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Trace elements in green turtle eggshells and coral sand sediments from the Xisha Islands, South China Sea

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ABSTRACT

This study investigated 12 trace elements in paired green turtle (*Chelonia mydas*) eggshell composites and coral sand samples to examine within-habitat heavy metal pollution from 40 nesting sites in the Xisha Islands. The concentrations of the elements ($\mu g \cdot g^{-1}$) found in the eggshells ranged as follows: Sr (41.3) > Zn (20.3) > Cu (12.8) > Fe (4.92) > Al (4.37) > Se (2.44) > Mn (0.91) > Cr (0.81) > Ba (0.44) > Pb (0.14) > As (0.08) > Cd (0.02). Significant correlations were observed between the levels of Cd and Se and the levels of Zn, Cu, and Pb in eggshells. The concentrations of Mn, Zn, Se, As, Cd, and Pb in *C. mydas* eggshells were significantly correlated with those in coral sand sediments. Cu concentrations in the eggshells exceeded the toxic reference value for bird eggs and Se concentrations were between the worst- and best-case scenario hazard quotients.

Metallic elements are among the most common pollutants in marine ecosystems. Their toxicity and non-biodegradable characteristics mean that they pose a significant threat to marine wildlife (Baptista et al., 2016; Finlayson et al., 2016). Sea turtles express a wide geographic distribution, longevity, and site fidelity (Broderick et al., 2007); they can bioaccumulate contaminants through the food chain and are noted as ideal bio-indicators of marine contaminants (Finlayson et al., 2016).

Bird and reptile eggs are useful bio-indicators of contaminants as they are relatively easily sampled compared to animal tissue. Moreover, reptiles are more sensitive to toxicants in their early life stages compared to mammals and egg exposure may interfere with or disrupt embryonic development (Trinchella et al., 2010; Perrault et al., 2013). Eggs provide further insight into the toxicokinetics of non-essential and essential elements for sea turtles during their breeding season (Guirlet et al., 2008). Adult females can sequester and pass on a surplus of certain trace elements, particularly non-essential ones, into eggshells (Burger, 1994; Preez et al., 2018a). Element accumulation in biological tissues involves mutual interactions between ions, and there may be interelemental correlation and competition between essential and non-essential metals to share uptake pathways given their similar chemical properties (Klaassen et al., 1999). Numerous studies have investigated the concentrations of trace elements in sea turtle eggshells; however, few have analyzed the relationship between them and their possible migration pathways (Sakai et al., 2000; Páez-Osuna et al., 2010; Agostinho et al., 2020). Concentrations of certain elements in eggshells are positively correlated with their levels in the tissues and eggs of female great tits (*Parus major*; Dauwe et al., 2005), and rook (*Corvus frugilegus*) eggshells are indicative of contaminant levels in their habitats (Orowski et al., 2016). Maternal transfer is also a significant source of potentially toxic substances in reptile embryos (Guirlet et al., 2008), and the transfer of elements between eggshells and the soil or sand at nesting sites represents another potential source (Brasfield et al., 2004; Marco et al., 2004). Therefore, monitoring trace elements in sea turtle eggshells and coral sand sediments may reflect the population-wide exposure and can provide information about pollutant levels and mobilization in foraging and nesting sites.

The South China Sea hosts the largest populations of sea turtles, most of which are green turtles (*Chelonia mydas*), in China; however, harvesting and habitat degradation have led to a marked decline in the sea turtle population over the last century (Chan et al., 2007). The Qilianyu cluster within the Xisha Islands hosts the largest nesting population of *C. mydas* in China (Jia et al., 2019), and studies have indicated that concentrations of certain heavy metals are increasing in the South China Sea (Xu et al., 2016). Therefore, it is important to investigate the baseline concentrations of trace metals in *C. mydas* and at their nesting sites. This study aimed to elucidate the pollution patterns of and potential

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risks from trace elements in *C. mydas* eggshells and coral sand sediments at their nesting sites and to explore the possible migration pathways of elements into eggshells.

