Ecotoxicology and Environmental Safety 106 (2014) 62–67

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Bioaccumulation of heavy metals in fish and Ardeid at Pearl River Estuary, China

C.K. Kwok [a,d], Y. Liang [a,d,*], H. Wang [c,d], Y.H. Dong [c,d], S.Y. Leung [a], M.H. Wong [a,b,d,**]

[a] Croucher Institute for Environmental Sciences, and Department of Biology, Hong Kong Baptist University, Hong Kong, China
[b] Department of Science and Environmental Studies, Hong Kong Institute of Education, Hong Kong, China
[c] Institute of Soil Science, Chinese Academy of Sciences (ISSAS), Nanjing, Jiangsu, PR China
[d] Joint Open Laboratory on "Soil and Environment" of Hong Kong Baptist University and Institute of Soil Science of Nanjing, PR China

ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form
11 April 2014
Accepted 15 April 2014
Available online 14 May 2014

Keywords:
Bioaccumulation factor
Bioaccumulation
Heavy metals
Mai Po
Ardeid
Eggshell

ABSTRACT

Sediment, fish (tilapia, *Oreochromis mossambicus* and snakehead, *Channa asiatica*), eggs and eggshells of Little Egrets (*Egretta garzetta*) and Chinese Pond Herons (*Ardeola bacchus*) were collected from Mai Po Ramsar site of Hong Kong, as well as from wetlands in the Gu Cheng County, Shang Hu County and Dafeng Milu National Nature Reserve of Jiangsu Province, China between 2004 and 2007 (n=3–9). Concentrations of six heavy metals were analyzed, based on inductively coupled plasma optical emission spectrometry (ICP-OES). Significant bioaccumulations of Cd (BAF: 165–1271 percent) were observed in the muscle and viscera of large tilapia and snakehead, suggesting potential health risks to the two bird species, as the fishes are the main preys of waterbirds. Significant ($p < 0.01$) linear relationships were obtained between concentrations of Cd, Cr, Cu, Mn, Pb and Zn in the eggs and eggshells of various Ardeid species, and these regression models were used to extrapolate the heavy metal concentrations in the Ardeid eggs of Mai Po. Extrapolated concentrations are consistent with data in the available literature, and advocate the potential use of these models as a non-invasive sampling method for predicting heavy metal contamination in Ardeid eggs.

1. Introduction

Inner Deep Bay Mai Po Ramsar site (Mai Po) is the largest wetland in Hong Kong (1500 ha). This area was designated as a wetland of international importance (also called Ramsar site) under the Ramsar Convention in 1995 (under the management of World Wide Fund, Hong Kong (WWFHK) (2006)), in order to protect some endangered species such as the Black-faced Spoonbill (*Platalea minor*). However, environmental deterioration of Mai Po Ramsar site, caused especially by heavy metals and other pollutants discharged from the Pearl River Delta, has received much public concern over the past decades (Liang and Wong, 2003). An earlier study indicated that sediments from this Ramsar site were in general rather toxic based on results of four toxicity tests (Microtox, solid-phase test, *Daphnia* mortality test and algal test using *Microcystis aeruginosa)* (Kwok et al., 2011). Sediments (Liang and Wong, 2003; Man et al., 2004) and different aquatic organisms including gastropods (Lai et al., 2005), fish and shrimps (Cheung and Wong, 2006) from Mai Po were found to be contaminated by heavy metals.

Due to waterbirds' longevity and top position in the food chains in wetland ecosystems (Furness, 1993), they are susceptible to heavy metal contamination. Given the high bioaccumulation of contaminants in their bodies (e.g., organochlorines, Hg and Cd) from an extensive geographical area over a long period of time (Khan et al., 2013; Zhang and Ma, 2011), waterbirds could therefore potentially serve as useful bioindicators of environmental pollution. A number of recent studies reported bioaccumulation of non-essential elements (e.g., Cd, Hg, and Pb) in different organs and tissues of Ardeid, such as feathers (Abdennadher et al., 2011; Zhang et al., 2006), liver, kidney, muscle (Jayakumar and Muralidharan, 2011; Kim and Koo, 2011) and eggs (Boncompagni et al., 2003; Zhang et al., 2006). Feathers and eggshells were commonly sampled (as a non-invasive sampling approach) for evaluating heavy metal contamination in birds (such as Fu et al., 2014; Malik and Zeb, 2009; Muralidharan et al., 2004). Nevertheless, analyses of contaminants in bird eggs can provide invaluable information on risks posed to both the young and adult birds (Lam et al., 2008), which cannot be acquired from feathers or eggshells.

