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Trace element exposure of whooper swans (*Cygnus cygnus*) wintering in a marine lagoon (Swan Lake), northern China

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ABSTRACT

Trace element poisoning remains a great threat to various waterfowl and waterbirds throughout the world. In this study, we determined the trace element exposure of herbivorous whooper swans (*Cygnus cygnus*) wintering in Swan Lake (Rongcheng), an important swan protection area in northern China. A total of 70 samples including abiotic factors (seawater, sediments), food sources (seagrass, macroalgae), feathers and feces of whooper swans were collected from the marine lagoon during the winters of 2014/2015 and 2015/2016. Concentrations of Cu, Zn, Pb, Cr, Cd, Hg and As were determined to investigate the trace element exposure of whooper swans wintering in the area. Results showed that there was an increasing trend in sediment trace element concentrations, compared with historical data. The trace element concentrations in swan feces most closely resembled those of *Zostera marina* leaves, especially for Cd and Cr. The Zn and Hg concentrations in the swan feces (49.57 and 0.01 mg/kg, respectively) were lower than the minimum values reported in the literature for other waterfowls, waterbirds and terrestrial birds. However, the concentrations of the other five trace elements fell within the lower and mediate range of values reported for birds across the world. These results suggest that the whooper swans wintering in Swan Lake, Rongcheng are not suffering severe trace element exposure; however, with the increasing input of trace elements to the lagoon, severe adverse impacts may occur in the future, and we therefore suggest that the input of trace elements to this area should be curbed.

1. Introduction

Trace element poisoning remains a great threat to various waterfowl and waterbirds, and has been well documented throughout the world (Blus, 1994; Honda et al., 1990, Nam and Lee, 2011; Carravieri et al., 2014; Newth et al., 2016). Nearly 10,000 swans of six species or subspecies had died from Pb poisoning at the end of the last century (Blus, 1994). At one locality in North Carolina, 7200 tundra swans died from lead (Pb) poisoning over five winters (Blus, 1994). Trace elements like cadmium (Cd), mercury (Hg) and Pb can adversely affect the health of wild animals by causing anemia (Goyer and Clarkson, 1996), cancer and nephrotoxicity (Binkowski and Sawicka-Kapusta, 2015), damaging organs, altering their metabolism or causing behavioral changes (Heinz and Hoffman, 1998; Wolfe et al., 1998). Arsenic (As) could adversely affect the behavior, reproduction and development of passerine birds (Sánchez-Virosta et al., 2015). Excessive copper (Cu) and zinc (Zn) can also be toxic to kidneys and impair reproduction (Heinz et al., 1989; Carpenter et al., 2004). It had been proved that excessive chromium (Cr) could increase the mortality of broiler chicks (Kim et al., 1996). Poor health may negatively affect wild waterfowl survival, breeding propensity and reproductive success (Sanderson and Bellrose, 1986; Haramis et al., 1986). Lead poisoning can also affect the developing immune systems of nestlings and underlie other diseases which occur secondarily to trace element-induced immunodeficiency (Cracknell, 2004; Vallverdú-Coll et al., 2015).

Whooper swans (*Cygnus cygnus*) are classified as being of 'least concern' by the IUCN (BirdLife International, 2012) and are a Class II protected species in China. Recent decades have seen a steady increase in numbers and a spread in the swans' distribution across the temperate Eurasian region (Albertsen and Kanazawa, 2002; Boiko, 2010; Boiko et al., 2014; Hall et al., 2012; Nilsson, 2014). While trace element poisoning, especially Pb poisoning, has been studied in a range of free-living waterfowls, waterbirds and terrestrial birds (Sears, 1988; O'Halloran et al., 2002; Day et al., 2003; Takekawa et al., 2002; Eeva et al., 2009; Koivula et al., 2011), few studies have focused on *C. cygnus* (O'Connell et al., 2008; Newth et al., 2016). Recent *C. cygnus* mortalities in Scotland (Spray and Milne, 1988), Japan (Honda et al., 1990) and South Korea

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(Nam and Lee, 2011) have raised concern about the lack of information on this species.

Swan Lake (also known as Moon Lake), a typical seagrass meadow in northern China (Zhou et al., 2015; Zhang et al., 2015; Xu et al., 2017), historically has been home to one of the largest wintering whooper swan populations in northeast Asia. These birds breed in areas ranging from the tundra in the far east of Russia to the desert wetlands of northeast China and Mongolia (Syroechkovski, 2002). Swan Lake was designated as a National Primary Wildlife Protection Area in 1988 (Gao, 1998). Moreover, it has become an important national swan reserve in China since 2007. However, environmental deterioration of Swan Lake caused by excessive nutrient and trace element loading has received much public concern over the past decades (Gao et al., 2013a; Gao et al., 2013b; Huang et al., 2013; Zhang et al., 2014). Pollution sources include fluvial transport, direct domestic discharge and industrial effluents (Huang et al., 2013). Wastewater discharge doubled from 1992 to 2007 and reached 129.9 t a^{-1} (Wang and Li, 2009). A recent study conducted in Swan Lake and the surrounding coastal sea showed that the concentrations of Pb, Zn, Cd, Cu and Cr in sediments had increased in the past decade and Pb in sediments could adversely affect the benthic organisms (Huang et al., 2013).

