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Chinese Birds 2010, 1(2):124–131

ORIGINAL ARTICLE

DOI 10.5122/cbirds.2010.0004

Effect of colonial breeding of Chinese Egret (*Egretta eulophotes*) on the heavy metal accumulation in heronry soil

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Abstract We measured the concentrations of heavy metal elements, i.e., V, Cr, Mn, Ni, Cu, Zn, Cd, Se and Pb and the semi-metal element As in feces of Chinese Egret (*Egretta eulophotes*) on Caiyu Island in Zhangpu County, Fujian Province, China using inductively coupled plasma-mass spectrometry (ICP-MS) and compared the differences between the topsoil of their nesting and non-nesting areas before and after breeding. The results show that no Se and Cd was detected in any of the samples, including feces and soil, while heavy metal concentrations in the non-nesting soil were not significantly different before and after breeding ($p > 0.05$), but the differences in concentrations of Zn and Pb in the nesting soil were highly significant before and after breeding ($p < 0.01$). A comparison of the concentrations of the elements in the nesting and non-nesting soils also reveals that before breeding, the concentration of Zn in these two soils were significantly different ($p < 0.01$). After breeding, concentrations of Cu, Zn, As and Pb in the nesting and non-nesting soil were significantly different ($p < 0.01$) while V and Ni concentrations showed merely significant differences ($p < 0.05$). These findings indicate that the colonial breeding activities of *E. eulophotes* play an important role in the transfer of heavy metals between wetland and island ecosystems and that such activities may, over time, result in heavy metal contamination of the heronry soil on the island.

Keywords Chinese Egret (*Egretta eulophotes*), heavy metal, island, heronry, soil

Introduction

As a consequence of the excessive discharge of pollutants from industrial production and other human activities, large amounts of pollutants have made their way into the environment. These accumulate in wetland ecosystems through the effect of runoff and are then transmitted along the food chain through the trophic relations of organisms (Furness and Greenwood, 1993; Kim and Koo, 2007). Recently, colonial waterbirds have attracted much interest because of their capacity to introduce large amounts of wetland-derived nutrients to land (Anderson and Polis, 1999; Garcia et al., 2002; Ellis, 2005). Ardeid birds are the top-level predators in wetland ecosystems, exhibiting behavioral characteristics of colonial breeding and perhaps exerting an enriching effect on pollutants.

Previous studies have found that during the course of colonial breeding, the feces of parent birds and their offspring, food dropped while parent birds feed their offspring, the bodies of dead birds, feathers being shed and the shells of abandoned eggs carry organic matters to the breeding grounds, causing changes in soil properties of the colonial breeding grounds (Hogg and Morton, 1983; Bukacinski et al., 1994; Li et al., 2005). When heavy metal contamination of nesting soils exceeds the limit of tolerance of

Received 20 January 2010; accepted 15 April 2010

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plants, the absorption and metabolism of these plants will be disrupted and some contaminants will end up inside the plants, affecting their growth, cause serious soil contamination and may even result in the death of plants (Anderson and Polis, 1999). Furthermore, heavy metal contamination of soils may drive plant species turnover in the nesting habitat after a few years, forcing birds to change their breeding location (Vidal et al., 2000; Li et al., 2005). However, not much research has been conducted on the accumulation of heavy metal contamination in soils as a result of colonial breeding in birds (Headley, 1996; Otero, 1998).

In this study, we aim specifically to investigate: 1) what are the concentrations of the heavy metals (V, Cr, Mn, Ni, Cu, Zn, Cd, Se and Pb) as well as of semi-metal element (As) in the feces of Chinese Egret (*Egretta eulophotes*, Plates I and II), which has been listed as a vulnerable species with an estimated global population of 2600–3400 birds (IUCN, 2009); 2) whether differences can be found between the topsoil of their nesting and non-nesting areas on islands before and after breeding and 3) if a colonial breeding of *E. eulophotes* is likely to have an effect on the accumulation of heavy metals in its heronry soil.

