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RESEARCH ARTICLE

A method for heavy metal exposure risk assessment to migratory herbivorous birds and identification of priority pollutants/areas in wetlands

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Abstract Wetlands are important habitats for migratory birds but have been degraded by many anthropogenic factors including heavy metal contamination. Birds inhabiting wetlands are exposed to pollutants. In this study, a method for exposure risk assessment of migratory herbivorous birds and identification of priority pollutants/areas was developed and employed in East Dongting Lake wetland (EDT). Four heavy metals (Cr, Cu, Pb, and Cd) in sedge and soil samples from ten lesser white-fronted goose (*Anser erythropus*) habitats in EDT were investigated. Results showed that negative effect of Cr and Pb on lesser white-fronted goose may occur while the concentrations of Cu and Cd are considered to be relatively safe. Prioritize threats were decreased in the following sequence: Cr > Pb > Cu > Cd. Cr and Pb were considered to be the priority pollutants. Spatial interpolation based on geostatistical methods showed that Spring Breeze Lake should draw much attention. Furthermore, regions with high hazard index were identified to be priority areas of EDT for risk management.

Keywords Heavy metal · Exposure risk assessment · Migratory birds · Priority pollutants/areas · East Dongting Lake wetland

Introduction

Wetlands are one of the three ecosystems in the world, providing appropriate ecological environment for a wide variety of flora and fauna, including endangered species. With the aggravation of population growth and resource consumption, wetland ecosystem has been increasingly affected by pollutant emission from human activities. These harmful anthropogenic contaminants may pose a risk to species (Zeng et al. 2013). Heavy metal contamination in the environment has been attracting much attention because of toxicity, persistence, extensive sources, and non-biodegradable properties. Heavy metals can be accumulated by species through the food chain in wetland ecosystem, resulting in adverse effect (Fimreite 1971; Larison et al. 2000; Mora 2003).

Many studies have shown the severity of heavy metal contamination in wetland habitats for birds (Liang et al. 2015b; Salamat et al. 2014). As an important part of wetland ecosystem, birds have been widely used as an indicator of wetland pollution status. Previous studies mainly focused on the effect of Hg exposure using stable isotopes and were conducted by collecting eggs, feathers, blood, and even the whole carcasses (Fort et al. 2014; Lavoie et al. 2015; Ofukany et al. 2012). Reducing the use of experimental animals and ethical treatment of animals will be an inevitable trend for evaluating toxicity of hazardous materials. Modeling can provide a non-destructive way for exposure risk assessment instead of causing harm to organisms. Human health risk assessment model has been widely used (Man et al. 2010; Yi et al. 2011; Tang et al. 2013; Zhao et al. 2014), but information on exposure

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model of birds seems scarce. Nichols et al. (1995) developed a bioenergetics-based model for bioaccumulation of PCBs in nestling tree swallows. Norstrom et al. (2007) validated a model for bioaccumulation of POPs in herring gulls. Lieske et al. (2014) assessed the distribution of seabirds at-sea with species distribution models and identified core areas. However, few studies have quantitatively evaluated heavy metal exposure risk to birds. In the present study, an improved model was employed as a nondestructive method for evaluating bird exposure risk to heavy metals in wetlands. Moreover, considering pollution-pathway-receptor, exposure risk combined with receptor population distribution can make assessment and management more effective (Li et al. 2015). Fang et al. (2012) identified ecological hotspots for risk management in wetlands based on the normalized difference vegetation index and potential ecological risk index. A method for identifying priority pollutants and priority areas was proposed by the combination of bird population distribution and exposure model.