Here, we determined the trace element content in seawater, sediments and seagrass (*Zostera marina*) to establish the availability of trace elements in Swan Lake and the adjacent terrestrial environment to allow an informed discussion of the potential for swan exposure to trace elements via ingestion. We also analyzed Cu, Zn, Pb, Cr, Cd, Hg and As concentrations in feces and feathers from whooper swans wintering in Swan Lake to determine current exposure of swans to trace elements. The data obtained were compared with results of similar research in other bird species across the world. The present study would be helpful to understand the trace element exposure and excretion processes of whooper swans.

2. Materials and methods

2.1. Study area

The survey was conducted in Swan Lake (Fig. 1), a small marine lagoon, in the southeast of Chengshan Town, Rongcheng, northern China. It is the biggest swan lagoon in China, with an area of about 4.8 km^2. The lagoon is connected to the Yellow Sea by a quite narrow inlet. Swan Lake retards winds and waves, creating a quiet low-wind environment, an ideal overwintering habitat for wetland birds, such as whooper swans. Swan Lake is also a typical seagrass meadow in northern China, with eelgrass (*Zostera marina*) meadow accounting for one third of the lagoon areas (Zhou et al., 2015). Eelgrass in the lagoon is an important food source for swans (Wang et al., unpublished data). Every year approximately ten thousand swans overwinter in the lagoon and in the nearby area. The substrate along the banks of the lagoon is sandy or muddy, with an average water depth <2 m.

2.2. Sample collection

The swans defecate mostly in the water, but occasionally leave droppings on the shallow shore and these terrestrial samples were collected. A total of 12 fresh swan fecal samples were collected during the low tide in the morning in the middle of December in 2014 and 2015, with 6 samples collected each year (Table 1; Fig. 1). We also collected eelgrass (N = 6), macroalgae (*Chaetomorpha linum*; N = 6) within the eelgrass beds in Swan Lake and swan feathers (N = 6) in the middle of December in 2015. Three swans were captured and breast feathers were collected from each bird. In laboratory, the seagrass samples were divided into leaf, sheath and rhizome. All organism samples were stored at −18 °C prior to analyses.

Samples of water (N = 22) and sediments (N = 6) were collected from three sites of the lagoon (Fig. 1) on December 15th and 16th of 2015. Two sediment samples were collected at depths varying from 0 to 10 cm at each site (W1, W3 and W6; Fig. 1). Each sample was homogenized within a polyethylene container. Surface water samples of low tide (N = 6; W1-3; Fig. 1) and high tide (N = 16; W1-8) were collected when the water depth was lower than 0.5 m or over 1.5 m, respectively. At each site 2 samples were collected in 50-mL acid-washed polyethylene sample bottles. Each sample was homogenized and filtered through GF/F glass fiber filters in laboratory in 4 h after sampling. All water and sediment samples were stored at −18 °C prior to analyses.

Prior to analyses, all the primary producers and feathers were washed vigorously in deionized water to remove any external contamination. All samples of the sediment, feces, seagrass, macroalgae and

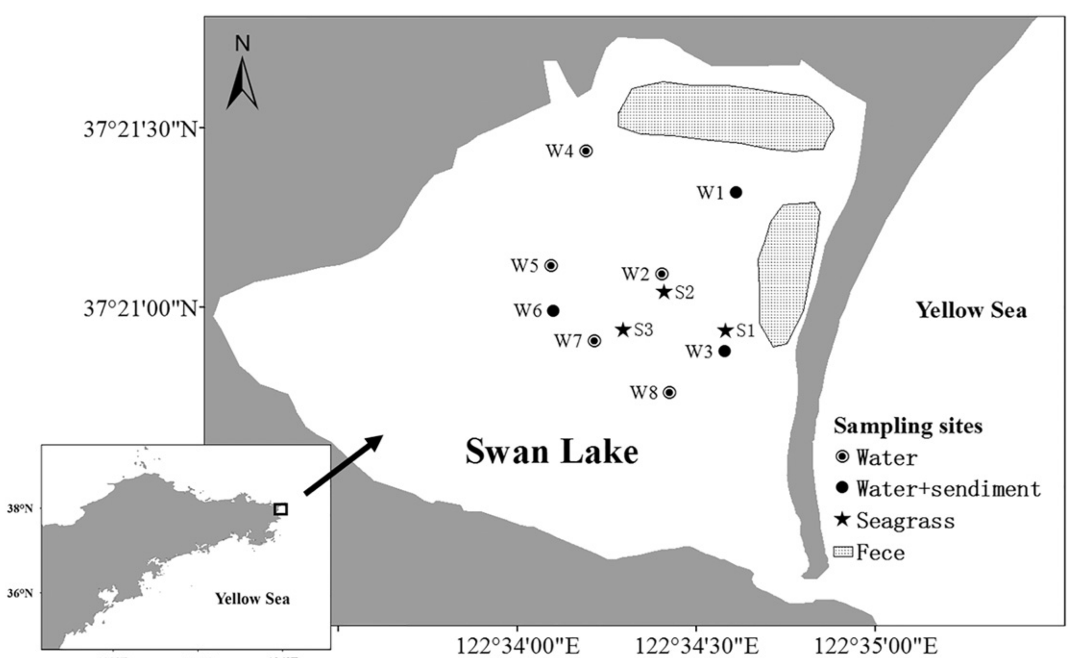

Fig. 1. Location of study area (Swan Lake).