Paired *C. mydas* post-hatch eggshells, and coral sand sediments were collected from 40 nests in the Qilianyu Cluster, within 3–4 days of incubation, during the nesting season (July–October 2019; Fig. 1). Ten to fifteen pieces of eggshell were collected from each nest and the hatching rate was calculated based on the method described by Jia et al. (2019). The eggshells were rinsed with ultrapure water to remove any particulate matter, after which the post-hatch eggshells and sediments were lyophilized and ground to <0.125 mm. Approximately 0.25 g of sediment sample was digested (CEC MARS6, USA) with 8 mL nitric acid (HNO_3, 65%), and 0.25 g of the eggshell sample was digested with 2 mL hydrogen peroxide (H_2O_2, 30%) and 6 mL HNO_3 (65%). The digestion process was as follows: 0–15 min to 120 °C, 15–30 min to 200 °C, and 30–60 min at 200 °C. The digestion solution was evaporated to >1 mL and then diluted to 25 mL with ultrapure water for analysis. Trace elements including Al, Cr, Fe, Mn, Cu, Zn, As, Se, Sr, Cd, Ba, and Pb were determined using inductively coupled plasma mass spectrometry (ICP-MS, Saimofi iCAP-Q, USA). The standard reference material, offshore marine sediment sample GB W07314, was obtained from the Center for National Standard Reference Material of China and used to test the analysis method. The recoveries of all 12 trace elements were 100 ± 10%.

The differences in element concentrations in *C. mydas* eggshells were assessed using a one-way analysis of variance (ANOVA), followed by a post-hoc Tukey's test. The relationships between the concentrations of elements in the eggshells and coral sand sediments were evaluated using Pearson's correlation analysis; for the former, principal component analysis (PCA) was also used. Statistical calculations were performed using SPSS (version 23.0, International Business Machines Corp., USA), and significance was set at $p = 0.05$.

Concentrations ($\mu g \cdot g^{-1}$) of elements in coral-sand sediment ranged from 11.7 to 192 for Al, 0.55 to 5.44 for Cr, 2.60 to 91.0 for Fe, 1.82 to 12.5 for Mn, 0.34 to 7.36 for Cu, 1.63 to 66.7 for Zn, 0.06 to 0.77 for As, 0.24 to 1.32 for Se, 3530 to 8630 for Sr, 0.02 to 0.17 for Cd, 3.28 to 11.8 for Ba, and 0.08 to 1.55 for Pb (Table 1). The concentrations of elements in coral sand sediments were lower than the first-grade limit values of the GB (18668–2002) national standard in China (AQSIQ, 2002) and less than the threshold effect levels developed by Long et al. (1995). Sr is generally present in marine environments, with their highest content being in coral sand sediments. The proportions of trace element contents in coral sand sediments, apart from Sr, descended in the following order: Al (54.3%) > Fe (18.7%) > Ba (7.6%) > Zn (6.8%) > Mn (6.7%) > Cr (1.8%) > Cu (1.0%) > Se (0.9%) > Pb (0.5%) > As (0.2%) > Cd (0.06%).