East Dongting Lake wetland (EDT) is one of the first batches of six wetlands in China registered in the List of Wetland of International Importance in 1992 and the IUCN Green List of Protected Areas in 2014. Migratory birds arrive at EDT as wintering habitats each year. With rapid industrialization and urbanization of surrounding cities, heavy metal contamination has become a serious environmental problem in EDT in the last 10 years (Li et al. 2013; Liang et al. 2015b; Qian et al. 2005; Yao 2008). Under long-term exposure to heavy metals, birds may be at risk of lethal and sublethal effects (Aazami et al. 2010). Previous bird studies in EDT were mainly focused on assessment of landscape structure, human disturbance, and habitat suitability (Liang et al. 2015a; Yuan et al. 2014), but little information is available on the impact of heavy metals on birds. This study was carried out to meet the following objectives: (1) to estimate heavy metal (Cr, Cu, Pb, and Cd) exposure risk to lesser white-fronted goose in EDT with improved model, (2) to prioritize threats from highest to lowest among the candidate metals, and (3) to identify priority pollutants and areas combining bird population density with integrated exposure risk.

Materials and methods

Study area

EDT is a part of Dongting Lake, which is the second largest freshwater lake in China. It is located on the middle and lower reaches of Yangtze River, Yueyang City, Hunan Province, central China. It covers about 1900 km^2 (approximately 112° 43′~113° 14′ E, 29° 00′~29° 38′ N), including core area of 290 km^2 and buffer area of 364 km^2 (Wu et al. 2015). EDT lies in the subtropical monsoon climate zone with an annual rainfall of 1100~1400 mm, average annual temperature of 16.4~17.0 °C, and frost-free period of 259~277 days (Wu et al. 2013). Wet season lasts from May to October and dry season from November to March. During wet season, the water level rises, and the water body overwhelms the vegetation. While in dry season, the whole lake shrinks to just 18 % and looks like a prairie with tracts of sedge and reed, providing abundant food for birds.

Due to the special geographical position and unique climate conditions, EDT provides an ideal habitat for birds to migrate, inhabit, and winter. Statistics of the East Dongting Lake National Nature Reserve Administration Bureau show that about 340 species of birds have been recorded in EDT, with 39 species listing in IUCN Red List of Threatened Species. Seven species belong to the First-Grade State Protection animals: Siberian crane (*Grus leucogeranus*), hooded crane (*Grus monacha*), oriental stork (*Ciconia boyciana*), black stork (*Ciconia nigra*), great bustard (*Otis tarda*), Chinese merganser (*Mergus squamatus*), and white-tailed sea eagle (*Haliaeetus albicilla*). More than 70 % of lesser white-fronted geese (*Anser erythropus*) in the world overwinter in EDT each year. In this study, the representative herbivorous lesser white-fronted goose has been chosen for a case study. Sedge is the main food for lesser white-fronted goose.

Bird survey and sample collection

Bird synchronization survey was carried out throughout the study area in January 13~15, 2015. The survey method is point counting. If bird populations are within 500, the number is exact to the individual. There are 50 birds in a telescope lens when more than 500. Birds were observed by monocular (Swarovski, ATS80HD, 25~50 × 80) and binocular (Nikula, 10 × 42). The location of each site was also recorded with a handheld global positioning system (BHCnav, K20) (Liang et al. 2015a; Yuan et al. 2014).

Sedge and soil samples were collected from ten selected main bird habitats in EDT, where lesser white-fronted geese are widely distributed according to the results of bird survey (Fig. 1). Four samples of newly grown sedge and the top soil (5 cm in thickness) were collected in a 50-m^2 area from each site. The samples were placed into plastic bags, refrigerated with car-carried refrigerator, taken back to the laboratory, and stored at −20 °C prior to analysis.

Analytical methods and quality control

Sedge samples were washed three times to remove external surface contamination, dried in an oven at 70 °C until constant weight, and then powdered with a high-speed grinder. Preprocessed samples of 0.5 g were accurately weighed and transferred to airtight Teflon vessels, added