Table 1
Trace element concentrations in seawater (μg L^{-1}), sediment, food sources, excrements and feathers (mg kg^{-1} DW) of whooper swans overwintering in Swan Lake, Rongcheng, China.

Material		As	Cd	Cr	Cu	Pb	Zn	Hg	Reference
Sediment	Means	5.95	0.35	72.35	24.98	46.82	68.86	0.020	Present study
N = 6	SD	1.13	0.04	4.26	1.24	6.44	4.76	0.004	
		–	0.32	40.1	21.7	34.6	59.6	–	Huang et al., 2013
		–	0.15	36.1	13.1	6.7	17.2	–	Yang et al., 2001
GB18668-2002 Level I		20	0.5	80	35	60	150	0.2	SEPA, 2002
Seawater									
Low tide	Means	0.53^a	0.05^a	0.26^a	0.81^a	0.21^a	15.54^a	0.102^a	
N = 6	SD	0.03	0.02	0.05	0.13	0.01	4.46	0.001	
High tide	Means	0.53^a	0.06^a	0.25^a	1.45^a	0.18^b	20.58^a	0.105^a	
N = 16	SD	0.35	0.03	0.06	0.71	0.02	4.74	0.053	
GB3838-2002 Level I		20	1	50	5	1	20	0.05	PRC EPM and
Level II		30	5	100	10	5	50	0.20	PRC AQSIQ, 2002
Z. marina									
Rhizome	Means	3.67^a	2.17^b	26.83^a	21.4^a	9.58^a	30.84^b	0.131^b	
N = 6	SD	0.24	0.12	2.41	1.41	0.67	2.40	0.010	
Sheath	Means	0.64^b	1.95^b	11.11^b	7.81^c	3.23^c	17.24^c	0.062^c	
N = 6	SD	0.05	0.18	0.21	0.16	0.41	0.42	0.004	
Leaf	Means	0.90^b	5.52^a	12.80^b	15.84^b	4.24^b	50.78^a	0.148^a	
N = 6	SD	0.04	0.38	0.52	1.68	0.29	5.10	0.006	
C. linum	Means	4.81	0.25	11.63	8.95	5.21	31.71	0.074	
N = 6	SD	0.30	0.02	0.13	0.10	0.03	2.79	0.007	
Feather	Means	0.30	0.067	6.57	21.96	3.64	103.49	0.196	
(N = 6)	SD	0.11	0.011	0.59	5.33	1.13	3.29	0.012	
Feces									
2014 (N = 6)	Means	1.16	5.67	10.93	12.32	8.78	55.32	0.011	
	SD	0.20	1.30	3.14	3.42	1.76	18.32	0.004	
2015 (N = 6)	Means	1.42	4.89	13.65	11.05	7.76	43.82	0.010	
	SD	0.21	0.85	1.83	1.40	1.42	9.86	0.005	
2014 + 2015 (N = 12)	Means	1.29	5.28	12.29	11.68	8.27	49.57	0.010	
	SD	0.24	1.13	2.83	2.58	1.62	15.26	0.005	

Values with different letters in the same row were significantly different from each other ($p < 0.05$).

feathers were dried in the air; then the sediment, feces and macroalgae samples were smashed, sieved and sealed in plastic containers for later analyses. The feather samples were cut into small pieces with plastic scissors before smashed.

2.3. Analysis for trace elements

2.3.1. Sediment

0.1 g (dry weight) of sediment were digested in a mixed acid with 4 mL HF, 5 mL HNO_3 and 1 mL $HClO_4$ at 140–220 °C, then the residual was dissolved with 1 mL HNO_3, and diluted to 25 mL with Millipore water. The solution was then detected by inductively coupled plasma-mass spectrophotometer (ICP-MS; Thermo Fisher Icap-Qc) for Cd analysis and inductively coupled plasma-optical emission spectrometry (ICP-OES; Perkin-Elmer 7300 DV) for Cu, Zn, Cr and Pb analysis.

For Hg analysis, 0.1 g (dry weight) of sediment were digested in 2 mL HNO_3 (1.42 g/mL) and 6 mL HCl (1.19 g/mL) at 100 °C for 1 h and 1 mL 1% liquor potassii permanganatis was added. The residual was then diluted to 25 mL with 1% oxalic acid solution and held overnight. Then, 2 mL of the supernatant was decanted for the analysis of Hg by atomic fluorescence spectroscopy (Jitian AFS-930).

