TRACE ELEMENTS IN FEATHERS AND EGGSHELLS OF TWO TROPICAL SEABIRDS FROM MALAYSIA

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ABSTRACT

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Seabird feathers and eggshells are used as bio-monitors for trace metal contamination. Eleven trace element concentrations in wing primaries (n = 21) and eggshells (n = 8) are reported herein for two resident seabirds, Bridled Tern *Onychoprion anaethetus* and Black-naped Tern *Sterna sumatrana*, at Pulau Ling and Pulau Tokung Burung, Malaysia. Metal concentration followed the pattern Zn > Cr > Mn > Sr > Cu > Se > Ba > Pb > Cd > Ga > Li. Significant positive correlations existed among all trace metals, except for Cr in Black-naped Tern and Li and Cd in Bridled Tern feathers. We also analyzed metal concentrations in eggshells, and for Bridled Tern the concentration pattern was Zn > Mn > Se > Sr > Ba > Pb > Co > Cu > Cd, whereas for Black-naped Tern it was Cr > Zn > Mn > Ba > Sr > Se > Cu> Co > Pb. A pairwise positive correlation was significant among most trace metals in feather samples, indicating synergistic effects of two or more elements. More studies are needed to build a baseline database of trace metal concentrations in seabirds of Southeast Asia, as little research on this subject has been conducted in this region.

Key words: trace metals, seabird feathers, bioaccumulation, South China Sea

INTRODUCTION

Birds can accumulate large amounts of chemicals from the environment due to their position in the food web, which is why they are often used as bio-monitors of environmental pollution (García-Fernandez 2013, Borghesi *et al*. 2016). Trace elements are classified into two groups: essential elements (required for biological processes), which include copper (Cu), chromium (Cr), nickel (Ni), selenium (Se), strontium (Sn), and zinc (Zn); and non-essential elements (not required for biological processes), which include mercury (Hg), cadmium (Cd), and lead (Pb). Non-essential trace elements, including mercury (Mg), can be toxic, even at low concentrations (Moura *et al.* 2018).

Sources of trace metals in tropical marine food webs are similar to those in temperate regions and include terrestrial runoff from rivers and streams, urban areas, and agricultural areas (Sebastiano *et al*. 2016). Landfills can leach directly or indirectly into shallow water tables, streams, and the coastal zone, particularly on porous limestone islands (Hussein *et al*. 2021). Owing to widespread industrial use and subsequent leakage into the environment, trace metals are bioaccumulated and magnified in marine biota tissues (Sun *et al*. 2019).

Many published studies on trace metal contamination in seabirds have been conducted in temperate oceans, but only a few have been conducted in tropical marine environments. It is widely speculated that trace metal pollution levels in the tropics should be lower than in temperate zones. However, this hypothesis may not hold true across all tropical regions (Burger *et al*. 1992), as several countries in the tropics have acquired industrialized areas and lack clean production practices, strict regulations, and enforcement.

Trace element concentration in a seabird feather reflects accumulation of that element through dietary and environmental exposure during a bird's lifespan (Borghesi *et al.* 2016; Dolci *et al.* 2017; de Assis Padilha *et al.* 2018). For instance, Abbasi *et al*. (2015) investigated trace metal levels in tropical seabird feathers wherein contamination originated from sediments where these birds foraged. Bird feathers are chemically and physically stable, resistant to heat and deterioration, and are therefore easily stored over time (de Assis Padilha *et al.* 2018). Unlike blood, which reflects the bird's short-term exposure to trace metals (Furness 1993), trace metal levels in feathers reflect long-term exposure because trace metal accumulation continues throughout feather growth. Therefore, feathers serve as an archive for food-based bioaccumulative compounds (Burger & Gochfeld 2000).

Eggshells have also been used extensively to monitor element accumulation in birds (Xu *et al*. 2011, Dolci *et al*. 2017, van Aswegen *et al*. 2019), but fewer data are available from tropical seabirds.