Study area and methods

Study area

Samples of feces and soil were collected from the heron colony of the endangered Chinese Egret on Caiyu Island in Zhangpu County, Fujian Province, China (23°46'N, 117°39'E). The distance of the uninhabited Caiyu Island from the mainland is about 8 km. This uninhabited island is an ideal place for studying the impact of the colonial breeding of birds on the concentration of heavy metals in its soils since the island is relatively far off the coast and the concentration of elements in its soil is not subject to the impact of agriculture and other human activities. The nearby mainland areas of this island are characterized by several artificial wetland habitats, including seawater culture ponds of shrimp and fish, rice fields and various sloughs and ditches. The breeding waterbird species at Caiyu Island are *Egretta eulophotes* and *E. sacra* of the Ardeidae family, *Larus crassirostris* of the Laridae family and *Sterna anaethetus* and *S. sumatrana* of the Sternidae family. *E. eulophotes* nests on trees in the center of the island and the other four species nest on the rocky areas around the edge of the island, thus making the *E. eulophotes* to form a separate heron colony.

Sample collection

Sampling was conducted on Caiyu Island in mid-April, 2008 before the Ardeidae birds started breeding and in mid-August of the same year after the parent birds and their offspring had left their nests. Feces samples of *E. eulophotes* were collected from the trunks of 10 nesting trees on the island during the 2008 breeding season.

For soil sampling, we designated 10 randomly selected breeding nest trees at the *E. eulophotes* nesting habitat as 10 sample locations. These 10 trees largely covered the entire nesting habitat. Nine soil sampling points were established under each tree according to a checkerboard distribution method for soil extraction. The checkerboard was 100 cm long and 100 cm wide. At each soil sampling point, a small stainless steel spade was used to extract approximately 0.25 kg of topsoil (about 0 to 5 cm deep). Each soil sample was sealed in a numbered bag and taken back to the laboratory. We also selected 10 non-nesting sample locations, corresponding to nesting sample locations in terms of properties (elevation, soil color, soil grain size and vegetation cover) and conducted sampling using the same method.

Element analysis

The instruments used included a Muffle furnace (Tianjin Northern China Lab Instruments Company), a high-pressure digestion vessel, an oven, PE DRC-e Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), a constant-temperature drying oven and ultra-pure water equipment (Millipore, France). The reagents used were a mixed standard solution, nitric acid (GR), ultra-pure water (Millipore), ultra-pure water and acetone.

Soil samples were spread on clean sheets of kraft paper for drying and removal of gravel, root residues and other impurities. After being compacted with a mallet, the samples were extracted using a quartering method (Li et al., 1998). Samples were then ground in a mortar before being screened with a 20-mesh nylon sieve. Afterwards, the soil samples were spread and grouped into a few cells. Then, the samples, totaling approximately 200 g, were taken from multiple points using a medicine spoon. The samples

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Plate I Chinese Egret (*Egretta eulophotes*). Photos by Qingxian Lin on summer at Caiyu Island, Fujian Province, China. (**a**) colonial breeding; (**b**) breeders in flight; (**c**) adult Chinese Egret in breeding plumage.

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Plate II   Chinese Egret (*Egretta eulophotes*). Photos by Qingxian Lin on summer at Caiyu Island, Fujian Province, China. (**a**) incubating of Chinese Egret at nest; (**b**) feeding of adult Chinese Egret in coast wetland; (**c**) nesting of Chinese Egret.

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were ground again and screened with a 100-mesh nylon sieve before being sealed in a bag. The *E. eulophotes* feces was dried in the constant-temperature drying oven and screened with a 100-mesh nylon sieve.

In the digestion of soil samples, 0.1 g soil samples were placed inside the high-pressure digestion vessel, where 5 mL HNO_3 and 1 mL $HClO_4$ were added. The vessel was tightly recapped and placed in the oven, where it was heated at 100°C for an hour and 120°C for four hours. Afterwards, the tightly-sealed digestion vessel was reopened and the samples were removed and placed into clean 50 mL PET bottles. The digestion vessel and its cover were cleansed in ultra-pure water three to four times and the cleansing solution was put into the volumetric flask to a level of 50 mL. A sample was also prepared for purposes of contrast.

In the digestion of feces samples, 0.2 g samples were placed into a crucible and 2 mL of HNO_3 was added to the crucible, which was then placed into the Muffle furnace for heating at 120°C for four hours. Afterwards, the samples were removed and another 2 mL HNO_3 was added. The samples were then placed in a clean 50 mL volumetric flask where they were rinsed with ultra-pure water; in the end, the samples were filtered and the flask filled to a level of 50 mL.