Concentrations ($\mu g \cdot g^{-1}$) of elements in *C. mydas* eggshells ranged from 0.76 to 15.1 for Al, 0.05 to 1.54 for Cr, 1.27 to 17.9 for Fe, 0.90 to 1.96 for Mn, 6.00 to 23.0 for Cu, 1.67 to 45.2 for Zn, 0.03 to 0.21 for As, 0.72 to 4.98 for Se, 11.1 to 164 for Sr, 0.001 to 0.15 for Cd, 0.10 to 0.84 for Ba, and 0.02 to 0.33 for Pb (Table 2). Our study showed significant inter-clutch differences in metal concentrations (Kruskal–Wallis one-way ANOVA, df = 40, $p < 0.001$), corroborating the results reported by Guzmán et al. (2020). The concentration of Sr was the highest in eggshells. Sr is an alkali metal element that participates in metabolic processes with Ca (Moiseenko et al., 2008). Sr seems to accumulate in eggshells owing to the high Ca content of the latter. The proportions of trace elements in the *C. mydas* eggshells, apart from Sr, descended in the following order: Zn (40.9%) > Cu (28.5%) > Fe (9.43%) > Al (7.94%) > Se (5.09%) > Mn (1.97%) > Cr (1.70%) > Ba (0.94%) > Pb (0.29%) > As (0.18%) > Cd (0.05%). The concentrations and proportions of essential elements (e.g., Zn, Cu, Fe, Se, and Mn) were much higher than those of non-essential elements (e.g., Pb and Cd). A previous study has shown that the maternal transfer of essential elements occurs freely, whereas this process is relatively limited in terms of toxic elements (Páez-Osuna et al., 2010).

The measured concentrations of trace elements in sea turtle eggshells

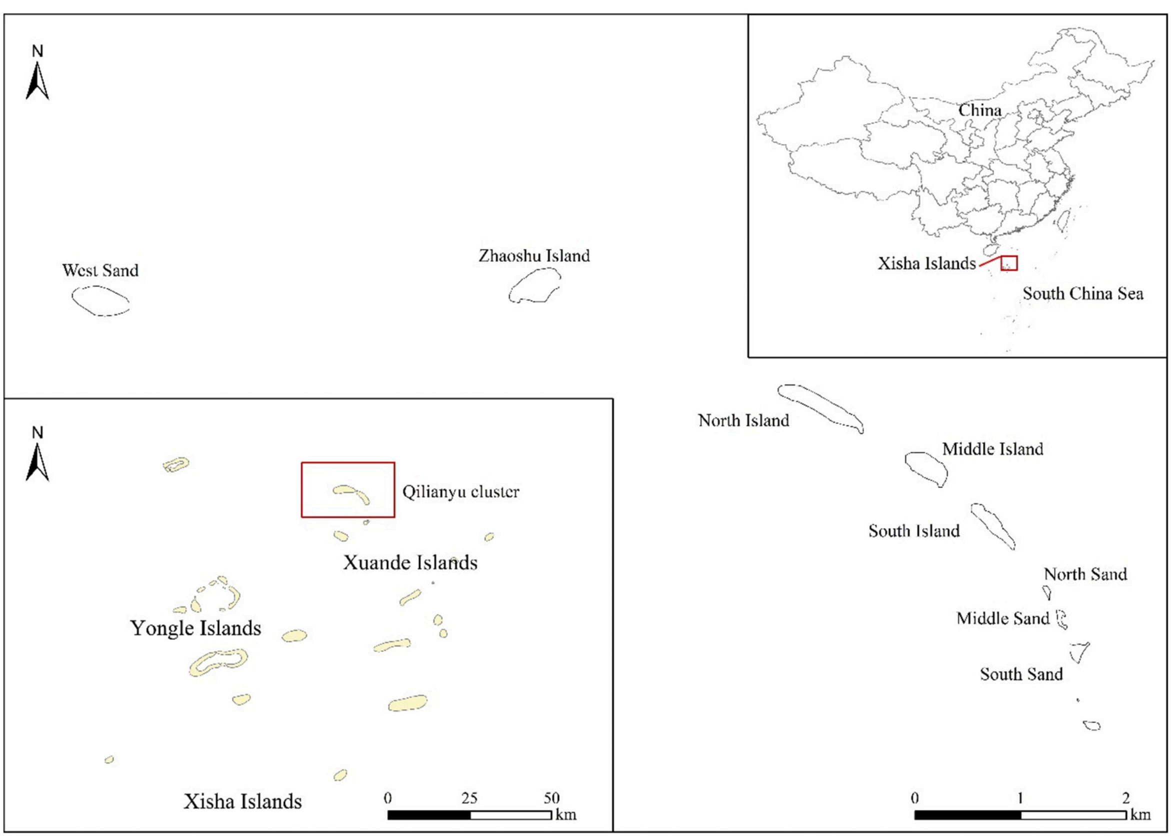

Fig. 1. Location of *Chelonia mydas* eggshell collection site: the Xisha Islands, China.