Most of the trace metal studies in tropical areas have focused on prey species that are caught commercially, such as fish and shellfish (e.g., Tengku Nur Alia *et al*. 2019, Poong *et al*. 2020). This study presents the first baseline data on 11 trace metal concentrations in feathers and eggshells of two tropical resident seabird species, Bridled Tern *Onychoprion anaethetus* and Black-naped Tern *Sterna sumatrana.*

METHODS

Description of sampling sites

Pulau Ling, also known as Pulau Chipor, is located within the more extensive Redang archipelago (05°43.6′N, 103°01.0′E) in the northeastern waters of Peninsular Malaysia. It is a bare rocky outcrop, rising ~11 m above sea level (asl), having a surface area of 0.0157 km^2 and a perimeter of 443 m (Fig. 1). The island hosts a breeding colony of Bridled Tern and a smaller colony of Black-naped Tern (Hamza *et al*. 2016). Samples were collected during monitoring of breeding terns along the east coast of Peninsular Malaysia.

Pulau Tokong Burung Besar (02°47.0′N, 103°57.6′E) is one of three small islands located southwest of Pulau Tioman, Pahang, Malaysia. The island of 0.36 km^2 is composed of granite boulders 54 m asl. Only Bridled Tern breed on the island (Hamza & Wong 2020).

Sample collection

Twenty-one feathers and eight eggshells were collected randomly from colonies of the two study species from a small part of each island. Molted wing primaries were found near nests; others were collected from recently deceased birds found in the colony. Two Bridled Tern eggshells from Pulau Tokong Burung Besar and six Black-naped Tern eggshells from Pulau Ling were analyzed. Both species are resident species (MNS Bird Conservation Council 2015). Sampling was conducted during May 2016 and May 2017. Samples were packed carefully in separate labelled plastic bags until laboratory analysis.

Analytical methods

Feather specimens were washed three times with acetone to minimize external contamination, then rinsed with distilled water (see Espín *et al*. 2014). The feathers and eggshells were rinsed with deionized water (Milli-Q), following Hashmi *et al*. (2013). Samples were then dried and homogenized using an agate mortar (Manjula *et al*. 2015). Feathers were analyzed for concentrations (μg/g dry weight) of the following trace metals: Cd, Cr, Cu, Mn, Ba, Co, Li, Pb, Se, Sr, and Zn. Trace metal detection was conducted at the Institute of Oceanography and Environment (INOS), Universiti Malaysia Terengganu. Sample acid digestions were carried out in closed Teflon vessels in an oven following Manjula *et al*. (2015).

Aliquots of feathers (0.01 g) and eggshells (0.05 g) were weighed and placed in clean Teflon containers; 1.5 mL of nitric acid (HNO_3) was added. Vessels were capped and placed in stainless steel jackets and then heated to 110 °C for 7 hr. The digested samples were then diluted with 10 mL of deionized water (Milli-Q) and stored in polypropylene test tubes. Trace metal concentrations were determined using inductively coupled mass spectrometry (ICP-MS; Perkin Elmer Elan 9000 ICP-MS). The ICP-MS was calibrated for a low concentration range of 0.1–100 μg/L in 1% (v/v) HNO_3 prepared from multi-element standard solution (ICP-MS Multi-Element Standard 2 Accutrace^{TM}, Perkin Elmer). To determine the accuracy of the acid digestion procedure and the ICP-MS measurement, standard reference materials (SRM; certified argillaceous Limestone, SRM 1d; and bovine liver, SRM 1577; National Institute of Standards and Technology [NIST]) were analyzed following the same procedure that was used for the samples. The recovery range for trace metals was between 95% and 113% (Table 1). Analytical blanks consisted of acidified Milli-Q water.