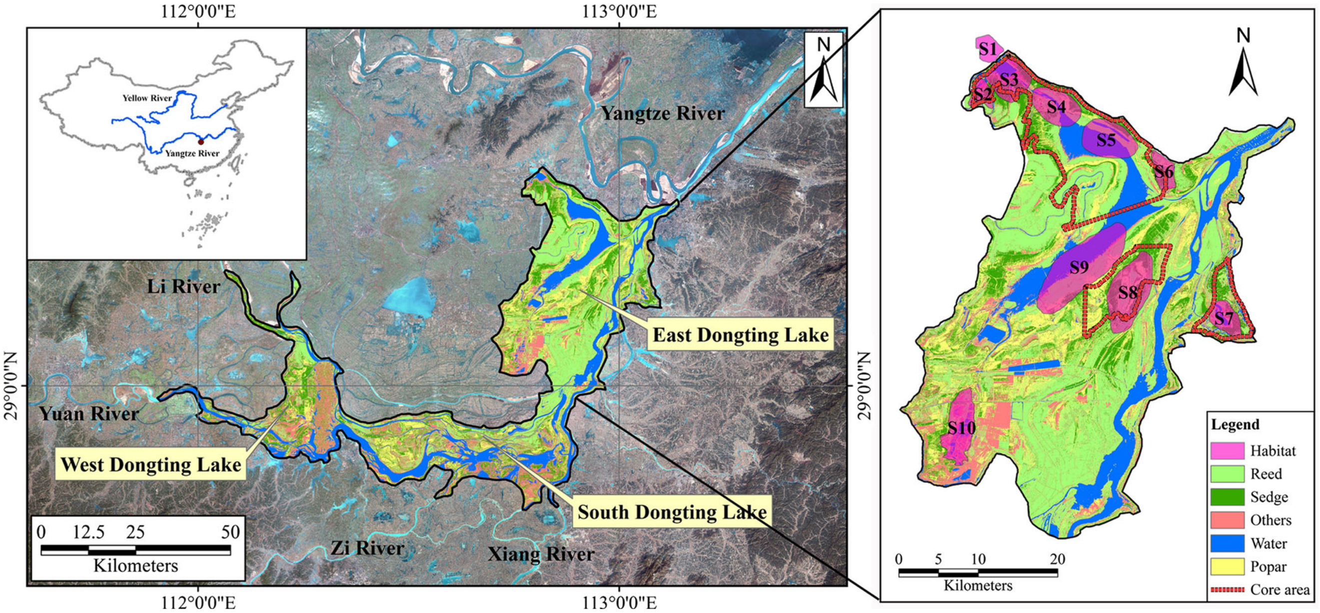


Fig. 1 Geographical location, vegetation classification, main bird habitats, and corresponding sampling sites of EDT. *S1* Caisang Lake, *S2* Small West Lake, *S3* Big West Lake, *S4* Three Dam, *S5* Dingzi Dyke, *S6* Junshan Back Lake, *S7* Spring Breeze Lake, *S8* Red Flag Lake, *S9* White Lake, *S10* Lu Lake

with 12 mL mixed acids ($HNO_3/HClO_4 = 3:1$) for digestion using Graphite Digestion System (SISP DS-360, China) (Sun et al. 2014). Soil samples were ground gently, sieved with 100-mesh sieve for homogenization. Precise 0.5-g preprocessed samples were transferred to airtight Teflon vessels, added with 10 mL HCl and then 13 mL mixed acid ($HNO_3/HF/HClO_4 = 5:5:3$) for digestion using Graphite Digestion System. The digested samples were diluted to a final volume of 50 mL with 2 % HNO_3 and then filtrated through a 0.45-μm membrane for heavy metal detection. Flame Atom Absorption Spectrophotometer (FAAS, PE AAnalyst 700, USA) was used to analyze Cr, Cu, Pb, and Cd (Li et al. 2015; Liu et al. 2015).

Ultrapure water was used for sample preparation, and all containers were soaked overnight in 5 % dilute nitric acid before using. Sample duplicates, method blanks, and standard reference materials were used to validate the results of each batch of samples. The analytical precision was conducted with repetitive rate of 10 %.

