam	0.04	ND	1.02	2.50	0.09	0.48	1.64	0.60	64.17
	Silver carp	0.15	ND	0.98	2.26	0.70	1.06	0.54	1.05	3.08
Little Egret	Silver carp	0.10	ND	0.64	1.57	1.45	0.35	1.50	4.28	0.03
	Shrimp	0.02	ND	0.64	0.09	1.61	0.33	1.75	3.13	0.18
Weever	Silver carp	1.58	ND	0.80	0.38	0.59	0.61	1.42	0.52	0.84
	Shrimp	0.28	ND	0.79	0.02	0.66	0.58	1.67	0.38	5.39
Catfish	Silver carp	1.01	ND	1.04	0.42	1.02	1.25	0.51	1.04	1.51
	Shrimp	0.18	ND	1.03	0.02	1.13	1.18	0.59	0.76	9.65
Javelin goby	Silver carp	3.88	ND	0.63	1.39	0.75	0.50	0.68	1.07	0.11
	Shrimp	0.68	ND	0.62	0.08	0.84	0.47	0.80	0.78	0.68

Generally, the concentrations of As, Mn, Cu, Cd, Ni, and Zn are higher in invertebrates, while Cr, Pb, and Hg are higher in fish, according to the values corrected for trophic level (Table 3). Marin-Guirao et al. (2008) also presented that the concentrations of Mn, Cu, and Cd are much higher in invertebrate muscles than that in fish from the Mar Menor coastal lagoon in Mediterranean area. Farag et al. (2007) presented that more heavy metals of As, Hg, Cu, Cd, and Zn are accumulated in invertebrates than that accumulated in fish in the Boulder River watershed from Motana. This can be caused by the differences of metal accumulation and detoxification mechanisms, e.g., metallothioneins (MTs) which can bind and sequester toxic and excess heavy metals (Laura, 2009).

4.3. Heavy metal concentration in water birds

Heavy metals in water birds are highly species-specific and related to trophic position, migration, and molt (Lavoie et al., 2010). The difference of heavy metal concentrations in different birds which lie in the highest trophic levels in the study area is identified. Resident bird such as Spot-billed Duck, which lives in the YRD during the whole year, is able to reflect the local heavy metal bioaccumulation. However, migratory birds, e.g., Purple Heron, Saunders's Gull, and Little Egret will move to the south in winter, the bioaccumulation of heavy metals will vary with different habitats, reflecting the differences of feeding habits at local and other places. Mn concentrations in three kinds of migratory birds are all lower than that in Spot-billed Duck, indicating that Mn in local environment is higher than that in other places.

Saunders's Gull is a globally threatened species. It is listed as 'Vulnerable' due to its small population (7100–9600 individuals), and it also listed on Appendix I of the Convention on Migratory Species (CMS, 2007). Cd in Saunders's Gull is lower, while Cu, Hg, Mn, and Zn are higher than that in Audubon's Shearwater of Reunion Island (Kojadinovic et al., 2007). Moreover, Cu in Saunders's Gull of the YRD is similar to, while Pb and Cd are lower than that of Black-headed Gull in the dam reservoir in south-western Poland (Orlowski et al., 2007). However, by comparing the heavy metal concentrations in Saunders's Gull with that in other three kinds of birds, the difference is not obvious. This suggests that heavy metal is not the major reason to lead the Saunders's Gull to be endangered.

Cd, Cu, Pb, and Zn in Spot-billed Duck of the YRD are lower, while Mn is higher than that in the Mallard of wetlands in Szczecin, Poland (Kalisinska et al., 2004). As and Cu in Purple Heron and Little Egret of the YRD are lower, while Cr, Mn, Zn, and Hg are higher, respectively,

Table 8
Heavy metal concentrations (μg g^{-1} DW) in whole organisms from references.

Sources	Names	As	Cd	Cr	Cu	Mn	Pb	Zn	Hg
MAFF, 2000 [a]	Fish	ND	0.80	ND	80.00	ND	8.00	80.00	1.20
	Invertebrate	ND	ND	ND	120.00	ND	40.00	400.00	1.00
Ikemoto et al., 2008	Fish	0.51–2.10	0.01–0.06	0.47–11.00	2.27–4.00	8.76–47.90	0.08–0.36	54.90–122.00	0.11–0.99
	Invertebrate	1.60–2.80	0.04–1.47	0.27–4.80	54.50–161.00	17.30–96.90	0.09–0.41	49.10–145.00	0.04–0.06
Saha et al., 2006	Fish	ND	ND	0.65	8.50	13.25	ND	21.25	0.35
Yi et al., 2008 [a]	Common carp	0.09	0.07	0.04	3.41	ND	0.08	28.80	0.06
	Weever	0.05	0.01	0.02	11.00	ND	0.04	27.32	0.04
Kojadinovic et al., 2007	Audubon's shearwater	ND	4.55	ND	21.00	1.83	ND	73.00	0.38
Ribeiro et al., 2009	Razorbill	3.18	0.06	8.22	20.29	2.39	0.37	42.84	2.67
Orlowski et al., 2007	Black-headed Gull	ND	0.39	ND	19.88	ND	3.59	ND	ND
Kalisinska et al., 2004	Mallard	ND	0.02	ND	23.60	2.40	4.36	49.00	ND
Horai et al., 2007	Grey heron	0.28	0	0.24	49.30	1.71	ND	74.00	0.35
	Great white egret	1.48	0.01	0.45	17.40	1.83	ND	62.10	0.24

[a] Wet weight for organisms, corresponding to 4 times of dry weight.

than that in Grey heron and Great white egret in the Kanto area, Japan (Horai et al., 2007), suggesting that there are different exposure pathways for different bird species. The interspecific differences in heavy metal concentrations may be induced by a variety of dietary habits. For example, Saunders's Gull mainly feeds small fishes, shrimps, and clam worms (Gao et al., 2009). The diet of Purple Heron is mainly composed of small fishes, frogs and invertebrates (Montesinos et al., 2008). Spot-billed Duck is polyphagous and their main foods are plants and clam. The diet of Little Egret mainly includes animals such as small fishes, shrimps, and frogs, but they also eat a small amount of plant such as grain (Gounter and Furness, 1997).