For As analysis, 0.1 g (dry weight) of sediment were digested with 10 mL *aqua regia* (HNO_3: HCl: water = 1:3:4, V:V:V) at 100 °C for 1 h. The residual was then diluted to 25 mL with Millipore water and held overnight. Then, 2 mL of the supernatant was added with 10 mL diluted HCl (HCl:water = 1:1, V:V) and thiourea-ascorbic acid reducing agent (5 g thiourea and 5 g ascorbic acid dissolved in 100 mL water). The residual was then diluted to 100 mL with Millipore water and held overnight. Then, 2 mL of the supernatant was decanted for the analysis of As by atomic fluorescence spectroscopy (Jitian AFS-930).

The detection limits of the different elements were 0.000001 μg/g (Hg), 0.00001 μg/g (As), 0.0004 μg/g (Cu), 0.001 μg/g (Pb), 0.0002 μg/g (Zn), 0.0002 μg/g (Cr) and 0.000002 μg/g (Cd). All detection limits are based on a 98% confidence level (3 standard deviations).

2.3.2. Organism

0.1 g (dry weight) of organic samples including seagrass, macroalgae, feces and feathers were digested in a mixed acid with 5 mL HNO_3 and 1 mL $HClO_4$ at 140–220 °C, then dissolved with 1 mL HNO_3 (1:1, V/V), and diluted to 25 mL with Millipore water. Samples were then detected by ICP-MS (Thermo Fisher Icap-Qc) for Cd, Cu, Zn, Cr and Pb analysis.

For Hg analysis, 0.1 g (dry weight) of samples were digested in 10 mL HNO_3 and 1 mL $HClO_4$ (GR) at 140–160 °C, then dissolved with 5 mL HCl, and diluted to 50 mL with 1% oxalic acid solution. 2 mL of the supernatant was decanted for the analysis of Hg by atomic fluorescence spectroscopy (Jitian AFS-930).

For As analysis, 0.2 g (dry weight) of samples were digested in 10 mL HNO_3 and 1 mL $HClO_4$ at 160 °C for 1 h, then dissolved with 25 mL diluted H_2SO_4 (H_2SO_4:water = 1:9, V:V) and 5 mL thiourea-ascorbic acid reducing agent (5 g thiourea and 3 g ascorbic acid dissolved in 100 mL water). The residual was then diluted to 50 mL with Millipore water and held overnight. 2 mL of the supernatant was decanted for the analysis of Hg by atomic fluorescence spectroscopy (Jitian AFS-930).

The detection limits of the different elements were 0.000001 μg/g (Hg), 0.00001 μg/g (As), 0.000011 μg/g (Cu), 0.000001 μg/g (Pb), 0.000111 μg/g (Zn), 0.000006 μg/g (Cr) and 0.000002 μg/g (Cd). All detection limits were based on a 98% confidence level (3 standard deviations).

2.3.3. Seawater

10 mL filtered seawater was diluted to 100 mL with Millipore water, and subjected to ICP-MS (Icap-Qc) for Cd, Cu, Zn, Cr and Pb analysis.

For Hg analysis, 100 mL filtered seawater was added 2 mL H_2SO_4 (1.84 g/mL) and 5 mL potassium peroxodisulfate (50 g/L) to digest for 24 h. 2 mL hydroxylamine hydrochloride solution (100 g/L) was added. The digested sample was then detected by atomic fluorescence spectroscopy (Jitian AFS-930).

For As analysis, 100 mL filtered seawater was added 10 mL HCl and 2 mL thiourea-ascorbic acid reducing agent (5 g thiourea and 3 g ascorbic acid dissolved in 100 mL water). The digested sample was then detected by atomic fluorescence spectroscopy (Jitian AFS-930).

The detection limits of the different elements were 0.001 μg/L (Hg), 0.01 μg/L (As), 0.011 μg/L (Cu), 0.001 μg/L (Pb), 0.111 μg/L (Zn), 0.006 μg/L (Cr) and 0.002 μg/L (Cd).

2.3.4. Quality control

All the chemicals used in the experiment were guaranteed reagent grade. All laboratory ware were soaked in 10% HNO_3 for 72 h, then rinsed three times with ultrapure water, and dried at 60 °C for 24 h prior to use. For plant analysis, *Laminaria japonica* was chosen as certified reference material (GBW 08517), and Yellow Sea sediment was selected as certified reference material for sediment analysis (GBW 07333).The recovery rates for all the elements were 90.8–104.1% and the measurement precision was under 3% relative standard deviations. Reagent blank and replicate samples were also carried out in the same way. The results showed that there was no contamination during analyses and the relative standard deviations of all replicate samples were <5% for all the trace elements.

2.4. Statistical analysis

Results are presented as mean ± SD. Trace element concentrations are provided on dry weight basis. Difference in trace element concentrations within swan feces between 2014 and 2015 were analyzed using ANOVA. Difference in trace element concentrations within water between low tide and high tide were also analyzed using ANOVA. Prior to analysis, data were examined for homogeneity of variances (Levene's test). Difference among groups (feathers, feces, water, sediments, seagrass, macroalgae) were tested with LSD test and Dunnett test. Pearson correlations in trace element concentrations among different materials were also conducted. A statistical significance level of 0.05 was used.