Before the analysis of the elements, standard curves were formulated. Mixed standard solutions were progressively digested with ultra-pure water to 10, 20 and 40 ppb to obtain a series of mixed standard solutions. A series of blank and standard solutions was collected under optimized instrumental conditions, with the instrument automatically drawing standard curves. The correlation coefficient of the standard curves of the elements, i.e., *r*, was greater than 0.9995 in all cases.

The concentration of heavy metals was measured using inductively coupled plasma-mass spectrometry (ICP-MS). We established the following ICP-MS analytical procedure: given the property of self-absorption of the PFA micro-flow centrifugal atomizer, the digestion solution of samples was atomized and then the plasma was entered. The analytical procedure was then activated to measure the signal strength of V, Cr, Mn, Ni, Cu, Zn, Cd, As, Se and Pb and their corresponding concentrations were measured using the standard curves.

Statistics analysis

Statistical tests were performed using SPSS software, version 13.0. One-way ANOVA was used to detect any differences in the mean concentrations of heavy metals and other elements in the soil. Following the ANOVAs, Student-Newman-Keuls (SNK) tests were used for pairwise multiple comparisons. Significance was accepted at the 0.05 probability level.

Results

Heavy metal concentrations and changes in soil

We did not detect any Se or Cd in the nesting or non-nesting soils before or after breeding. As shown in Table 1, a comparison of the data from the non-nesting soils before and after breeding reveals no

Table 1 Comparison of elements in the nest and non-nest soil of *Egretta eulophotes* (unit: $\mu g \cdot g^{-1}$)

Sample	V	Cr	Mn	Ni
Before-non	0.678±0.191	32.334±37.333	92.484±24.407	3.498±3.712
After-non	0.641±0.089a	27.176±28.830	82.929±17.043	7.097±12.395a
Before-nest	0.781±0.244	39.273±37.642	87.116±19.096	20.591±45.894
After-nest	0.868±0.190b	121.653±303.890	102.349±40.525	68.105±112.834b
Sample	**Cu**	**Zn**	**As**	**Pb**
Before-non	0.647±0.576	29.888±7.392a	0.365±0.350	12.012±1.563a
After-non	0.708±0.481a	33.351±2.455ab	0.245±0.245	12.739±1.637a
Before-nest	1.874±0.930	39.248±2.240c	0.498±0.267	12.871±1.759ab
After-nest	3.136±3.002b	45.742±2.351d	0.756±0.383	15.309±1.500c

Note: "Before-non" stands for non-nest soil before breeding, "after-non" for non-nest soil after breeding, "before-nest" for nest soil before breeding, and "after-nest" for nest soil after breeding. The values with different letters in rows for means (± SD), represent significant differences ($p < 0.05$).

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significant difference in heavy metal concentrations in the non-nesting soils before and after breeding. Another comparison from the nesting soil before and after breeding shows that all elements in the soil had a higher concentration after breeding than they did before breeding and that there was a highly significant difference ($p < 0.01$) in the concentrations of Zn and Pb before and after breeding.

A further comparison of the data from the nesting and non-nesting soils during the same period indicated that the nesting soil contained higher concentrations of heavy metals (except Mn) than non-nesting soils, both before and after breeding. Before breeding, the concentration of Zn was significantly greater in the nesting soil ($p < 0.01$) than in the non-nesting soil, while after breeding, the concentrations of V, Cu, Zn, As and Pb were considerably greater in the nesting soil ($p < 0.01$) than in the non-nesting soil. The concentration of Ni was also significantly greater in the nesting soil ($p < 0.05$) than in the non-nesting soil.

Heavy metal concentration in feces

No Se or Cd was detected in *E. eulophotes* feces in this study. The concentrations of V, Cr, Mn, Ni, Cu, Zn, As and Pb in *E. eulophotes* feces are shown in Fig. 1. The highest concentration of Zn reached 424.292 ± 268.727 μg·g^{-1}, while the concentration of Mn was 218.590 ± 112.298 μg·g^{-1}, that of Pb 26.333 ± 9.370 μg g^{-1}, of Cr 20.000 ± 37.223 μg·g^{-1}, of Cu 7.038 ± 2.569 μg·g^{-1}, of Ni 6.968 ± 5.245 μg·g^{-1}, of V 0.037 ± 0.0562 μg·g^{-1} and the concentration of As was 0.014 ± 0.0439 μg·g^{-1}.