Improved exposure risk assessment model

The models utilized in the present study are mainly based on Toxicological Benchmarks for Wildlife: 1996 Revision and Ecological Risk Assessment (Second Edition) (Sample et al. 1996). Generally, receptors are exposed mainly through three pathways: ingestion, dermal contact, and inhalation. When estimating wildlife exposure, dermal contact and inhalation routes are usually ignored (Suter II 2011). Heavy metal exposure to birds through oral ingestion can be quantified by the following equations (Liu et al. 2015).

$$I_{df} = 0.648BW^{0.651} \tag{1}$$

where I_{df} is the food consumption rate (g/day, dry weight). Food consumption rates are estimated from allometric regression models (Nagy 1987; Sample et al. 1996). *BW*, bodyweight of selected species (g), was estimated to be 2000 g according to body weight measurement.

Soil attached to the plant leaves and roots can be ingested by herbivores when grazing, so it is a potential exposure pathway.

$$I_s = P \times I_{df} \tag{2}$$

where I_s is the soil consumption rate (g/day) and P is the proportion of soil accounted food. For lesser white-fronted goose, 8.2 % was chosen (Beyer et al. 1994).

In this study, exposure dose of heavy metal can be calculated by Eq. (3) (Suter II 2011).

$$E_j = \frac{\sum_{i=1}^{m} (I_i \times C_{ij})}{BW} \tag{3}$$

where E_j is the oral exposure dose of heavy metal (j) (mg/kg/day), m is the number of absorbing medium (in this study: food and soil), I_i is the consumption rate of medium (i) (g/day), and C_{ij} is the concentration of metal (j) in medium (i) (mg/kg).

Heavy metal exposure risk (risk of adverse effect) to lesser white-fronted goose is evaluated by comparing the intake dose to tolerable daily intake (*TDI*). *TDI* can be calculated by Eq. (4) (CCME 1998).

$$TDI_j = \frac{\left(LOAEL_j \times NOAEL_j\right)^{0.5}}{UF} \tag{4}$$

where TDI_j is the tolerable daily intake of heavy metal (*j*) (mg/kg/day), $LOAEL_j$ is the lowest observed adverse effect level of heavy metal (*j*) (mg/kg/day), $NOAEL_j$ is the no observed adverse effect level (mg/kg/day), and *UF* is the uncertainty factor. *TDI* is an estimate of a substance that is not anticipated to result in adverse effect. *LOAEL* and *NOAEL* were obtained from avian toxicity tests (Sample et al. 1996). According to Protocol for the Derivation of Canadian Tissue Residue Guidelines for the Protection of Wildlife that Consume Aquatic Biota, *UF* was used to account for the uncertainty of risk model and differences in sensitivity among species. The total *UF* applied for the derivation of a *TDI* may not be less than 10 in order to extrapolate to a long-term exposure concentration without an effect. The *UF* selected may be higher than 10 depending on the substance, type, amount, and quality of data available. The value of 10 was chosen in this study (CCME 1998; Morrissey et al. 2005).

Heavy metals often exist as mixtures, and the toxicity to the environment has been little investigated (Cobbina et al. 2015). Imitating human health risk assessment model (USEPA 1989), hazard quotient (*HQ*) has been employed to estimate each heavy metal exposure risk to birds intuitively.

$$HQ_j = \frac{E_j}{TDI_j} \tag{5}$$

where HQ_j is the hazard quotient of heavy metal (*j*). In this study, *HQ* was divided into three levels: no risk ($HQ<1$), low risk ($1<HQ<2$), and high risk ($HQ>2$), respectively.

Hazard index (*HI*) was used to analyze the combined risk of heavy metals to birds at each habitat. *HI* is equal to the sum of *HQ*.

$$HI_n = \sum HQ_j \tag{6}$$

where HI_n is the hazard index of sampling site (*n*). *HI* represents the combined *HQ* and can be used to estimate risk of multiple potentially hazardous elements. This approach assumes that simultaneous subthreshold exposures to several chemicals could result in an adverse health effect. It also assumes that the magnitude of the adverse effect will be proportional to the sum of the ratios of the subthreshold exposures to acceptable exposures. When *HI* exceeds unity, there may be concern for potential health effects. While any single chemical with an exposure level greater than the toxicity value will cause *HI* to exceed unity, for multiple chemical exposures, *HI* can also exceed unity even if no single chemical exposure exceeds its *TDI* (USEPA 1989).

