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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:

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

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

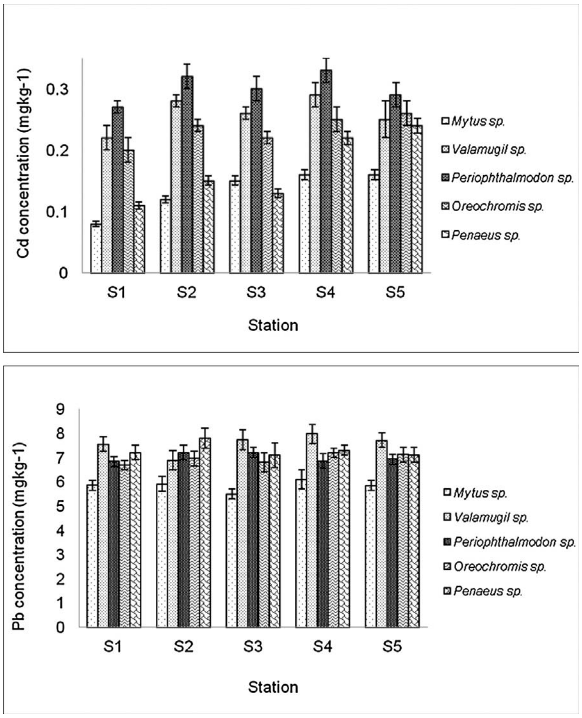

Fig. 1. Cadmium (a) and lead (b) levels (mg kg^{-1}) in the biological samples collected from the Milky Stork foraging sites.

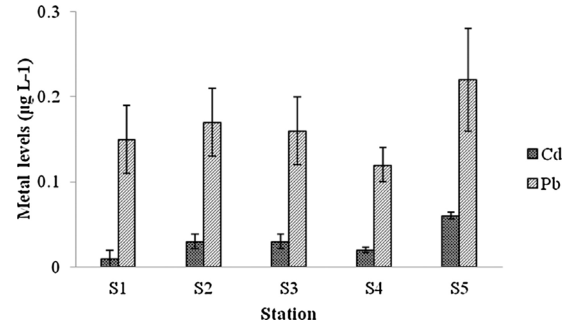

Fig. 2. Average Cd and Pb levels in water (µg L^{-1}) obtained from the different stations (1–5) (± SD).

r = 0.94, p = 0.00; Pb: r = 0.75, p = 0.01) and *Mystus* sp. (Cd: r = 0.82, p = 0.00; Pb: r = 0.77, p = 0.01). High correlation between the metals and length were also found in *Valamugil* sp. (Zn: r = 0.87, p = 0.00; Cd: r = 0.94, p = 0.00; Pb: r = 0.90, p = 0.00), *Penaeus* sp. (Zn: r = 0.98, p = 0.00) and *Mystus* sp. (Zn = 0.90, p = 0.02). The findings suggest that weight and length are important factors in determining metal levels on the species analyzed.

The total metal levels in the biological samples collected range from 0.08–0.33 mg kg^{-1} and 5.5–7.98 mg kg^{-1} for Cd and Pb respectively. Moreover, the metals levels among similar species only vary slightly between the different foraging sites (Fig. 1). Variation among the different species from the same water body suggests that the accumulation may be species dependent [17], possibly due to the differences of feeding habits and bioaccumulation factor [18]. However, Cd levels in Site 3, 4 and 5 were generally higher that Site 1 and this could be due to the increasing anthropogenic activity in these areas. Certain species like *Penaus* sp., *Mystus* sp. and *Oreochromis* sp. in Site 4 and 5 in particular have also been found to accumulate high level of Cd. Multiple anthropogenic activities in these sites i.e. fisheries, tourism activity and residential could have led to the observed pattern. Nonetheless, no adverse effects were observed on the samples. A recent study by Renieri et al. [19] found that metal accumulation in fish exposed to high levels of toxic metal like Cd can be gradual or progressive, resulting in less adverse effects on the fish. No comparison with other studies was done for the biological samples as most of them only use muscle tissue. As for the water (Fig. 2), the metals levels range from 0.001–0.06 for Cd and 0.15–0.22 for Pb. Site 5 had the highest Cd (0.06 mg kg^{-1}) and Pb (0.22 mg kg^{-1}) levels and they are significantly different when compared to the other sites ($P < 0.05$). Table 3 highlights the average Cd and Pb levels in the water in the study area and nearby region.

Table 3
Average Cd and Pb levels in water (µg L^{-1}) in the study area and nearby region.

	Location	Cd	Pb	References
1	Juru River, Malaysia	0.12–0.28	0.13–0.47	Alkarkhi et al. [33]
2	Langat River, Malaysia	0.01–0.53	0.01–6.99	Lim et al. [34]
3	Jejawi River, Malaysia	0.05–0.28	0.15–0.39	Alkarkhi et al. [34]
4	Port Dickson, Malaysia	0.03–0.42	0.97–5.2	Shazili and Mohamed [35]
5	Gulf of Thailand, Thailand	0.01–0.26	0.20–1.13	Cheevapron and Menasveta [20]
6	Kuala Gula, Malaysia	0.001–0.06	0.15–0.22	This Study

Table 4
Predicted exposure doses and the total exposure dose of Cd and Pb.