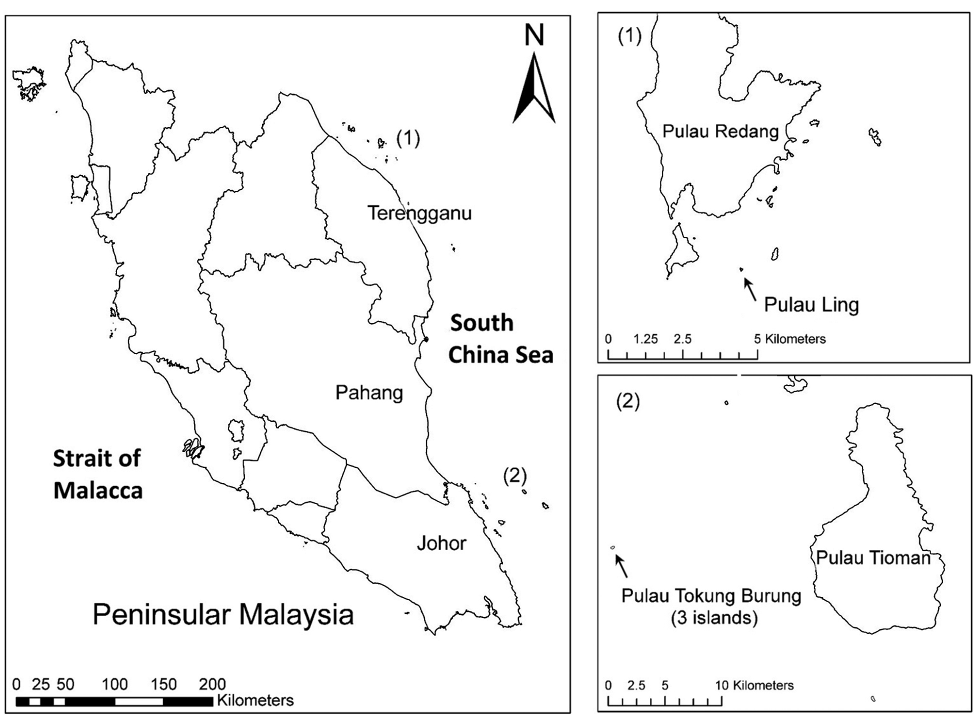

Fig. 1. Map showing the location of (1) Pulau Ling, located within the Redang archipelago in the northeastern waters of Peninsular Malaysia, and (2) Pulau Tokong Burung, located southwest of Pulau Tioman, Pahang, Malaysia.

TABLE 1
Certified, mean observed, and recovery values (%) of trace metal concentrations in standard reference materials

Trace element	Certified value (µg/mg)		Present study (Mean) (µg/mg)		Recovery (%)	
	1577c Bovine Liver	1d Limestone	1577c Bovine Liver	1d Limestone	1577c Bovine Liver	1d Limestone
Li	0.012	na	0.014	0.665	113	na
Cr	0.053	na	0.059	33.916	111	na
Mn	10.46	na	10.640	1.626	102	na
Co	0.3	na	0.287	1.093	96	na
Cu	275	na	285	2.092	103	na
Zn	181	na	172	12.3	95	na
Se	2.031	na	2.087	–0.186	103	na
Sr	0.095	na	0.103	1.690	109	na
Cd	0.097	0.3	0.094	0.277	97	92
Ba	na	na	0.594	17.787	na	na
Pb	0.063	na	0.063	0.609	100	na

Statistical analysis

Data were not normally distributed, even after logarithmic and square root transformation (Kolmogorov-Smirnov test). Therefore, the nonparametric Mann-Whitney U test was used to compare trace metal concentrations in Bridled Tern feathers from Pulau Ling and Pulau Tokung Burung. Pairwise correlations among trace metal concentrations were calculated using Spearman's rho correlation. Due to the small sample size, no statistical tests were conducted on egg trace metal concentrations. The statistical significance was defined at $P < 0.05$. All statistical analyses were conducted using SPSS, version 25.

RESULTS AND DISCUSSION

Trace metal concentrations in feathers of Bridled Tern

Trace metal concentrations in feathers of Bridled Tern were compared against standard reference materials (Table 2). The mean concentrations of trace metals in feathers of Bridled Tern from Pulau Tokong Burung Besar and Pulau Ling followed the pattern Zn > Cr > Sr > Mn > Cu > Se > Ba > Pb > Co > Cd > Li, whereas the pattern at Pulau Ling was Zn > Cr > Mn > Sr > Cu > Se > Ba > Pb > Co > Cd > Li (Table 2). The trend in trace metal concentrations was similar in both Bridled Tern populations, except in the order of Sr and Mn.

At both sites, in Bridled Tern feathers, Zn and Cr were present at the highest concentrations, whereas Pb, Co, Cd, and Li were present at the lowest concentrations. The median trace metal concentrations were higher in feathers from Pulau Tokong Burung Besar compared to Pulau Ling. A Mann-Whitney Test indicated that these differences were significant ($P < 0.05$), except for Cr and Ba ($P > 0.05$).