Statistics and geostatistical methods

To make the data visualization, statistical analyses were performed using OriginPro 8.0 software, and geostatistical methods were employed as well. Remote sensing image (Landsat Thematic Mapper image, spatial resolution of 30 m) in January, 2015, of the study area was downloaded, and supervised classification based on spectral characteristics was conducted by ENVI 4.7. The inverse distance weighted interpolation and overlay methods were performed to spatially analyze the integrated heavy metal exposure risk (*HI*) in ArcGIS 9.3, combined with bird population distribution in EDT.

Results and discussion

Statistics of bird survey and heavy metal concentrations

Bird survey results by point counting showed that there were approximately one hundred thousand birds inhabiting EDT. The dominant species were 34,004 bean goose (*Anser fabalis*); 28,774 falcated duck (*Anas falcata*); 10,628 teal (*Anas crecca*); and 10,024 lesser white-fronted goose (*Anser erythropus*). These species of birds are all *Anatidae*, which are accounting for 80 % of the total population in EDT.

Table 1 Descriptive statistics of heavy metal concentrations in sedge and soil from EDT (mg/kg, dry weight, $n=40$)

		Cr	Cu	Pb	Cd
Sedge	Min	0.30	10.60	4.20	0.10
	Max	3.05	18.85	12.60	1.60
	Mean	1.33	16.02	8.09	0.58
	SD	1.00	2.75	2.49	0.44
	CV (%)	75.27	17.19	30.76	75.89
Soil	Min	96.40	55.54	32.95	0.72
	Max	134.75	88.01	124.09	1.33
	Mean	108.99	64.24	68.16	1.06
	SD	11.99	10.26	35.29	0.17
	CV (%)	11.00	15.98	51.78	16.34
	BV	83.92	25.00	27.75	0.23

SD standard deviation, *CV* coefficient of variation, *BV* the background values of heavy metals in soil from Dongting Lake, according to the Environment Quality Report of Hunan Province (2011)

Descriptive statistics of studied heavy metals in sedge and soil from EDT are shown in Table 1, along with the background values of soil in Dongting Lake. The concentration ranges (mg/kg, dry weight) of Cr, Cu, Pb, and Cd were as follows: 0.30~3.05, 10.60~18.85, 4.20~12.60, and 0.10~1.60 for sedge and 96.40~134.75, 55.54~88.01, 32.95~124.09, and 0.72~1.33 for soil. The mean concentrations of elements in soil all exceeded the corresponding background values at 1.30, 2.57, 2.46, and 4.61 times, respectively. It demonstrated that there was a certain degree of heavy metal pollution, which was consistent with previous studies (Li et al. 2014; Liang et al. 2015b). High coefficients of variation were found for Cr and Cd of sedge and Pb of soil, indicating their high inhomogeneity, which might be ascribed to the impact of human activities. In contrast, Cu showed a relatively small spatial differentiation.

Heavy metal exposure to lesser white-fronted goose

Selected toxicity parameters and calculated *TDI*s are shown in Table 2. Heavy metal exposure doses to lesser white-fronted goose were calculated based on Eqs. (1)~(3), and the results are presented in Fig. 2. Sedge exposure dose of Cr was lower than the corresponding *TDI*, but the total was higher than *TDI* because of high exposure from soil. The result indicates that Cr contamination in soil may have a great influence on lesser white-fronted goose. Therefore, it is incomplete when estimating bird exposure merely considering food ingestion. Soil consumption should also be taken into account. The total exposure dose of Cu was below the corresponding *TDI*, indicating no negative effect on population. Compared with sedge ingestion dose and *TDI*, soil exposure of Cu was very low, and the effect could be ignored.