Several earlier studies investigated the relationship between heavy metal contents in bird egg content and eggshells (Abduljaleel et al., 2011; Dauwe et al., 1999; Mora, 2003), and generally demonstrated

* Corresponding author at: Croucher Institute for Environmental Sciences, and Department of Biology, Hong Kong Baptist University, Hong Kong, China.
** Corresponding author at: Department of Science and Environmental Studies, Hong Kong Institute of Education, Tai Po, Hong Kong, China.
E-mail addresses: yliang@hkbu.edu.hk (Y. Liang), minghwong@ied.edu.hk (M.H. Wong).

http://dx.doi.org/10.1016/j.ecoenv.2014.04.016
0147-6513/

that inorganic elements could accumulate in eggshells, and thus eggshells could be used as indicators of heavy metal pollution. However, these studies were based on domestic poultry, passerine birds and tits, and to date, there has been no relevant research in this area on waterbird species. In order to use contamination in eggshells to predict contamination in waterbird eggs, it is critical to establish a quantitative relationship between eggshell and egg content contamination for these species.

The major objectives of this study were to (1) study the current status of heavy metal contamination in the main prey of two species of Ardeid (Little Egrets [*Egretta garzetta*] and Chinese Pond Herons [*Ardeola bacchus*]), and in the eggs and eggshells of these two bird species; (2) establish preliminary quantitative relationships between metals in the eggs and eggshells, in order to use the models constructed as convenient tools for using the metal concentrations of eggshells to estimate the contamination in the eggs. Using these models, the associated risks of metal contamination to the Ardeid could be evaluated without sacrificing their eggs. This approach is especially useful for the Ramsar site in Hong Kong, which is managed under very stringent regulations.

Little Egrets and Chinese Pond Herons, as well as their common prey (fish: tilapia [*Oreochromis mossambicus*] and snakehead [*Channa asiatica*]) were studied at Mai Po wetland, because of their common occurrence in wetlands in Hong Kong and China (Viney et al., 2005). Tilapia is known to be a major prey item for the Ardeid, accounting for up to 69 percent of their diet (Muda, 1988), while snakehead is occasionally consumed (local observation). Heavy metals (namely, Cd, Cr, Cu, Mn, Ni, Pb and Zn) in fish, egg content and eggshells were determined. It is hypothesized that the metal concentrations in eggshells could reflect the metal concentrations in the eggs of the two species of Ardeid.

2. Materials and methods

2.1. Sample collection and pretreatment

Tilapia and snakehead ($n=9$ and 3 respectively) as well as surface sediment (0–5 cm, $n=3$) were collected in shrimp ponds (local name “gei wais”, gei wai 14 and 24a; Fig. 1a) at Inner Deep Bay Mai Po Ramsar site between 2004 and 2006 (between June and December). Eggshells of Little Egrets and Chinese Pond Herons were collected at heronries around Mai Po ($n=3$). Eggs of these two species ($n=3$ for each species) were haphazardly collected from different nests around fish ponds of Gu Cheng (GC) County, Shang Hu (SH) County and Dafeng Milu National Nature Reserve (DF) of Jiangsu Province in China between 2005 and 2007 (Fig. 1b). Bird eggs were only collected in Jiangsu Province, China, but not Hong Kong, because the bird populations were smaller and under more stringent wildlife protection regulations in Hong Kong. It is acknowledged that the sample sizes of the collections are relatively small, but is inevitable due to adherence to the regulations in the protected areas and nature reserves. All sediment and biota samples were stored in air-tight plastic bags immediately after sampling, and were kept at 4 °C in an ice box before reaching the laboratory for further treatment.

In the laboratory, sediment samples were weighed (wet weight) using a top-loading balance, wrapped with aluminum foil and frozen at −20 °C overnight before freeze-drying. Freeze-dried sediments were weighed again (dry weight), sieved (63 μm) and wrapped with aluminum foil and stored in a desiccator before determination of heavy metals.

The lengths and wet weights of fish samples were recorded using a ruler and a top-loading balance respectively. Individuals of tilapia were separated into three size classes according to their weights, including small (30–50 g), medium (180–350 g) and large (460–560 g). Fish of these sizes (<600 g) have been reported to be consumed by waterbirds similar to the Ardeid (Skoric et al., 2012). Tilapia of each size class was in triplicates. Snakeheads collected from Mai Po were of similar sizes and weighed between 180 and 350 g. Muscle and viscera of the fish were dissected and weighed again before freeze-drying. These two biological compartments were selected for analysis as they have considerable contribution to fish biomass, and bioaccumulation of pollutants in these compartments is also commonly investigated in similar research (Liang et al., 1999, 2007). The egg content of Little Egrets and Chinese Pond Herons was weighed and then poured into a beaker for freeze-drying. All sediments, fish samples, freeze-dried egg and eggshells (with shell membrane removed) fish samples and sediment were separately homogenized and then stored in a desiccator before metal analysis. The experiment is approved by the Committee on the use of human and animal subjects in teaching and research (HASC) of Hong Kong Baptist University. Guidelines from HASC were strictly followed in the experiment.