Table 1
Trace element concentrations (μg·g^{-1}) in coral sand sediments from nesting sites.

Elements	Min	Max	Mean	SD	Threshold values[a]	TEL[b]	PEL[b]
Al	11.7	192	61.5	48.8	–	–	–
Cr	0.55	5.44	1.51	1.10	80	81	370
Fe	2.60	91.0	17.3	14.9	–	–	–
Mn	1.82	12.5	5.67	2.03	–	–	–
Cu	0.34	7.36	0.79	1.09	35	34	270
Zn	1.63	66.7	6.95	10.8	150	150	410
As	0.06	0.77	0.17	0.15	20	8.2	70
Se	0.24	1.32	0.70	0.24	–	–	–
Sr	3530	8630	5800	1110	–	–	–
Cd	0.02	0.26	0.05	0.05	0.5	1.2	9.6
Ba	3.28	11.8	6.35	1.81	–	–	–
Pb	0.08	1.55	0.41	0.27	60	46.7	218

[a] Environmental quality standards for marine sediment of China (grade I, GB18668-2002).
[b] Sediment quality guidelines for coastal and marine waters developed by Long et al. (1995).

Table 2
Trace element concentrations (μg·g^{-1}) in *C. mydas* eggshells.

Elements	Min	Max	Mean	SD	CV%
Al	0.76	15.1	4.37	3.00	68.6%
Cr	0.05	1.54	0.81	0.37	46.4%
Fe	1.27	17.9	4.92	2.82	57.3%
Mn	0.90	1.96	0.91	0.33	36.6%
Cu	6.00	23.0	12.8	4.12	32.0%
Zn	1.67	45.2	20.3	9.85	48.5%
As	0.03	0.21	0.08	0.04	48.8%
Se	0.72	4.98	2.44	1.30	53.3%
Sr	11.1	164	41.3	26.5	64.3%
Cd	0.001	0.15	0.02	0.03	137%
Ba	0.10	0.84	0.44	0.19	43.1%
Pb	0.02	0.33	0.14	0.07	50.4%

were compared with previous results from other regions (Table 3). In general, the mean concentrations of Cu, Sr, and Zn in *C. mydas* eggshells from Xisha were higher than those recorded in most other regions. The Cu content was 1.5–10 times higher than the results from all other areas except Turkey (Çelik et al., 2006) and South Africa (Preez et al., 2018a, 2018b). The Zn content was 1.2–10 times higher than those found in all other regions apart from the coast of Japan (Sakai et al., 2000). The mean concentration of Sr in this study (41.29 μg·g^{-1}) was considerably higher than that of the only other comparable location, Hong Kong (0.02 μg·g^{-1}; Lam et al., 2006). However, the concentrations of Fe, Al, Pb, and Cr in the present study were similar to or lower than those in South Africa and Brazil (Agostinho et al., 2020). Some possible reasons for the different concentrations of elements in sea turtle eggshells observed in this study and those from other regions include the following. Generally, the bird and reptile eggs could be used to investigate recent exposure in current habitat, and the trace elements in *C. mydas* eggshells may reflect the current levels of pollution at foraging or nesting sites. The slightly higher concentrations of Cu, Zn, and Sr in this study may also be due to contamination by these metals in the areas around the Xisha Islands, this requires further investigation. In addition, adult *C. mydas* feed mainly on algae and seagrass (Cardona et al., 2009), and some typical forage of *C. mydas*, such as *Enhalus acoroides* and *Thalassia hemprichii*, accumulate high concentrations of Cu and Zn (Zheng et al., 2018). The trace elements in *C. mydas* forage are consistent with sediment geochemistry in that coastal bay forage has higher Fe, Mn, Cu, and Zn contents whereas forage from coral cays has higher Sr levels (Thomas et al., 2020). Moreover, given that post-hatch eggshells were used in this study, the concentration of contaminants may be attributed to the adsorption of metals from surrounding matrices after incubation (Marco et al., 2004).