Several significant positive correlations ($P < 0.05$) were found among trace metal concentrations for both seabirds. These positive correlations were between Li–Sr; Mn and each of Co and Cu; and between Cd–Pb. In Black-naped Tern feathers, positive correlations existed between Li and each of Ba–Pb, Cr–Se, Co–Cd, and Co–Ba. Positive correlations were found among trace metals in Bridled Tern feathers between Mn and each of Sr, Cd, and Pb. Similarly, Cu was positively correlated with Zn, Se, Sr, Cd, and Pb. On the other hand, Se–Sr were positively correlated with Cd–Pb and Cr–Li. The inter-correlation among these trace elements can exert several chemical and physiological effects, especially when their concentrations are higher than usual.

Trace metal concentrations in feathers of Black-naped Tern

The mean concentrations of trace metals in feathers of Black-naped Terns from Pulau Ling followed the pattern Cr > Zn > Cu > Mn > Sr > Se > Ba > Co > Pb > Cd and Li. This pattern deviates somewhat from that for Bridled Tern feathers from the same site, specifically in the order of Cr, Zn, Cu, Sr, Co, and Pb (Table 2). However, in both species from Pulau Ling, Cr and Zn concentrations were the highest, whereas Co, Pb, Cd, and Li were present at the lowest concentrations. It is also notable that Black-naped Tern showed lower concentrations of all accumulated metals at Pulau Ling compared to Bridled Tern (Table 2). Spatial differences in foraging areas between the two species may explain such variations. The Black-naped Tern is a nearshore forager that depends on shallow-water fish; therefore, it may be exposed to more anthropogenic pollutants through its prey than Bridled Tern, which tend to forage in oceanic waters farther offshore (Hamza *et al*. 2016).

High concentrations of Cr and Zn were also present in feathers of the two species at all sites. A similar trend was found in other tropical seabirds, such as the Magnificent Frigatebird *Fregata magnificens* (Sebastiano *et al*. 2016), whereas other studies found that Pb and Cd concentrations were highest in the Bridled Tern (Burger & Gochfeld 1991) and Herring Gull *Larus argentatus* (Abbasi *et al*. 2015). Similar feeding guilds can cause these similarities in metal concentration.

Trace metal concentrations in eggshells

Bird eggs reflect the female's diet before egg-laying (Muçoz Cifuentes *et al*. 2003), and foraging at different sites can result in variations in exposure to trace metal contamination within the same population. Females can also eliminate trace metals through the formation of the yolk and albumen (Burger 1994). The metal concentrations in the eggshells of Bridled Tern from Pulau Tokong Burung followed the pattern Zn > Mn > Se > Sr > Ba > Pb > Co > Cu > Cd > Li and Cr (Table 3). In our study, the concentrations of Cu, Cr, Sr, and Se were lower, and the concentrations of Zn, Mn, Cd, Co, and Pb were much higher, compared to Bridled Tern eggshells from Hong Kong (Lam *et al*. 2005). Such differences can be attributed to differences in bird age, exposure time, and the bioaccumulation process. Our results for Se, Pb, Co, and Cd concentrations were much lower than those reported for Persian Gulf Bridled Terns (Khademi *et al*. 2015).

The mean concentrations of trace metals in eggshells of Black-naped Tern from Pulau Ling followed the pattern Cr > Zn > Mn > Ba > Sr > Se > Cu > Co > Pb > Li > Cd. Cr concentrations were higher in Black-naped Tern eggshells compared to Bridled Tern eggshells (Table 3).

TABLE 2
Trace element concentrations (μg/g dry weight) in feathers of Bridled *Onychoprion anaethetus* and Black-naped *Sterna sumatrana* terns from Pulau Ling and Pulau Tokong Burung Besar, Malaysia, with concentrations ranked in decreasing order for each site