Discussion

Headley (1996) found that the feces of seabirds in the Arctic contained high levels of heavy metals and therefore drew the conclusion that the feces of seabirds was the primary reason for heavy metal contamination in breeding grounds. The concentrations of Zn, Mn and Cu in the feces of *E. eulophotes* in our study (424.292 ± 268.727, 218.590 ± 112.298 and 7.038 ± 2.569 μg·g^{-1}, respectively) were higher than those in feces of *Larus hyperboreus* as measured by Headley (76.0, 39.3 and 6.3 μg·g^{-1}). The concentrations of Zn, Mn and Pb (26.333 ± 9.370 μg·g^{-1}) in our study were also higher than those in the feces of *Rissa tridactyla* as measured by Headley (the concentration of Zn, Mn and Pb stood at 176.0, 24.1, and 21.6 μg·g^{-1}, respectively). However, the concentration of Ni (6.968 ± 5.245 μg·g^{-1}) in our study was lower than that of *L. hyperboreus* (9.9 μg·g^{-1}), while the concentrations of Cu and Ni in this study were also lower than those of *Rissa tridactyla* (51.2 and 8.4 μg·g^{-1}). Compared with the heavy metal concentrations in *Larus cachinnans* on Cies Island (Otero, 1998), the concentrations of Zn and Cr (20.000 ± 37.223 μg·g^{-1}) in the feces of

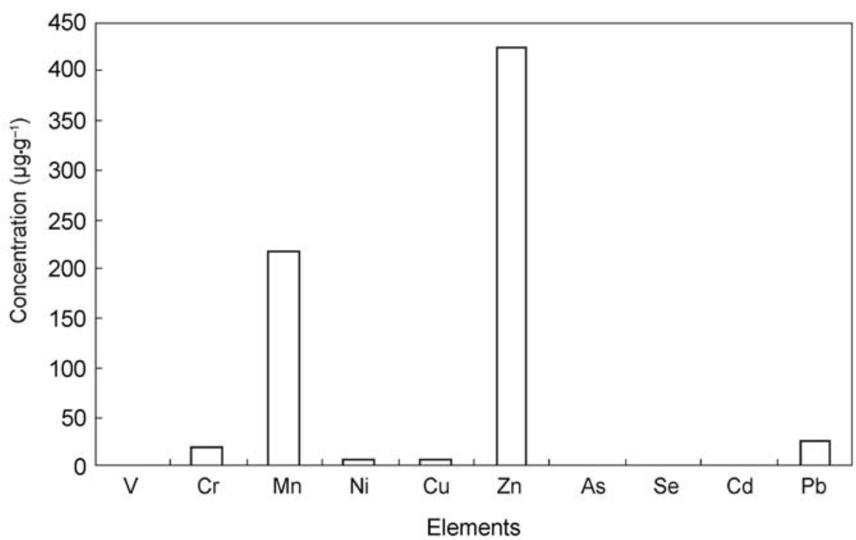

Fig. 1 Comparison of elements in feces of *Egretta eulophotes*

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E. eulophotes were higher than those in the feces of *L. cachinnans* (305.1 ± 158.5 and 9.8 ± 5.1 $\mu g \cdot g^{-1}$), whereas the concentrations of Cu and Pb in the feces of *E. eulophotes* were lower than those in the feces of *L. cachinnans* (60.1 ± 33.3 and 39.9 ± 5.8 $\mu g \cdot g^{-1}$, respectively). Some studies suggest that heavy metal concentrations in birds are related to their feeding habits, offspring rearing behavior and physiological characteristics and that heavy metals in feces are useless metals which have accumulated in their internal organs and are eventually expelled by various physiological means (Norheim, 1987; Rainbow, 1990; Elliot et al., 1992). The feces of *E. eulophotes* in this study contained higher levels of Zn, Cr and Mn, lower levels of Ni and average levels of Cu and Pb compared with previous reports (on the heavy metal concentrations in the feces of seabirds in the Arctic and *Larus cachinnans* on Cies Island). Whether these differences were caused by food, metabolism or other factors requires further study.