Multivariate PCA and Pearson's correlation analyses were conducted to determine the relationships between the levels of trace element in *C. mydas* eggshells. The Kaiser–Meyer–Olkin value (0.641) and the significance of the Bartlett's test ($p < 0.0001$) confirmed that the PCA results were valid. Fig. 2 shows a two-dimensional component plot. The first principal component (PC1) explained 37.6% of the total variance and had a strong positive correlation with Cr, Fe, Zn, Ba, and Pb (a loading factor > 0.7), whereas the loading factors of Al, Mn, Cu, and Cd were relatively lower. The second principal component (PC2) explained 15.6% of the total variance and had a strong positive correlation with As, Se, and Cd (a loading factor > 0.6). The concentrations of elements heavily loaded by the same component (PC1 or PC2) were significantly correlated in most cases ($p < 0.05$, Table 4). Significant correlations between the levels of metals in organisms are due to common sources or intake pathways related to the proteins that bind metals (Andreani et al., 2008). This study found positive associations between essential and non-essential metals pairs, such as Se and Cd, Fe and Pb, and Pb and Zn. Eggs from females exposed to Pb exhibited higher concentrations of

Table 3
Comparisons between the levels of trace elements in *C. mydas* eggshells in this study and others (μg·g^{-1}).

Species	Location	Al	Cr	Mn	Fe	Cu	Zn	As	Se	Sr	Cd	Ba	Pb	Reference
L. olivacea	Oaxaca, Mexico[a]	–	–	–	–	7.48	12.4	–	–	–	0.47	–	–	Páez-Osuna et al., 2010
C. mydas	Hongkong, China[b]	<0.15	0.47	0.27	2.30	1.30	1.20	0.22	2.50	0.02	0.016	3.50	0.11	Lam et al., 2006
E. imbretica	Qeshm Island, Iran[a]	–	–	–	–	6.64	5.56	–	–	–	0.36	–	4.16	Ehsanpour et al., 2014
C. mydas	Oman[a]	–	–	–	–	6.85	5.32	–	–	–	0.40	–	4.25	Sinaei and Bolouki, 2017
D. coriacea	South Africa[a]	26.0	11.0	0.73	375	8.60	5.80	0.10	11.0	–	0.001	–	0.25	Preez et al., 2018a,
C. caretta		44.0	38.0	2.00	390	16.0	16.0	0.10	5.80	–	0.001	–	0.77	2018b
C. caretta	Mediterranean[a]	–	–	–	17.75	5.29	5.00	–	–	–	0.649	–	0.633	Kaska and Furness, 2001
C. mydas	Japanese Coastal Waters[b]	–	–	1.33	1.98	0.78	20.30	–	–	–	<0.03	–	<0.03	Sakai et al., 2000
C. mydas	Turkey[a]	–	0.56	–	49.16	18.44	–	ND	–	–	0.58	–	0.04	Çelik et al., 2006
C. mydas	Rocas Atoll, Brazil[b]	21.39	0.17	0.20	7.81	3.12	2.90	0.30	–	–	0.04	0.55	0.11	Agostinho et al., 2020
C. mydas	Qilianyu, China[a]	4.37	0.81	0.91	4.92	12.8	20.3	0.08	2.44	41.3	0.024	0.44	0.14	Present Study
	Qilianyu, China[b]	2.77	0.51	0.58	3.11	8.10	12.9	0.05	1.54	26.1	0.02	0.28	0.09	

Values are expressed as *a*, mean dry weight; *b*, mean wet weight; –, not reported; ND, not detected.