The total exposure dose of Pb was beyond *TDI*, and sedge exposure dose of Pb roughly reached the value of *TDI*. It implied that lesser white-fronted geese were sensitive to Pb contamination and more likely to have an adverse effect. Attentions should be drawn on controlling potential sources of Pb. Cd exposure doses were all far below *TDI*, suggesting a safer level. It is inconsistent with previous work, which showed that the pollution risk of Cd is the most serious compared with other heavy metals. It is probably due to its higher value of *TDI* for lesser white-fronted goose. In general, Cr and Pb are most likely to have adverse effect on lesser white-fronted goose in EDT, while the concentrations of Cu and Cd are considered to be relatively safe. Cu, Pb, and Cd had similar exposure characteristics with higher sedge exposure doses than soil. Effect of soil exposure of Cu and Cd is relatively small. In general, sedge ingestion pathway can be identified as the main route of heavy metal exposure.

Table 2 Selected toxicity parameters and calculated *TDI*s in the study (mg/kg/day)

	NOAEL	*LOAEL*	*TDI*
Cr	1	5	0.22
Cu	11.7	15.4	1.34
Pb	1.13	11.3	0.36
Cd	1.45	20	0.54

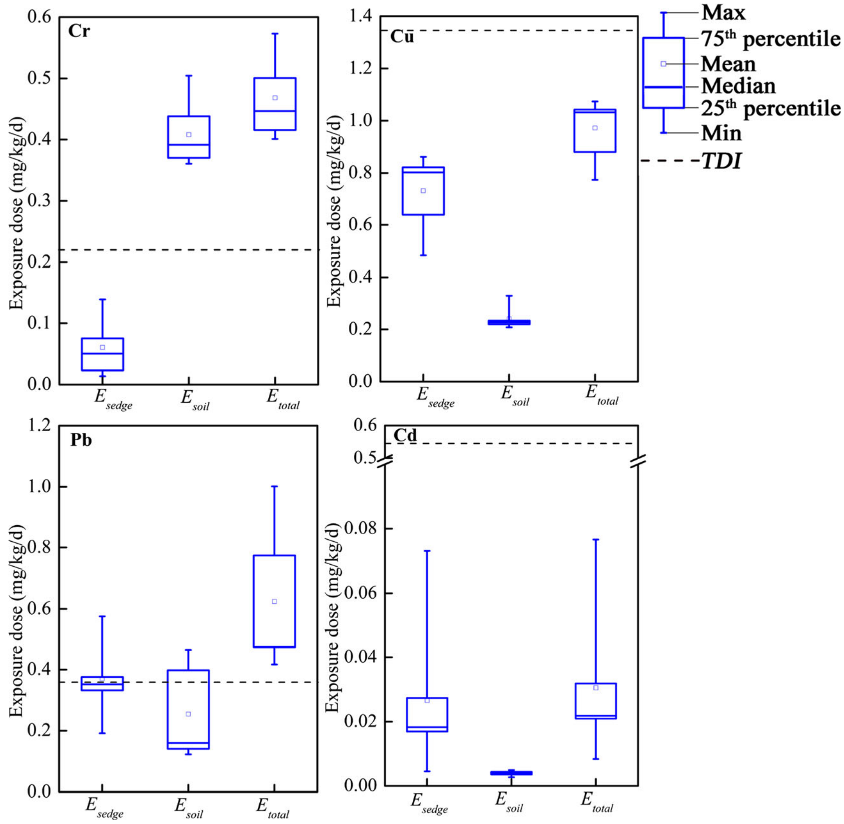
The corresponding *HQ* of heavy metal exposure to lesser white-fronted goose is presented in Fig. 3. Prioritize threats from highest to lowest among the candidate metals were decreased in the following sequence: Cr > Pb > Cu > Cd. According to the grades of *HQ* assigned in this study, Cr had the highest risk with $HQ > 2$ and Pb was in a low risk with $1 < HQ < 2$. No risk was found for Cu and Cd with $HQ < 1$. Kertész and Fáncsi (2003) found that duckling malformation rate was 30 % after Mallard (*Anas platyrhynchos*) eggs soaked in a trivalent chromium solution of 50 μg/L for 30 min. Pb can cause anemia and damage to the central nervous system and tissue (Park et al. 2008). On the whole, Cr and Pb were considered to be the priority pollutants.