3. Results

Trace element concentrations varied markedly among the studied materials (Table 1). Cr (72.35 ± 4.26 mg kg^{-1}) and Zn (68.86 ± 4.76 mg kg^{-1}) were the most abundant elements in the sediments, followed by Pb, Cu, As, Cd and Hg. In seawater, the concentration of Zn was higher than those of other trace elements by one or two orders of magnitude. All trace element concentrations except Zn at the high tide were <1 μg L^{-1}. There were no significant differences in concentrations of As, Cd, Cu and Zn in seawater between the high tide and the low tide ($p < 0.05$; Table 1). However, the Pb concentration at low tide was significant higher than that at the high tide ($p < 0.05$).

In *Z. marina*, the concentrations of As, Cr, Cu and Pb were highest in the rhizomes ($p < 0.05$), while the highest Cd, Zn and Hg concentrations were detected in leaves ($p < 0.05$; Table 1). As with seagrass and sediment samples, Zn and Cr were found in the highest concentrations in *C. linum*, followed by Cu, Pb, As, Cd and Hg.

The highest trace element concentrations detected in whooper swan feathers were Zn and Cu. Unlike the above biotic and abiotic materials, Cd was found in the lowest concentrations in feathers, rather than Hg.

The average concentrations of Cu, Zn, Pb, Cr, Cd, Hg and As in swan feces were 11.68 ± 2.58 mg kg^{-1}, 49.57 ± 15.26 mg kg^{-1}, 8.27 ± 1.62 mg kg^{-1}, 12.29 ± 2.83 mg kg^{-1}, 5.28 ± 1.13 mg kg^{-1}, 0.010 ± 0.005 mg kg^{-1}, and 1.29 ± 0.24 mg kg^{-1}, respectively. There was no significant difference in trace element contents within feces between 2014 and 2015 (for Cr, F = 3.373, $p = 0.096$; for Cu, F = 0.709, $p = 0.419$; for Zn, F = 1.834, $p = 0.206$; for Cd, F = 1.520, $p = 0.246$; for Pb, F = 1.215, $p = 0.296$; for Hg, F = 0.093, $p = 0.767$; for As, F = 4.668, $p = 0.056$). The trace element concentrations in feces decreased by the order: Zn > Cr > Cu > Pb > Cd > As > Hg, which resembled those of *Z. marina* leaves; and Hg was two orders of magnitude lower than other trace elements.

The correlation relationship in the trace element contents among different materials were shown in Table 2. There was significant correlation in trace element contents between sediments and rhizomes ($p = 0.009$), leaves and water ($p = 0.001$), feces and leaves ($p < 0.001$). The correlation relationship among leaves, sheathes, and rhizomes were also significant (Table 2). The feather trace element concentration was extremely significantly correlated with water, *Z. marina* leaves, *C. linum*, and feces (all $p < 0.01$; Table 2).

4. Discussion

4.1. Trace element contamination in seawater, sediment and primary producers from Swan Lake

No trace element concentrations in sediments from Swan Lake exceeded Level I of the China National Environmental Quality Standards for Sediment (Table 1; Level I for nature reserve, mariculture, endangered species, GB18668-2002). Previous studies conducted on Swan Lake waters and sediments have also indicated a lack of trace element pollution in this area (Yang et al., 2001; Gao et al., 2013a; Huang et al., 2013). However, there has been a clear increasing trend in trace element concentration of the sediment since the end of the 20th century (Table 1; Yang et al., 2001; Huang et al., 2013). Yang et al. (2001) reported that Cd, Cr, Cu, Pb, and Zn in 1998 in Swan Lake sediments were 0.15, 36.1, 13.1, 6.7, and 17.2 mg kg^{-1}, respectively (Table 1); and these values are much lower than those determined in 2009 (Huang et al., 2013), i.e. 0.32, 40.1, 21.7, 34.6 and 59.6 mg kg^{-1}, respectively; and the latter are also lower than those determined in the present study, especially for the three metals of Cr, Pb and Zn.

Larger particles or grit are usually used by waterbirds or waterfowls to break up and grind food in their gizzard (Gionfriddo and Best, 1999). They often intake some sediments or soils incidentally, and soil or sediment derived Pb has been identified as an important Pb source together with lead shot and lead fishing weight in the past (Martinez-Haro et al., 2010, 2011; Aloupi et al., 2015). Sediment ingestion rates of mute swans (*Cygnus olor*) typically feeding on submerged aquatic vegetation have been estimated between 3% and 5% (Beyer et al., 1998a, 2008). Tundra swans (*C. columbianus*) whose diet consists mainly of mollusks and winter cereals ingest between 9% and 15% sediment in their diet (Beyer et al., 1998b, 2008). The Pb concentrations in the sediment of Swan Lake were much lower than the values of lowest effect level calculated for mute swans (530 mg/kg, 95% confidence interval: 370–690 mg/kg; Beyer et al., 2000). Effect thresholds of other trace elements for swans are not currently available.