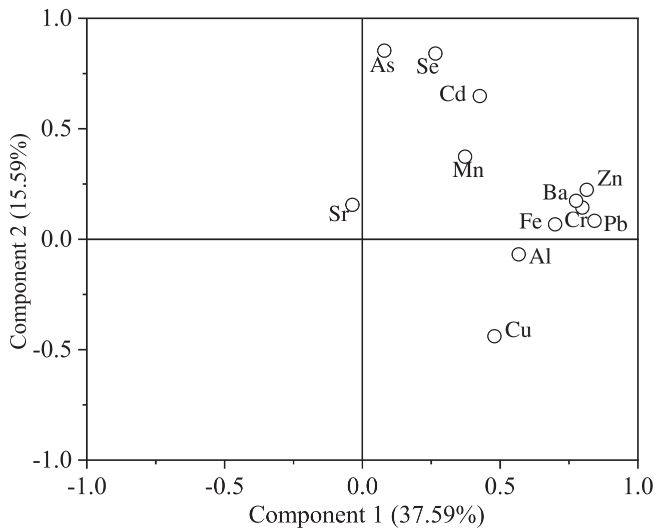

Fig. 2. Principal component loads of the trace elements in *Chelonia mydas* eggshells.

ovotransferrin because the latter binds Pb^{2+} and may thus enable egg detoxification, thereby protecting the embryo (Chatelain et al., 2016). Pb also binds tightly to Zn sites in proteins (Godwin, 2001). Guirlet et al. (2008) suggested that the toxicokinetics of Se and Cd differed from those of Zn, Cu, and Pb during maternal transfer. Previous studies have found positive relationships between Se and Cd in the tissues of various organisms and indicated that co-exposure to Se and Cd could reduce Cd toxicity (Gardner et al., 2006; Barraza et al., 2019).

The concentrations of Mn ($r = 0.438$), Zn ($r = 0.439$), As ($r = 0.551$), and Cd ($r = 0.688$) in eggshells were significantly correlated with those in coral sand sediments at the $p < 0.01$ level. Meanwhile, the concentrations of Se ($r = 0.341$) and Pb ($r = 0.339$) were significantly correlated with those of the sediments at the $p < 0.05$ level. Essential elements for embryonic development can migrate from maternal tissues to embryos (Páez-Osuna et al., 2010), and eggs may also adsorb elements from surrounding matrices (Marco et al., 2004). Eggs of many oviparous species, including sea turtles, have soft and leathery shells that are permeable to metals (Marco et al., 2004; Guirlet et al., 2008; Preez et al., 2018b). Marco et al. (2004) showed that lizard eggs can adsorb As from contaminated environments. The compositions of crocodile eggshells have been found to be strongly associated with the concentrations of metals (particularly, Ni, Co, and As; Preez et al., 2018b) found at their nesting sites. Therefore, the leathery, semi-permeable shells of *C. mydas* eggs may allow the migration of bioavailable trace elements (Marco et al., 2004; Guirlet et al., 2008). However, the kinetics of the transfer of metals from *C. mydas* females or coral sand sediments into the eggs is unclear and requires more comprehensive research.

The ecotoxicological profiles of metals in the sediment were evaluated using the adverse effect index (AEI) and potential ecological risk index (PERI). AEI values were calculated using the following equation (Celis-Hernandez et al., 2018):

$$AEI = \frac{[MC]}{[SQG]}$$

where MC is the metal concentration in the coral sand sediment and SQG is the sediment quality guideline. This study used the threshold effect level (TEL) quality guideline developed by Long et al. (1995). An AEI < 1.0 indicates that the concentration of metals is not high enough to produce adverse effects in biota; conversely, an AEI ≥ 1.0 indicates that the concentration of metals could produce adverse effects.

