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Full Length Article

Exposure of the endangered Milky stork population to cadmium and lead via food and water intake in Kuala Gula Bird Sanctuary, Perak, Malaysia

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ABSTRACT

The Milky stork is listed as an endangered species endemic to the Southeast Asia region. In Malaysia, the population is currently being reintroduced back into the wild. However, the increase of anthropogenic activity throughout the coastal area might expose the population to hazardous chemicals such as heavy metals. This study highlights the contamination of cadmium (Cd) and lead (Pb) in the Milky stork's diet. Additionally, this is the first time an integrated exposure model being used to assess heavy metal exposure risk to the population. Lead level (5.5–7.98 $mg\ kg^{-1}$) in particular was relatively high compared to Cd (0.08–0.33 $mg\ kg^{-1}$). This was probably related to the different niches occupied by the species in the aquatic environment. The results further show that the predicted exposure doses (through intake of both food and water) for all metals are much lower than the Tolerable Daily Intake (TDI) values. The total exposure dose for Cd was 0.11 $mg\ kg^{-1}\ d^{-1}$ with TDI value of 0.54 $mg\ kg^{-1}\ d^{-1}$ while Pb total exposure dose was 0.31 $mg\ kg^{-1}\ d^{-1}$ with TDI value of 0.64 $mg\ kg^{-1}\ d^{-1}$. Several possible factors that could lead to the observed pattern were discussed. In conclusion, there is an urgent need to improve the current habitat quality to protect the endangered species. The authors also emphasized on the protection of remaining Milky stork's habitats i.e. mudflats and mangroves and the creation of buffer zone to mitigate the negative impacts that may arise from pollution activity.

1. Introduction

Milky stork (*Mycteria cinerea,* Raffles 1882) is a large waterbird with a restricted distribution in the coastal areas of the Southeast Asia. However, due to its rapid population decline it has been listed as an endangered [1]. Currently, the species is undergoing a re-introduction program in Kuala Gula, Malaysia. Kuala Gula is one of the important bird areas including stopover for migratory shorebirds in the East-Asian Australian pathway. Thus it holds a critical link to the Milky stork and other migratory birds' survival in the northern part of the peninsular. Nevertheless, the recent increase of anthropogenic activity in Kuala Gula has changed its coastal area into a massive fishery industry. This led to an increase of certain heavy metals like cadmium (Cd) and lead (Pb) in its aquatic environment [2].

Heavy metals like Cd and Pb can be toxic to organism even at low levels [3]. They can even affect our physiology including the endocrine system at environmentally relevant levels [4]. Furthermore, increasing pollution in the coastal area can cause the waterbirds to be more susceptible to health impairment and death as they are at the top of the food-chain. High level of heavy metals has been found to cause severe impairment and even death in waterbirds. Behavioral changes, increased susceptibility to diseases and reproductive dysfunction are some of the possible consequences of the exposure to sub-lethal dose [5–7] which could hamper any effort to conserve endangered species. Furthermore, these metals are not only damaging to the adults but also highly nephrotoxic to newly born chicks [8], affecting embryonic development [9] and causing renal and hematological toxicity [10]. In addition, these metals particularly Cd has been found to negatively affect the reproductive parameters of fish, reducing their fertility rate [11]. Therefore, there is a need to assess the level of these metals in the diet or preys consumed by the Milky stork as it has never been reported before. Moreover, the use of an integrated exposure model which includes metals intake from both food and water allowed us to assess the heavy metals exposure risk to the Milky stork population in Kuala Gula, Malaysia.

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Table 1
Coordinates and description of the study site.

Site	Coordinates (Latitude, Longitude)	Area description
1	4.934019, 100.487891	Newly developed shrimp farms surrounded by mangrove forest
2	4.940662, 100.468779	A small strip of mangrove forest with heavy anthropogenic activity i.e. boating
3	4.955036, 100.488715	Mangrove forest turned into shrimp farm
4	4.925130, 100.461756	Mangrove forest turned into shrimp farm
5	4.937456, 100.468060	Intertidal mudflat surrounded by residential, jetties and fishery activity

2. Materials and methods

2.1. Descriptions of Kuala Gula Bird Sanctuary

The study was conducted in Kuala Gula, part of the larger Matang Mangrove Forest in Malaysia. The area is regarded as one of the important stopovers in the peninsular and as sanctuary to both migratory and resident birds. We sampled several fish species and shrimp from five different sites commonly visited by the Milky storks between the year 2014 and 2015. The descriptions of the areas are mentioned in Table 1.