All trace element concentrations in water samples were well below Level II of the China National Environmental Quality Standards for Surface Water (the standard for habitat protection of aquatic organisms, spawning grounds of fish and shrimp and food grounds of juvenile fish) (PRC EPM and PRC AQSIQ, 2002).

In *Z. marina*, the leaves and rhizomes are able to absorb trace elements from ambient and interstitial water, respectively (Faraday and Churchill, 1979; Lyngby et al., 1982). Compared with the leaves, higher concentrations of As, Cr, Cu and Pb were detected in rhizomes, which implies that these trace elements were mainly absorbed from the interstitial water. The highest Cd, Zn and Hg concentrations were detected in leaves, indicating that these trace elements were mainly derived from ambient water. Higher Cd and Zn contents in above-ground parts of plants than below-ground parts has also been reported in Limfjord, Denmark (Lyngby and Brix, 1982; Brix et al., 1983). Laboratory experiments have also shown the higher uptake rate of these two metals by leaves (Lyngby and Brix, 1982).

All trace elements except for As in *C. linum* were lower than those in *Z. marina* rhizomes or leaves. Fourqurean and Cai (2001) also found that the As content in leaves of *Thalassia testudinum* was generally lower

Table 2
Pearson correlation among the trace element contents of different materials.

	Rhizome	Sheath	Leaf	*C. linum*	Feather	Fece	Sediment	Water
Rhizome	1							
Sheath	0.964[b]	1						
Leaf	0.808[a]	0.923[b]	1					
C. linum	0.851[a]	0.937[b]	0.97[b]	1				
Feather	0.708	0.844[a]	0.982[b]	0.953[b]	1			
Fece	0.784[a]	0.908[b]	0.991[b]	0.974[b]	0.981[b]	1		
Sediment	0.881[b]	0.85[a]	0.657	0.758[a]	0.693	0.567	1	
Water	0.625	0.789[a]	0.954[b]	0.932[b]	0.965[b]	0.988[b]	0.524	1

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is extremely significant at the 0.01 level (2-tailed).

than literature values of marine macroalgae and attributed this to the inability of this seagrass species to detoxify and accumulate As.

4.2. Trace element concentrations in whooper swan feathers and feces

The mean Pb concentrations in whooper swan feathers (3.64 mg/kg) were higher than those of heron (*Ardea cinerea*) and egret (*Egretta garzetta*) chicks in Pyeongtaek colony (2.05–2.65 mg/kg), as well as those of white-fronted geese (*Anser albifrons*) and spot-billed ducks (*Anas poecilorhyncha*) without Pb shot in their carcasses in Gimpo, Korea (1.96 and 1.69 mg/kg respectively) (Kim and Oh, 2014a, 2014b). However, the mean Cd contents of whooper swan feathers (0.067 mg/kg) were remarkably lower than the aforementioned birds in Korea (0.52–1.51 mg/kg) by about one or two orders (Kim and Oh, 2014a, 2014b), and the Cu and Zn contents were similar between the studies.

The fecal trace elements concentrations resembled those of *Z. marina* leaves, especially for Cd and Cr, which implies that the main food source of whooper swans wintering in Swan Lake is *Z. marina* leaves.

The Zn and Hg concentrations in the feces from whooper swans wintering in Swan Lake were lower than the minimum values reported in the literature for other waterfowls, waterbirds and terrestrial birds (Table 2). The herbivorous diet or lower trophic level of whooper swans may have contributed to these lower values. However, the content of the other five trace elements tested fell within the lower and mediate ranges of values reported for birds across the world (Table 3).

Compared to a study conducted by Beyer and Day (2004), the mean fecal Pb, Cd and Cr concentrations in the present study are higher, while Cu, Zn, As, Hg concentrations are lower than feces of mute swan feeding on contaminated sediments. In that study, none of the elements detected in the liver were considered toxic. In another laboratory study investigating the toxicity of lead-contaminated sediments to mute swans, Day et al. (2003) found that mute swans with fecal Pb, Cu, Zn, Cr and Cd concentrations of 1500, 77, 1500, 21 and 15 mg/kg, respectively, suffered a 24% decrease in mean body weight and nephrosis. The extraordinarily high levels of Pb in digesta (700 mg/kg) rather than Cr and Cd contributed to the pathological changes of mute swans (Day et al., 2003) (Table 3).

The fecal trace element contents of whooper swans in Swan Lake were also found to be similar or even lower than the red-footed booby (*Sula sula*) inhabiting the pristine Dongdao Island located in South China Sea, except for Pb (Liu et al., 2006, Yan et al., 2010). Emissions from industrial combustion constitute the major source of Pb during the last century (Goyer, 1996, Järup, 2003). The long distance from mainland China and other Southeast Asian countries may have contributed to the low Pb contents in the diets and feces of the red-footed booby. Low Pb contents have also been reported in the feces of yellow-legged gulls (*Larus michahellis*) colonizing Marinello Nature Reserve far from urban centers and other direct anthropogenic inputs (4.70 mg/kg, Signa et al., 2013), Gentoo penguins (*Pygoscelis papua ellsworthii*) in the Antarctic (<0.4 mg/kg, Metcheva et al., 2011) and great tits (*Parus major*) in rural areas of Foz, Portugal (0.86 mg/kg, Costa et al., 2012).