PERI was calculated using the following equation (Hakanson, 1980):

$$PERI = \sum_{i=1}^{n} E_r^i = \sum_{i=1}^{n} T_r^i \times c_f^i$$

$$C_f^i = c_s^i / c_n^i$$

where E_r^i is the potential ecological risk factor (ER_i) for individual heavy metals, T_r^i is the toxicity coefficient of heavy metal i (e.g., Cr = 2, Ni = 5, Cu = 5, Zn = 1, As = 10, Cd = 30, and Pb = 5), c_f^i is the contamination index of heavy metal i, c_s^i is the average concentration of metal i in the samples, and c_n^i is the local background value of heavy metal i (Zhang and Du, 2005). The following assessment grades were used to interpret the PERI values: (1) PERI < 150, low ecological risk; (2) 150 ≤ PERI ≤ 300, moderate ecological risk; (3) 300 ≤ PERI ≤ 600, considerable ecological risk; and (4) PERI > 600, very high ecological risk.

The AEI values for Cr, Cu, Zn, As, Cd, and Pb were < 1 in the coral sand sediments (Fig. 3a). Their average ER_i values were in the following order: Cd (8.70) > Cu (0.53) > As (0.18) > Pb = Zn (0.13) > Cr (0.08) (Fig. 3b). The PERI values for all coral sand sediments were lower than 150, indicating low ecological risk at the *C. mydas* nesting sites. Although the information available on the toxicological effects of metals and metalloids on sea turtles is limited (Finlayson et al., 2016), previous studies have used results based on avian eggs to predict the risk to sea turtle embryos (Lam et al., 2006; Preez et al., 2018a). The toxic reference values (TRV) in bird eggs for Cu and Se are 10–20 $\mu g \cdot g^{-1}$ and 8 $\mu g \cdot g^{-1}$ (dw, dry weight), respectively (Meyer et al., 2015). The worst- and best-case scenario hazard quotients (HQs) for Pb in *C. mydas* eggs are <0.1 and 0.2 $\mu g \cdot g^{-1}$ (ww, wet weight), and those for Se are 0.2 and 24.5 $\mu g \cdot g^{-1}$ (ww), respectively (Lam et al., 2006). The concentrations of Pb measured in this study ranged from 0.03–0.19 $\mu g \cdot g^{-1}$ (ww), with 28% of the samples between the worst- and best-case scenario HQs. The concentrations of Cu here ranged from 6.00–23.0 $\mu g \cdot g^{-1}$ (dw), with 5% of the samples exceeding 20 $\mu g \cdot g^{-1}$ and 75% of the samples exceeding 10 $\mu g \cdot g^{-1}$ (TRV). The contents of Se in eggshells ranged from 0.46–3.15

Table 4
Pearson's correlation coefficient (r) between trace element concentrations in *C. mydas* eggshells.

	Al	Cr	Mn	Fe	Cu	Zn	As	Se	Sr	Cd	Ba	Pb
Al												
Cr	0.299*											
Mn	0.370*	0.280										
Fe	0.667**	0.328*	0.533**									
Cu	0.029	0.288	−0.051	0.139								
Zn	0.235	0.706**	0.261	0.432**	0.367*							
As	−0.130	0.161	0.228	0.039	−0.111	0.335*						
Se	0.001	0.413*	0.219	0.097	−0.040	0.470**	0.804**					
Sr	−0.003	−0.187	0.202	0.145	−0.137	−0.016	−0.047	−0.020				
Cd	0.399*	0.340*	0.437**	0.468**	−0.223	0.310*	0.385*	0.535**	0.134			
Ba	0.172	0.697**	0.182	0.425**	0.288	0.691**	0.194	0.322*	0.208	0.416**		
Pb	0.382*	0.653**	0.143	0.468**	0.343*	0.734**	0.213	0.313*	−0.131	0.385*	0.653**	

** Correlation is significant at the 0.01 level (two-tailed).
* Correlation is significant at the 0.05 level (two-tailed).