2.2. Metals analysis

A total of 150 biological samples consisted of fish and shrimps were collected throughout the study period. The total length (TL) and body weight (BW) of the samples were measured. The samples were kept in ice before being transported to laboratory. In the laboratory, the samples were thawed to room temperature (~30 °C) and dried in an air-circulating oven at 60 °C for at least 72 h until constant dry weights (dw) were achieved. The dried samples were then crushed and homogenized using stainless steel heavy duty blender. About 1.0 g of the homogenized tissues were weighed and digested in 10 mL of concentrated nitric acid (AnalaR grade, BDH 69%). The tissues were subjected to total digestion method as described by Ismail and Ramli [12]. They were placed in digestion block at 40 °C for the first 1 h and then to 140 °C for the next 3 h. The digested samples were then diluted with 40 mL miliQ water and filtered through Whatman No. 1 filter papers. The filtrates were stored in polyethylene bottles at 4 °C until further analysis. Water samples were collected in triplicates in each site and kept in ice during transportation. The filtered samples were then stored in polyethylene bottles in the same manner as other filtrates prior to metal analysis.

Metals determination was done using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 880. All data are presented in μg/g dry weight. Standard solutions were prepared from 1000 mg/L stock solutions prepared for each metal (MERCK Titrisol). All apparatus were acid-washed (5% of nitric acid) for 24 h then rinsed with double distilled water before used. All solutions were prepared using double de-ionized water (USF Maxima, 18.2 MÙ cm^{-1}). Procedural blanks were analyzed once for every ten samples to check for sample accuracy. A quality control sample was routinely run through during the period of metal analysis. One-Way ANOVA with Tukey post-hoc test were used to test for the mean differences of metals between sites. Pearson's correlation was also employed to seek the relationship between the samples TL and BW and metals level. All statistical tests were done at 95% level of significance using Statistical Package for Social Science (SPSS) software version 17.

2.3. Exposure models

To assess the metals exposure of the Milky stork population, an integrated exposure model that accounts for external contamination through oral ingestion was used [13]. However, as the population' diet in the study mainly consist of fish and shrimp, soil consumption rate is not included. Thus, the exposure model to quantify heavy metals risk to the population used the following formula:

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

where E_j is oral exposure dose of heavy metal (mg kg^{-1} d^{-1}), m is the number of absorbing medium, food and water, I_i is the absorptivity of medium (i) (g d^{-1} or mL d^{-1}) and C_{ij} is the level of metal (j) in medium (i) (mg kg^{-1}) and BW is body weight of the bird. An average body weight of 2400 g was used for the Milky stork.

$$I_{df} = 0.648BW^{0.651}$$

where I_{df} is food consumption rate (g d^{-1}, dw) estimated from the allometric regression model [14].

$$I_w = 59BW^{0.67}$$

where I_w is water consumption rate (mL d^{-1}) also estimated from allometric regression model [15]. It is difficult to determine the critical threshold levels relevant to all species and thus TDI is used. The TDI is calculated from the results of avian chronic toxicity tests in which the substance was administered orally and sensitive endpoints were measured [16]. Thus, the metal-exposure model is compared with the tolerable daily intake using the formula:

$$TDI = (LOAEL \text{ x } NOAEL)^{0.5}/UF$$

where TDI = tolerable daily intake, LOAEL = lowest-observed-adverse-effect level, NOAEL = no-observed-adverse effect level, and UF = uncertainty factor. The no-observable adverse-effect-level and lowest-observable-adverse-effect level for suitable avian toxicity tests were obtained from the summary made by Sample et al. [16]. The TDI estimates in the study make use of the uncertainty factor of 10 to account for the lowest sensitivity amongst the population.

3. Results and discussion

3.1. Cd and Pb levels in biological and water samples

The different species collected including their length and weight are summarized in Table 2. In most cases, the metals levels were significantly correlated with the weights of the species ($p < 0.05$). Cadmium and lead levels show moderate to high correlation with the weight of *Oreochromis* sp. (Cd: r = 0.56, p = 0.04), *Valamugil* sp. (Cd:

Table 2
Summary of the weight, total length and metals (mg kg^{-1}) in the samples caught.

	Species	N	Weight (g)	Length (cm)	Cd	Pb
1	*Oreochromis* sp.	30	12.7 ± 4.8	10.3 ± 1.3	0.13 ± 0.03	5.84 ± 0.22
2	*Valamugil* sp.	30	18.1 ± 3.7	11.8 ± 3.0	0.26 ± 0.03	7.57 ± 0.41
3	*Penaeus* sp.	30	6.2 ± 2.5	12.6 ± 2.0	0.30 ± 0.02	7.01 ± 0.19
4	*Periophthalmodon* sp.	30	10.3 ± 2.8	14.0 ± 1.4	0.23 ± 0.02	6.96 ± 0.21
5	*Mystus* sp.	30	7.7 ± 2.4	9.6 ± 1.1	0.17 ± 0.06	7.30 ± 0.29