Trace element	Range	Median	Mean	Standard error
Bridled Tern (Tokong Burung Besar, *n* = 9)				
Zn	23.53–177.08	128.67	111.12	18.14
Cr	3.02–212.07	19.60	75.09	27.73
Sr	3.40–86.67	32.92	45.28	11.47
Mn	5.92–40.80	11.50	15.94	3.74
Cu	4.13–25.80	13.60	15.76	2.17
Se	1.00–20.96	10.40	11.64	2.10
Ba	1.95–9.42	2.87	3.90	0.80
Pb	0.46–3.97	1.74	2.23	0.45
Co	0.50–2.78	1.31	1.33	0.25
Cd	0.03–1.36	0.29	0.50	0.17
Li	0.02–0.27	0.15	0.14	0.03
Bridled Tern (Pulau Ling, *n* = 2)				
Zn	168.75–389.13	278.94	278.94	51.94
Cr	21.30–392.19	206.75	206.75	87.42
Mn	51.41–65.22	58.31	58.31	3.26
Sr	10.63–56.56	33.60	33.60	10.83
Cu	20.78–33.48	27.13	27.13	2.99
Se	5.26–17.50	11.38	11.38	2.88
Ba	5.86–8.30	7.08	7.08	0.58
Pb	4.16–4.93	4.55	4.55	0.18
Co	1.66–6.02	3.84	3.84	1.03
Cd	0.32–0.93	0.62	0.62	0.14
Li	0.08–0.18	0.13	0.13	0.02
Black-naped Tern (Pulau Ling, *n* = 9)				
Cr	18.49–768.42	118.90	198.75	73.16
Zn	118.11–212.96	184.20	166.23	10.78
Cu	8.85–46.67	18.18	23.66	4.68
Mn	6.03–53.43	9.25	22.24	5.75
Sr	1.51–34.41	4.74	10.65	3.98
Se	2.51–10.00	5.16	5.60	0.71
Ba	0.79–8.11	1.86	2.82	0.74
Co	0.52–3.51	0.89	1.70	0.38
Pb	0.43–6.42	0.86	1.67	0.60
Cd	0.04–0.47	0.10	0.14	0.04
Li	0.02–0.18	0.03	0.05	0.02

TABLE 3
Trace element concentrations (μg/g dry weight) in eggshells of Bridled Tern *Onychoprion anaethetus* from Pulau Tokong Burung Besar and Black-naped Tern *Sterna sumatrana* from Pulau Ling, Malaysia, with concentrations ranked in decreasing order for each site

Trace element	Range	Median	Mean	Standard error
Bridled Tern (Tokong Burung Besar, *n* = 2)				
Zn	9.74–16.77	13.25	13.25	3.52
Mn	5.79–15.32	10.55	10.55	4.77
Se	1.45–6.32	3.88	3.88	2.44
Sr	1.74–2.12	1.93	1.93	0.19
Ba	5.86–8.30	1.72	1.72	0.37
Pb	0.46–2.30	1.37	1.37	0.92
Co	0.49–0.64	0.57	0.57	0.08
Cu	0.36–0.63	0.49	0.49	0.13
Cd	0.06–0.15	0.11	0.11	0.04
Li	0.08–0.18	0.09	0.09	0.04
Cr	0.006–0.019	0.01	0.01	0.01
Black-naped Tern (Pulau Ling, *n* = 6)				
Cr	6.21–16.23	9.39	10.15	1.93
Zn	5.99–10.77	6.82	7.77	1.21
Mn	1.18–3.60	2.96	2.62	0.58
Ba	0.83–3.70	1.55	1.84	0.68
Sr	1.04–2.04	1.37	1.47	0.20
Se	0–3.56	1.29	1.46	0.76
Cu	0.90–1.67	0.56	1.13	0.16
Co	0.52–0.84	1.05	0.60	0.07
Pb	0.06–0.25	0.11	0.14	0.05
Li	0.05–0.09	0.058	0.06	0.01
Cd	0.01–0.03	0.01	0.01	0.00