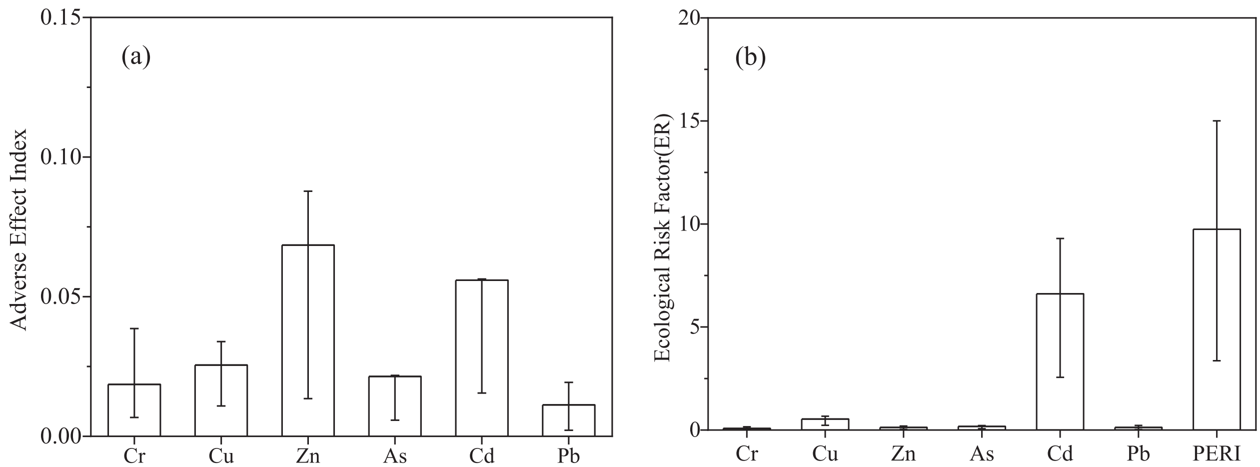

Fig. 3. Ecotoxicological profile of heavy metals in coral sand sediment used for *C. mydas* nests.
(a) Adverse effect index and (b) ecological risk factor and potential ecological risk index.

$\mu g \cdot g^{-1}$ (ww); this was less than the TRV value in bird eggs and fell between the worst- and best-case scenario HQs. Studies have also shown that the concentration of Se in sea turtle yolks is higher than that in eggshells (Lam et al., 2006; Preez et al., 2018a, 2018b), and there was a significant negative correlation between the concentration of Se in eggshells and hatching rate ($r = -0.39$, $p < 0.05$). However, no significant association was observed between Cu content and hatching rate, and the Cu content has been reported to be higher in eggshells than in albumen and yolk (Páez-Osuna et al., 2010). Further investigations are needed to supplement the preliminary results of this study on the ecological risks of trace elements.

In summary, this study examined trace element concentrations in *C. mydas* eggshells and coral sand sediments from nests on the Xisha Islands in the South China Sea. The concentrations of Cu, Zn, and Sr in the *C. mydas* eggshells from this region were slightly higher than those in eggshells from other regions. PCA showed that the levels of Se, Cd, and As in eggshells had significant associations with each other; the levels of Cr, Fe, Zn, Ba, and Pb were also significantly correlated with each other. Elements with similar loadings in the PCA components may have similar metabolic or migratory pathways during eggshell formation. There were significant correlations between the concentrations of Zn, Se, Mn, As, Cd, and Pb in eggshells and coral sand sediments, indicating a similar migration of these elements into or out of the eggs. The AEI values for Cr, Cu, Zn, As, Cd, and Pb were <1 in the coral sand sediments analyzed, and the PERI values for all coral sand sediments in *C. mydas* nests were lower than 150, indicating a low ecological risk. The concentrations of Cu in 75% of the *C. mydas* eggshells sampled exceeded the TRV value (10 $\mu g \cdot g^{-1}$) while the concentrations of Se fell between the worst- and best-case scenario HQs for *C. mydas* eggs.

CRediT authorship contribution statement

Li Jian: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Rui Guo:** Investigation, Methodology. **Xiaobo Zheng:** Formal analysis, Writing – review & editing. **Haitao Shi:** Conceptualization, Writing – review & editing. **Jichao Wang:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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