The fecal Hg contents of whooper swans were one order lower than values reported in non-herbivorous birds (Tiller et al., 2005), and resemble feces of mute swans in Chesapeake Bay, USA (e.g. Beyer and Day, 2004; Costa et al., 2012). The fecal Zn contents (49.57 mg/kg) were similar to feces from omnivorous glaucous gulls (76 mg/kg, Headley, 1996) and well below the median (314.2 mg/kg) of reported values. Generally, the feces of ichthyophagous and insectivorous birds contain higher levels of Zn than herbivorous and omnivorous birds.

Cd has long been used as an efficient anticorrosion agent and it is also present in phosphate fertilizers. Cd compounds are also commonly used in color pigments and rechargeable nickel–cadmium batteries (Järup, 2003). The fecal Cd contents of whooper swans were found to be at the median of the range of values reported for Cd contents of birds across the world. The use of fertilizers in nearby farmland and occasionally discarded nickel-cadmium batteries by local residents, workers and visitors may have contributed to the Cd contamination of this lagoon.

The anthropogenic sources of As include smelting activities of non-ferrous metals, combustion of fossil fuels and the use of arsenical pesticides, herbicides and wood preservatives (Järup, 2003). It has been found that the excrements of two songbirds, the great tit (*Parus major*) and the blue tit (*Parus caeruleus*) nesting near a metallurgic factory contain significantly higher As (16.02 mg/kg) than birds nesting 4 km farther east (1.32 mg/kg) (Dauwe et al., 2000). Similar and low fecal As contents (approximately 1 mg/kg) have also been reported in the red-footed booby (*Sula sula*) inhabiting Dongdao Island, the Chinese egret (*Egretta eulophotes*) breeding in the uninhabited Caiyu Island, Fujian, China, and in the great tit (*P. major*) living in a paper-and-pulp–industry area in Urso, Portugal.

Like As, the fecal Cu contents of whooper swans lie with the lower limit of global values. Elevated values have been reported both in pristine (Liu et al., 2006; Yan et al., 2010; Signa et al., 2013) and polluted (Mateo et al., 2006; Dauwe et al., 2000, Berglund et al., 2015) coastal as well as terrestrial ecosystems.

There have been few reports on the Cr contents of bird feces and the results of the present study fall within the range of reported values (5.0 to 27.6 mg/kg).

Overall, we can conclude that the whooper swans wintering in Swan Lake, Rongcheng, are not suffering severe trace element exposure; however, additional studies are still needed to evaluate potential effects of the trace element exposure on this species. The establishment of the Rongcheng Swan National Nature Reserve in China may contribute to the good habitat for swans.

Few trace elements (generally <5%) in seagrass, macroalgae and seagrass are available to swans (Turner and Hambling, 2012). The fecal trace elements analyses described here, using whooper swans, provide an alternative for long-term environmental monitoring using seagrass or benthic bivalves as indicator species of Swan Lake, Rongcheng. The data could reflect the relative levels of trace element pollutants in the ecosystem, without requiring detailed knowledge of the conditions of the swans, or their food supply network. Nevertheless,

Table 3
Trace element contents in feces (mg/kg; dry weight) defecated by terrestrial and water birds across the world.