In this study, the concentration of elements in non-nesting soil did not show any significant differences before and after breeding, indicating that during the breeding season of *Egretta eulophotes* on the island, no external factors (such as absorption of plants, rainfall and soil runoff) had caused changes in heavy metal concentrations in the soils of the island. On the other hand, heavy metal concentrations in the nesting soil after breeding were larger than those in the same soil before breeding, with the concentrations of Zn and Pb showing a highly significant difference ($p < 0.01$). As such, one year colonial breeding of *E. eulophotes* may cause an increase of heavy mental concentrations in the nesting soil.

Furthermore, the concentrations of heavy metals (except Mn) were greater in the nesting soil than in the non-nesting soil, both before and after breeding. After breeding, the concentrations of V, Cu, Zn, As and Pb were greater in the nesting soil ($p < 0.01$) than in the non-nesting soil, with the concentration of Ni ($p < 0.05$) significantly greater in the nesting soil than in the non-nesting soil. This indicates that during the colonial breeding of *E. eulophotes*, heavy metals resulting from microbiological degradation of the feces of parent birds and their offspring, dropped food, dead birds, shed feathers and abandoned egg shells increased the heavy metal concentrations in the nesting soil. This is consistent with the findings of previous studies (Headley, 1996; Otero, 1998; Garcia et al., 2002). Before breeding, only the concentration of Zn was significantly greater in the nesting soil ($p < 0.01$) than in the non-nesting soil. This is consistent with the high concentration of Zn in the feces of *E. eulophotes*, indicating that the high concentration of Zn in the feces had resulted in an increased difference in the concentration of Zn in nesting and non-nesting soils after years of colonial breeding and Zn accumulation.

Taken together, it is clear that colonial breeding activities of *E. eulophotes* play an important role in the transfer of heavy metals between wetland and island ecosystems. However, the heavy metal concentrations on the island as measured in this study do not exceed the level specified in the Grade 1 standard in China's Environmental Soil Quality Standards (GB15618-95) (Xia et al., 1995). This perhaps can be explained by the small size of the *E. eulophotes* population on Caiyu Island (approximately 200 birds per year) and by the short history of documented *E. eulophotes* breeding on the island (no documentation until 2003). As such, it is necessary to conduct long-term monitoring to identify changes in the size of the colonial breeding of *E. eulophotes* and in heavy metal concentrations in the nesting soil in order to provide a scientific basis for the formulation of management strategies of the nesting soil of *E. eulophotes*.

Acknowledgments This research was supported by the National Natural Science Foundation of China (Grant Nos. 40876077, 30970380) and the Fujian Natural Science Foundation of China (2008S0007, 2009J01195).

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黄嘴白鹭集群繁殖对营巢地土壤重金属积累的效应

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摘要：本研究采用电感耦合等离子质谱（ICP-MS）方法，测定福建漳浦菜屿岛黄嘴白鹭（*Egretta eulophotes*）排泄物的重金属元素（V、Cr、Mn、Ni、Cu、Zn、Cd、Se、Pb）及半金属元素（As）的含量，分析比较黄嘴白鹭繁殖之前和繁殖之后的集群营巢地、非营巢地的表层土壤的重金属元素及半金属元素含量的变化。结果显示：在本次测定的所有排泄物和土壤样本中均未检出元素Se和Cd；非营巢地土壤中各元素的含量在繁殖前后都没有显著差异（$p > 0.05$），营巢地土壤中的Zn和Pb元素的含量在繁殖前后有极显著差异（$p < 0.01$）。营巢地和非营巢地土壤之间的元素含量比较结果发现，繁殖之前两地土壤之间的Zn含量存在极显著差异（$p < 0.01$）；繁殖之后两地土壤之间的Cu、Zn、As、Pb存在极显著差异（$p < 0.01$），V、Ni存在显著差异（$p < 0.05$）。上述数据说明黄嘴白鹭的集群繁殖活动对湿地和海岛生态系统之间的重金属转移起着重要作用，长时间则可能造成海岛营巢地土壤的重金属污染。

关键词：黄嘴白鹭（*Egretta eulophotes*），重金属，海岛，营巢地，土壤

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