Spatial distribution of bird population and integrated exposure risk

With increased influence on eco-environment of EDT by anthropogenic activity, Hunan Province and the East Dongting Lake National Nature Reserve Administration Bureau have transformed the ecological environment and carried out closed-off management since 2006. Up to 2012, closed-off management has been implemented all over the core areas (Liang et al. 2015b). There are six habitats, Small West Lake (S2), Big West Lake (S3), Three Dam (S4), Dingzi Dyke (S5), Spring Breeze Lake (S7), and Red Flag Lake (S8), included in the existing core areas (Fig. 1). To identify the priority areas from the perspective of heavy metal exposure risk to birds, population density and spatial interpolation of *HI*s are shown in Fig. 4. *HI*s were performed by inverse distance weighted interpolation, and the spatial interpolation map of *HI*s was made with overlay of bird population distribution map.

In general, eastern area had higher *HI* than western, especially S7 and S8. There were relatively more birds at S7, and the integrated exposure risk of studied heavy metals was great in this area. Half of the total lesser white-fronted goose in EDT inhabited in Spring Breeze Lake according to bird survey data. It is recommended that priority conservation strategies should be developed in this area. It is urgently needed to identify heavy metal sources and strengthen control pollution measures. According to previous studies, mining, pesticides, chemical industries, and burning of fossil fuels were the main sources of heavy metal pollution in Dongting Lake. A moderate number of birds were presented at S6 and S9. Relatively few birds were found at S8, but the exposure risk cannot be ignored with high *HI*. According to the spatial interpolation map, high heavy metal risk areas were identified as priority

Fig. 2 Sedge, soil, and total exposure doses of heavy metals to lesser white-fronted goose in EDT. E_{sedge} exposure dose via food pathway, E_{soil} exposure dose via soil pathway, E_{total} exposure dose via both food and soil pathways



areas with $HI > 5$ (Fig. 4) for risk control and hierarchical management, including S6, S7, S8, and eastern part of S9.

Core area of the northwest including S2, S3, S4 and S5, which presented a low heavy metal exposure risk, could be ascribed to less pollution sources and close-off management. Other core areas were located in the priority areas assigned in this study. However, these areas are subject to intensive human activities, such as sand excavation, electric fishing, and inevitable leakage of oil from operating vessels. What is more, the only freshwater dolphin subspecies in the world, Finless Porpoise (*Neophocaena asiaorientalis asiaorientalis*), survives in the lake between the eastern two core areas. Moreover, S8 and S9 are the habitats of China's only natural wild elk populations. Heavy metal exposure to these species may exist according to this study of birds. It further highlights the significance of identified priority areas in the study considering most of the above-mentioned areas included.