2.2. Acid-digestion and metal quantifications

The standard method 3052 (Microwave-assisted total heavy metal digestion) (United State Environmental Protection Agency (USEPA), 1996) was employed for heavy metal digestion. Approximately 0.25 g of freeze-dried and homogenized sediment/biota sample was weighed and pre-digested overnight with mixture of acids (9 ml:3 ml:1 ml of concentrated nitric acid (69 percent), hydrofluoric acid (48 percent) and hydrochloric acid (37 percent) for sediments; 4.5 ml:0.5 ml of concentrated nitric acid and hydrogen peroxide solution (30 percent) for biota samples; all chemicals were purchased from Riedel-de Haën). For each digestion, triplicates of blank (pure solvents) and standard reference materials (National Institute of Standards and Technology: Standard Reference Materials 1944—New York–New Jersey waterway sediment for sediment sample; National Research Council of Canada: TORT-2 lobster hepatopancreas for biota sample) were used for checking the veracity of data. After pre-digestion, the samples were placed in a microwave oven (X-press model, CEM Corporation, USA) for further digestion for about 2 h (temperature raised to 175 °C and held for 35 min). After cooling, the extracts were filtered (using 5C filter paper), diluted to 50 ml using Milli-Q pure water and then kept in acid-treated plastic bottles. Heavy metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn) in the digested mixtures were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin-Elmer Optima 3000DV, USA). The recoveries of Cd, Cr, Cu, Pb and Zn in SRM 1944 and TORT-2 were >80 percent and 90 percent respectively.

2.3. Calculation of BAF and statistical analysis

Bioaccumulation factors (BAF) were calculated from the following equation (Barron, 1995), using triplicates of fish and the corresponding pond sediment data:

$$(C_{\text{biota}}/C_{\text{sediment}}) \times 100\%$$

where C_{biota} and C_{sediment} refer to heavy metal concentrations (μg g^{-1}, d.w.) in biota and sediment respectively. BAFs higher than 100 percent indicate bioaccumulation of the contaminant in the sample.

One-way ANOVAs and independent sample *t*-tests were used to compare any differences in heavy metal concentrations in the sediment, fish and bird samples. Normality of the data passed the Kolmogorov–Smirnov test, and the variances of the data were homogeneous according to Levene‘s test of homogeneity. No data transformation was required. Duncan‘s test was employed as the post-hoc test when significant differences in the mean values were revealed by the One-way ANOVAs.

Linear regressions were performed to evaluate the relationship between heavy metal concentrations in the eggs and eggshells of Ardeid, using the samples from this study, and data collected from other wetlands in China, including Hefei (Zhou et al., 2005), Fujian Province (Wang et al., 2009), Bailihe reserve (Zhou et al., 2006), Xiamen (Lin, 2007) and Hong Kong (Lam et al., 2004). Five species of Ardeid, including Little Egret, Chinese Egret (*E. eulophotes*), Cattle Egret (*Bubulcus ibis*), Chinese Pond Heron and Black-crowned Night Heron (scientific name), were included in the regressions. In constructing these models, considerable attention was given in selecting similar studies concerning heavy metal contaminations in Ardeid eggs in the southern part of China, aiming to enhance the representativeness of the data and applicability to the region. The software SPSS version 10 was employed for all statistical analyses.

3. Results and discussions

3.1. Bioaccumulation of heavy metals in fish from Mai Po

Heavy metal levels and calculated BAFs of Mai Po varied among sediments, fish muscle and viscera (Tables 1 and 2). Muscles of large tilapia contained significantly ($p<0.05$) higher concentrations of Cd, Cr, Cu and Zn (mean=8.55, 2.48, 8.73, 45.9 μg g^{-1}, d.w., respectively) than the small individuals (mean=2.21, 1.66, 2.76, 21.2 μg g^{-1}, d.w., respectively). The same pattern was also observed for Cd and Cr in the viscera of large and small tilapia (Table 2).

Bioaccumulation of Cd (BAF>100 percent) was observed in the muscles of snakehead and large tilapia (182–1118 percent) (Table 2). Similar bioaccumulation of Cd was observed in the viscera of snakehead and large tilapia, with BAFs ranging between