Metals	Exposure dose		Total exposure dose (mg kg^{-1} d^{-1})	TDI (mg kg^{-1} d^{-1})
	Water (mg L^{-1})	Fish (mg kg^{-1}, dw)		
Cadmium, Cd	0.01	0.10	0.11	0.54
Lead, Pb	0.01	0.30	0.31	0.64

3.2. Milky stork population exposures to Cd and Pb

Table 4 shows the predicted exposure dose of both Cd and Pb to the Milky stork population. In general, the predicted exposure doses (both food and water) for all metals are much lower than the TDI values. For cadmium, the combined dose in water and fish is five times lower (0.11 mg kg^{-1} d^{-1}) than that of the TDI (0.54 mg kg^{-1} d^{-1}). As for Pb, the total exposure of the metal in both water and food is two times lower (0.31 mg kg^{-1} d^{-1}) than that of the TDI value (0.64 mg kg^{-1} d^{-1}). In addition, the exposure doses for all metals are much lower in water compared to the food. This suggests that the Milky storks are more likely to accumulate higher amount of Cd and Pb through food intake (> 90% of exposure). The findings also suggest that the re-introduced population is not being exposed to high amount of metals through their diet. However, if the pollution levels continue to increase, prolong consumption of the preys (and water) in the foraging area should be a concern. For instance, the increasing pattern of Pb in Kuala Gula's aquatic environment need to be monitored as the current total exposure dose is at least 50% the TDI value. Exposure to toxic metals particularly through the food chain has been found to affect several normal metabolic processes in experimental animal [21]. If no protection is given to the Milky stork's foraging habitats, the observed pattern could be harmful to the population in the long run.

3.3. Other factors responsible for the increase in metal levels in Kuala Gula

Metals level in the sediment could be one of the important factors that contribute to their accumulation in the animals [22]. For instance, lead level in the surface sediment of Kuala Gula coastal area was reported to be between 12 and 29 ppm [23] and 28–47 ppm [2] which are considerably high. Although moderate correlations between metal levels in sediment and the samples collected were found, they were not significant ($p > 0.05$). In addition, the high percentage of Pb in oxidizable fraction (more than 30%) in the sediments of Kuala Gula suggest that its release into the aquatic environment may occur if the sediment is re-suspended and the sediment particles come into contact with oxygen-rich water [24]. Considering the recent increase of mangrove reclamation activity and development of land-based aquaculture in Kuala Gula coastal area, this pattern could be one of the important reasons for the increase in metals level in the area. The use of leaded petrol in boating activity still occur and has been reported to be contaminating the aquatic environment in the country [25]. Another possible reason for the high level of Pb recorded in this study is due to the use of the whole fish or shrimp instead of muscle-tissue only. This is important to reflect the actual metals uptake by the stork population when they consumed their prey. Thus, the metals levels should be higher in general as compared to the muscle only data as reported by other studies.

It is also important to note that high level of Pb was reported in several commercial fish in the Peninsular Malaysia. High levels of heavy metals in farmed fish and shrimps were reported in earlier studies in Malaysia [26,27] and nearby region including Thailand [20,28] and Sri Lanka [29]. Furthermore, Arai et al. [30] reported that Pb level in *Anguilla bicolor bicolor* in the west coast of Peninsular Malaysia to be between 2 and 200 times than other regions such as Vietnam and Japan. In addition, Yin et al. [31] also found that Pb level in *Monopterus albus* in the east coast has reached up to 22.7 μg/g dw in muscle alone, which is 2–3 times higher than the whole tissues used in this study. Apart from boating and other anthropogenic activities in the vicinity, uncontrolled uses of commercial feed pellets to feed cultured fish and shrimps may also contribute to the high level of Pb in the environment. Fish feeds may already possess some heavy metals and other contaminants in them which could bio-accumulate and bio-concentrate in the fish [32]. Hence, the presence of non-essential metals such as Cd and Pb in the pellet could cause detrimental effects to the environment in the long run and need to be monitored. Nevertheless, further monitoring and studies are required as the current findings suggest that the high daily exposure dose of Pb predicted can be harmful to the Milky stork population. Close and continuous monitoring of the Milky stork food quality is important as their foraging areas are currently under the influence of both direct and indirect anthropogenic activities.

4. Conclusion

We conclude that there is an emergent need to improve Kuala Gula habitat quality. This is important to ensure that the wildlife species including the endangered Milky storks are not being exposed to toxic pollutants such as the heavy metals. Responsible parties should closely monitor Kuala Gula's environment, conserve remaining mudflats and mangroves and provide adequate buffer zones (at least 100 m from anthropogenic activity) to help mitigate the negative impacts of pollution.

Conflict of interest

The authors declare that there are no conflicts of interest.

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