4.4. Biomagnification of heavy metals

The magnification of selected heavy metals in wetland food webs is not obvious (Table 5). Lower *R*-values of regressions indicate that the underlying relationships between logarithm of concentration and trophic level are non-linear. Jaeger et al. (2009) assumed that the tissue obtained from the prey that exposed to the contaminants is a good proxy for the total body burden. However, metal uptake and adsorption through the gills and body surfaces are important exposure pathways for invertebrates and fish (Costa et al., 2009). Most heavy metals are dynamic and are actively dependent on transport molecules and binding site competition (McGeer et al., 2003). Many physical and biological factors such as metallothionein, geography, sex, age, and body condition will influence metal deposition in animal tissues (Ilbäck et al., 2004; Laura, 2009). The length of trophic level varying from 1.50 to 3.80 is longer than that mentioned in literature, the longest food web is approximately 4 compared with that without water bird levels such as 3 (Camusso et al., 1998; Ikemoto et al., 2008) and 2 (Watanabe et al., 2008).

BMF is determined based on the comparison of heavy metals in predators and preys. It is assumed that both predators and preys involved in the comparison have a simple predator–prey relationship. However, this assumption may not be true in the complex wetland food webs. For example, Purple Heron and Little Egret have a diverse diet, and carnivorous fish are known to shift their diet with age and season and they have different prey selections based on sex (Syvaranta et al., 2009). The calculation of BMF also assumes that predators completely consume the preys. The results show that except for Zn and Hg (Cd was not detected in birds), other selected heavy metals are generally not biomagnified or biodiluted through the food webs in the YRD.

Hg is considered to be endogenous and bioavailable for organisms in food webs. For example, the Hg concentrations in 6 of 10 common edible marine fish consumed in Pearl River delta region exceed 0.2 mg kg^{-1} for at-risk groups proposed by WHO (1990) for uncontaminated fish (Cheung et al., 2008). In this study, Hg in sediments collected from site H is the highest in all sample sites (Table 2). However, Hg in fish from site H is not the highest, implying that Hg may not be bioavailable even if it is elevated in sediments. This result is consistent with the results presented by Roach et al. (2008). However, Hg concentration in organisms depends on TL (Dietz et al., 2000). Especially for this study, a positive linear relationship between TL and Hg was observed in the organisms of the wetland food webs (Fig. 5). This is similar with the results presented by Campbell et al. (2005). They found that Hg was biomagnified through the Northwater Polynya food web based on the significant positive relationships between log Hg concentrations vs. $\delta^{15}N$ in muscle and liver of fish and seabirds.

The biomagnification of Hg from the lower to the higher trophic levels is well known. In this study, the BMF of Hg from clam to Saunders's Gull, is 64.17 which is the highest among all of BMF values from prey to predator (Table 7). Similar result was also presented by Zhang et al. (2009). They found that Hg in mantis, which mainly feed on *Locusta migratoria manilensis* and *Acrida chinensis* in summer grassland, is 21.55 times and 3.54 times greater than that feed on *Locusta migratoria manilensis* and *Acrida chinensis*, respectively. Campbell et al. (2005) and Goodale et al. (2008) also presented that significant Hg was accumulated in special tissues of marine mammals and sea birds which are located at the top trophic levels in the aquatic food chains. From Table 7, one can see that Hg concentration in Catfish (Carnivorous fish) is obviously higher than that in other fish species (Omnivorous and herbivorous fish), implying that the feeding habits may affect Hg accumulation in the higher trophic levels (Lu et al., 2008; Zhang et al., 2008).

From this research, the trophic transfer is a predominant way for Hg accumulation in higher trophic levels. Wang et al. (2010) also pointed out that Hg in tilapia mainly came from the trophic transfer along the food webs. While a faster growth and shorter life history of animals might lead to less biomagnification of Hg even at the same trophic level. This can be derived from this study that BMFs of Hg in Catfish are higher than those in Javelin goby although they share the same prey organisms, i.e., Silver carp and Shrimp (Table 7). Moreover, the amount of Hg digested by organisms in each trophic level will also be different according to Hg forms in food webs. For example, Wang and Wong (2003) pointed out that the assimilation efficiency of Hg(II) and MeHg vary from 10% to 27% and from 56% to 95%, respectively, for 3 different preys (copepods, silverside, and brine shrimp).

5. Conclusions

Nine heavy metals in different media, i.e., water, sediments, and aquatic organisms in newly- formed wetlands of the YRD are

analyzed in this research. The results show that the length of food web is approximately 4 with the highest trophic level of birds based on the stable nitrogen and carbon isotope values. The content of heavy metals in the newly-formed wetlands is lower than that in other similar regions due to fewer exposures to contaminants. Only Cd, Hg, and Zn are found to have biomagnification effects based on FWMF. From BMF, there is a significant biomagnification effect from clam to Saunders's Gull, while no obvious effects are observed from Chinese shrimp to weever. The difference of heavy metal concentrations between endangered Saunders's Gull and other three kinds of birds is not obvious. The identification of heavy metal concentrations in season birds is more complicated due to their seasonal migration. Further investigations are required in order to provide sound basis for protecting wildlife and habitats in the YRD.

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