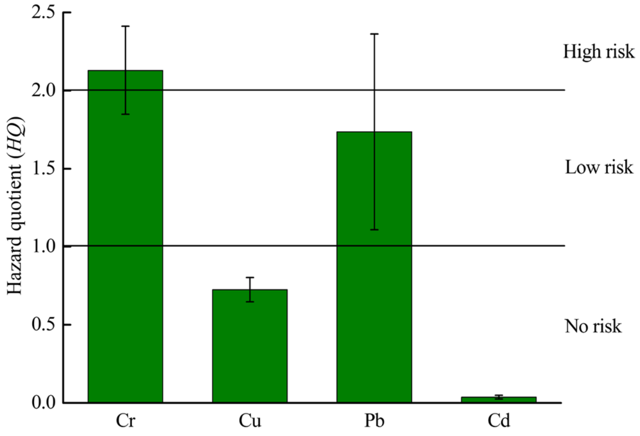


Fig. 3 *HQ* of selected heavy metals

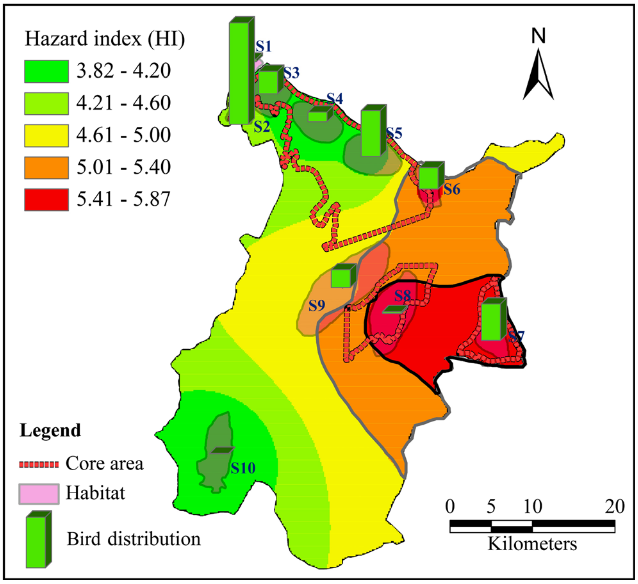


Fig. 4 Spatial interpolation of *HI* with the overlay of bird population density

Contamination transport analysis and model uncertainty evaluation

EDT plays an important role in Northeast Asia *Gruidae* flyway, East Asia *Anatidae* flyway, and East Asia-Australia wader flyway. Tens of thousands of migratory birds arrive at EDT as wintering habitats from Siberia and Mongolia (breeding habitats) around October each year, wintering until March the next year. Migratory birds can accumulate heavy metals in tissues at EDT (Fu et al. 2014; Morrissey et al. 2005; Salamat et al. 2014). Contamination during the wintering period can influence concentrations in birds at breeding habitats (Lavoie et al. 2014). When returning to the breeding habitats in spring, migratory birds may bring heavy metal contamination in these areas.

As a risk assessment model, uncertainty is the restrictive factor and should take into account, such as the choice of parameters and the model itself. Some assumptions are the premise during quantitative analysis. In this study, drinking water exposure, dermal contact, inhalation routes, food composition, and grit consumption were not considered. The annual migration and daily movement were ignored as well. The selections of these test and endpoint species are other similar species. Moreover, other heavy metals (Hg, As, Zn, Mn, etc.) and carnivorous birds were not studied. Although there are some uncertainties, the method is demonstrated to be effective for exposure risk assessment, and the results are considered to be useful for risk management in wetlands.

Conclusion

A model for estimating heavy metal exposure risk to lesser white-fronted goose in EDT has been employed and improved. Bird survey was carried out in EDT based on point counting to identify bird distribution in January, 2015. Sedge and soil samples were collected at the same time. The concentrations of Cr, Cu, Pb, and Cd in soil all exceeded the background in EDT. Cr in soil may have a great influence on lesser white-fronted goose, and lesser white-fronted geese were sensitive to Pb exposure. Negative effect of Cr and Pb on lesser white-fronted goose in EDT may occur while the concentrations of Cu and Cd are considered to be relatively safe. *HQ*s decreased in the order of $Cr > Pb > Cu > Cd$. Cr had a high risk with $HQ > 2$, and Pb was in a relatively low risk with $1 < HQ < 2$. Cu and Cd presented no risk with $HQ < 1$. Cr and Pb should be considered to be the priority pollutants.

Interpolation of *HI*s with overlay of bird population density was performed to identify the priority areas from the perspective of heavy metal exposure risk to birds. S7 with high *HI* and relatively more birds should draw much attention. Exposure risk of S8 with fewer birds but high *HI* cannot be overlooked. Priority areas with $HI > 5$ were identified for decision makers in risk management and developing bird conservation strategies. The presented method can also be used for exposure risk assessment of other pollutants to other wildlife and risk management around the world.

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Compliance with ethical standards The manuscript has not been submitted to more than one journal for simultaneous consideration.

The manuscript has not been published previously (partly or in full).

A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time.

No data have been fabricated or manipulated (including images) to support the conclusions.

No data, text, or theories by others are presented as if they were our own. Proper acknowledgements have been given, quotation marks are used for verbatim copying of material, and permissions are secured for material that is copyrighted.

Consent to submit has been received explicitly from all co-authors.

Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

Conflict of interest The authors declare that they have no conflict of interest.

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