Site	Pollution status	Species	Sampling	Trace element contents in feces (mg/kg)							Reference
			Period	Pb	Cu	Zn	As	Hg	Cr	Cd	
Coeur d'Alene River Basin (USA)	Polluted	Tundra swan	–	880	–	–	–	–	–	–	Beyer et al., 1998a, 1998b
Coeur d'Alene River Basin (USA)	Polluted	Mute swan	1995	1500	77	1500	–	–	21	15	Day et al., 2003
Chesapeake Bay (USA)	Polluted	Mute swan	1997	6.2	14	120	5.4	0.038	5.0	1.6	Beyer and Day, 2004
Lake Chatcole, Idaho (USA)	Unpolluted	Blue heron	1978	46	–	–	–	0.28	–	1.8	Fitzner et al., 1982
Vernita, Hanford Reach (USA)	Polluted	Blue heron	1996	3.5	13.2	259.0	–	0.1	6.7	0.6	Tiller et al., 2005
Antwerp (Belgium)	Unpolluted	Great tit	1998	2.34	36.16	400.4	1.37	–	–	5.72	Dauwe et al., 2000
	Polluted		1998	80.40	90.28	429.4	16.03	–	–	16.81	
	Unpolluted	Blue tit	1998	5.54	37.50	311.0	1.28	–	–	3.11	
	Polluted		1998	124.80	92.74	317.4	16.0	–	–	9.35	
Antwerp (Belgium)	Polluted	Great tit	1997	22.6	37.2	288.5	6.6	0.024	–	9.0	Dauwe et al., 2004
Zator and Milicz (Poland)	Polluted	Mallard	2006–2009	–	–	–	–	–	–	0.60[a]	Binkowski and Sawicka-Kapusta, 2015
		Coot	2006–2009	–	–	–	–	–	–	0.41[a]	
Harjavalta (Finland)	Polluted	Pied flycatcher	1992–2008	17	450[b]	–	–	–	–	11	Berglund et al., 2015
		Great tit	1992–2008	13	160[b]	–	–	–	–	11	
Harjavalta (Finland)	Polluted	Pied flycatcher	2009	4.80	197	324	9.85	–	–	4.43	Berglund et al., 2011
		Great tit	2009	3.58	133	233	11.3	–	–	2.16	
Harjavalta (Finland)	Polluted	Pied flycatcher	2014	9.92	221.8	361.1	6.54	0.15	–	6.40	Espín et al., 2016
	Unpolluted		2014	2.18	79.8	314.4	0.52	0.10	–	3.17	
	Polluted	Great tit	2014	6.21	149.9	284.4	7.08	0.12	–	4.01	
	Unpolluted		2014	2.20	72.3	272.9	0.54	0.08	–	1.68	
Harjavalta (Finland)	Polluted	Great tit	2004	5.1	232	389	12.9	–	–	3.8	Koivula et al., 2011
	Unpullted		2004	2.7	111	338	1.7	–	–	1.8	
Figueira da Foz (Portugal)	Polluted	Great tits	2009	1.10	98.5	392	1.26	0.35	–	1.08	Costa et al., 2012
	Unpolluted		2009	0.86	81.6	409.8	7.09	0.22	–	1.58	
Guadalquivir Marshes (Spain)	Polluted	Greylag geese	2001–2002	15	27	308	2.8	–	–	–	Mateo et al., 2006
Ebro Delta (Catalonia, Spain)	Polluted	Mallard	2007–2008	1103.8	–	–	–	–	–	–	Martinez-Haro et al., 2010
Medina lagoon (Spain)	Polluted	Mallard	2006–2008	3.78[b]	–	–	–	–	–	–	Martinez-Haro et al., 2011
		Coot	2006–2008	5.36[b]	–	–	–	–	–	–	
Entremuros, Doñana (Spain)	Polluted	Greylag geese	2004–2008	2.98	–	–	–	–	–	–	Martinez-Haro et al., 2013
		Purple gallinule	2004–2008	4.07	–	–	–	–	–	–	
Evros Delta (Greece)	–	Greater white fronted goose	2012–2014	3.49	–	–	–	–	–	–	Aloupi et al., 2015
		Lesser white fronted goose	2013–2014	8.16	–	–	–	–	–	–	
Kerkini Lake (Greece)	–	Greater white fronted goose	2012–2014	8.67	–	–	–	–	–	–	
		Lesser white-fronted goose	2013–2014	8.32	–	–	–	–	–	–	
Marinello ponds (Italy)	Unpolluted	Yellow-legged gull	2008–2009	4.70	28.37	171.60	10.01	0.89	27.66	0.33	Signa et al., 2013
Stuphallet, Spitsbergen (Norway)	–	Kittiwake	1994	21.6	51.2	176	–	–	–	–	Headley, 1996
		Glaucous gull	1994	30.0	6.25	76	–	–	–		
Northern Chile	Polluted	Humboldt penguin	2011–2012	5.39	139	236.83	3.35	0.61		37.14	Celis et al., 2014
Livingston Island (South Shetlands)		Gentoo penguin	2006–2007	<0.4	104	145	5.13			1.03	Metcheva et al., 2011
Dongdao Island (China)	Unpolluted	Red-footed booby	2003	1.6	21.1	419.4	–	0.11	–	6.34	Liu et al., 2006
Dongdao Island (China)	Unpolluted	Red-footed booby	2003	2.61	39.8	489	0.98	–	–	6.62	Yan et al., 2010
Caiyu Island (China)	Unpolluted	Chinese egret	2008	26.3	7.04	424	0.014	–	20.0	–	Fang et al., 2010
Swan Lake (China)	Polluted	Whooper swan	2014–2015	8.27	11.68	49.57	1.29	0.01	12.29	5.28	Present study

Tundra swan, *Cygnus columbianus*; mute swan, *Cygnus olor*; blue heron, *Ardea herodias*; mallard, *Anas platyrhynchos*; coot, *Fulica atra*; great tits, *Parus major*; blue tit, Parus caeruleus; pied flycatcher, *Ficedula hypoleuca*; greylag geese, *Anser anser*; purple gallinule, *Porphyrio porphyrio*; yellow-legged gull, *Larus michahellis*; kittiwake, *Rissa tridactyla*; glaucous gull, *Larus hyperboreus*; greater white fronted goose, Anser bifrons; lesser white fronted goose, *Anser erythropus*; Humboldt penguins, *Spheniscus humboldti*; Gentoo penguin, *Pygoscelis papua* ellsworthii; red-footed booby, *Sula sula*; Chinese egret, *Egretta eulophotes*; whooper swan, *Cygnus cygnus*.

[a] The value is given in median.

[b] The value is given in geometric mean; Pollution status, including polluted and unpolluted environments based on information from pertinent literature.

comprehensive food sources coupled with feces measurements should be carried out in the future to provide a more quantitative understanding of the implications of this monitoring technique.

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