Interpretation of trace metal concentrations

The concentrations of dissolved trace elements have been detected at several locations in the coastal and estuarine waters of the east coast of Peninsular Malaysia using water samples (Ariffin *et al*. 2019), animal tissues (Fuad *et al*. 2013, Rahouma & Shuhaimi-Othman 2013, Dabwan & Taufiq 2016), plant tissues (Kamaruzzaman *et al*. 2009), and sediments (Shazili *et al*. 2007). Antonina *et al*. (2013) found that the concentrations of Ba, Cd, Cu, Cr, Mn, and Pb were lower in sediment compared to oceanic crust, but that Zn was an exception to this trend. Zn concentrations in our study were also the highest among other tested trace metals for Bridled Tern feathers from both study sites, and Zn was the second-most abundant trace element (after Cr) in Black-naped Tern feathers from Pulau Ling. Zn and Cu are both regulated in a bird's body and do not merely reflect a simple bioaccumulation process (Adriano 2001). Both trace metals are preferentially deposited in the feathers only when their concentrations are high in the blood (Furness 1993). There are no known effects of high concentrations of Zn in biological systems. The threshold concentration for toxic effects of Zn in birds is 1 200 μg/g dry weight, which exceeds the concentrations reported in this study. Chromium was the second-most concentrated trace element in Bridled Tern feathers from both sites, also reflecting the baseline pollution from industrial activities, not necessarily near these sites but in the whole region. Chromium plays a vital role as an essential element; however, some studies have found that elevated concentrations of Cr can cause neurotoxic effects in seabirds. The Cr concentrations reported in this study, for both feathers and eggshells, exceed the known average range of this trace metal found in bird feathers (Burger 1993).

Manganese was present in higher concentrations in Bridled Tern feathers from Pulau Ling than Pulau Tukung Burung, and in both cases, the concentrations were above the known average concentrations in seabird feathers (Burger & Gochfeld, 2000). Strontium was also present at intermediate concentrations in all species/sites. Elevated concentrations of Sr in the eggshell, for example, could affect later-stage embryos, possibly by interfering with calcium metabolism and bone growth, resulting in reduced hatching success and potentially minor beak deformities (Mora *et al*. 2007). The concentrations of Sr in our data were far below those reported by Mora *et al*. (2007). Copper concentrations in Bridled Tern feathers from Pulau Ling were approximately eight times those from Tokong Burung, and lower concentrations were detected with Black-naped Tern feathers from Pulau Ling. Selenium is a trace metal that birds require, in minimal amounts, for biological functions (Ohlendorf & Heinz 2011). However, at high concentrations, Se can be very toxic and is subject to homeostatic regulation. In feathers, concentrations of 3.8–30 mg/kg (the concentration varies per species) results in severe adverse effects, such as bird embryo mortality (Heinz 1996). The concentration of Se in feathers was higher in Bridled Tern compared to Black-naped Tern, but it was within threshold concentrations for seabirds. Chronic Ba exposure has been associated with muscle hypertension and impaired cardiovascular regulation in experimental animals (Perry *et al*. 1989), and it is usually used to trace the fate of oil drilling chemicals at sea (Chow 1980). Although it was present at all sites in both feather and eggshells, Ba concentrations were low and beneath threshold concentrations for birds.

Lead is a neurotoxin that causes a decrease in growth, learning ability, and metabolism (Burger & Gochfeld 2000), although seabirds can often tolerate high Pb concentrations. The Pb concentrations detected in both the feathers and eggshells of both species of terns (Tables 2, 3) were below known concentrations in seabirds (Burger & Gochfeld 2000), indicating low Pb pollution in the Malaysian waters of the South China Sea. Cobalt is a bioactive metal and serves as an essential micronutrient. More recently, Xu *et al*. (2019) found that Co is transferred by seabirds from the ocean to terrestrial ecosystems via their guano, eggshells, and skeletons. Concentrations of Co in our study were within the known concentrations for seabirds. Cadmium is a very toxic element and may cause a reduction in growth rate, and it has lethal effects at lower concentrations compared to other harmful elements. Cadmium concentrations in the two species' feathers were above the known threshold limits for toxic effects, which can be considered a potential threat to the avian populations (Burger & Gochfeld 2000). Lithium is found naturally in the aquatic and terrestrial environment, but in small concentrations; when Li is ingested in excessive amounts, it primarily affects the gastrointestinal tract, central nervous system, and kidneys (Rasooli *et al*. 2018). No data is available on the concentrations or effects of Li on seabirds to enable comparison with our results.

Our study provides baseline data for two seabird species—Bridled Tern and Black-naped Tern—which are under-investigated globally. Future studies should evaluate the impact of the trace element concentrations in our study to the birds' physiology to understand whether the current environmental concentrations pose a threat. The use of stable isotopes may help to understand whether prey type or location affect the trace metal concentrations that we observed.

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