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Levels of trace elements, methylmercury and polybrominated diphenyl ethers in foraging green turtles in the South China region and their conservation implications[☆]

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ABSTRACT

Sea turtles are globally endangered and face daily anthropogenic threats, including pollution. However, there is a lack of ecotoxicological information on sea turtles, especially in the Asia-Pacific region. This study aims to determine pollutant levels of foraging green turtles (*Chelonia mydas*) in South China, including Hong Kong, Guangdong and Taiwan, as a basis for their conservation. Scute, liver and muscle tissues of stranded green turtles were analysed for levels of 17 trace elements and methylmercury (MeHg) ($n = 86$ for scute and $n = 14$ for liver) and polybrominated diphenyl ethers (PBDEs) ($n = 11$ for muscle and $n = 13$ for liver). Ten-fold higher levels of Pb, Ba, V and Tl and 40-fold greater Cd levels were measured in green turtle livers in South China relative to other studies conducted over 10 years ago. Measured PBDE levels were also 27-fold and 50-fold greater than those reported in Australia and Japan. These results warrant further investigation of potential toxicological risks to green turtles in South China and their source rookeries in Malaysia, Micronesia, Indonesia, Marshall Islands, Japan and Taiwan. Research should target monitoring pollutant levels in sea turtles within the West Pacific/Southeast Asia regional management unit spanning East Asia to Southeast Asia to fill in knowledge gaps, in particular in areas such as Thailand, Vietnam, Indonesia, Malaysia and the Philippines where less or no data is available and where foraging grounds of sea turtles have been identified.

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1. Introduction

Of the seven sea turtles species in the world, five species are found in the South China Sea: the green turtle (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), olive ridley (*Lepidochelys olivacea*), loggerhead (*Caretta caretta*), and hawksbill (*Eretmochelys imbricata*) turtles (Wang, 1993; Chan et al., 2007). These sea turtle

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species are migratory with circumtropical distribution, with adults traveling hundreds to thousands of kilometres between nesting beaches and foraging grounds. Among the five sea turtle species recorded in the South China region, the green turtle is the most common and is the only species that nests in the area (Wang, 1993; Chan et al., 2007; Wang and Li, 2008; Ng et al., 2014). Foraging grounds of green turtles are mainly distributed along the coasts of mainland China (Hainan Island and Guangdong Province), as well as of Taiwan, and on outlying islands in the South China Sea and East China Sea. Green turtles migrating for breeding and during the inter-nesting period usually stop foraging (Ng, 2015). Green turtles in the South China Region mainly forage on red algae and occasionally marine biota such as fishes and crabs (Ng et al., 2016). Their primary source rookeries are in Malaysia, Micronesia, Indonesia, the Marshall Islands, Japan and Taiwan (Ng et al., 2017). The globally endangered green turtle (IUCN, 2016) faces various anthropogenic threats, such as direct take and by-catch (Cheng and Chen, 1997; Wang and Li, 2008), trade pressure (Pilcher et al., 2009; Lam et al., 2011), habitat degradation (Wang and Li, 2008) and pollution and marine debris (Lam et al., 2006; Wabnitz and Nichols, 2010). Nesting populations of green turtles in South China have been dwindling for decades (Chan et al., 2007; Wang and Li, 2008).

Sea turtles are known to be exposed to trace elements and persistent organic pollutants (POPs) (Lam et al., 2004; Storelli and Marcotrigiano, 2003; Day et al., 2005; Van de Merwe et al., 2010). Some trace elements (e.g., Se, Zn), are essential to the health and biological function of organisms but can be detrimental above optimal levels; others such as As and Pb have no nutritional value and can be toxic at low doses (Hoffman et al., 2003). POPs such as the additive flame retardants polybrominated diphenyl ethers (PBDEs) remain intact for long time periods in the environment, are highly soluble in lipids in living organisms and can cause adverse impacts to humans and wildlife (Hites, 2004). Although the production and use of PBDEs (mainly the penta- and octa-BDEs) have been banned/restricted under the Stockholm Convention since 2004, PBDEs remain in the environment. PBDEs have been detected in marine microplastics and reported in abdominal adipose tissue of oceanic seabirds in the North Pacific Ocean (Hirai et al., 2011).

Coastal waters and habitats in South China are contaminated by organic pollutants and trace elements due to the recent rapid increase in industrialized activities in the region (Blackmore, 1998; Mai et al., 2005; Wong et al., 2006; Vane et al., 2009). Levels of PBDEs in two small cetaceans from the South China Sea, the Indo-Pacific humpback dolphin (*Sousa chinensis*) and finless porpoise (*Neophocaena phocaenoides*), increased from 1990 to 2000 (Isobe et al., 2007) as well as 1997–2007 (Lam et al., 2009). Studies on trace elements in biota in South China have mostly focused on benthic fauna (Che and Cheung, 1998; Wong et al., 2005) and birds (Burger and Gochfeld, 1993; Connell et al., 2002). Toxicology studies in sea turtles in China are limited to measurement of levels of selected trace elements in muscle and organs of stranded green turtles (Lam et al., 2004) and in green turtle eggs (Lam et al., 2006) in Hong Kong, and trace elements (including As, Cd, Cr, Cu, Hg, Ni, Pb and Se) in blood of stranded, by-catch or captive sea turtles and nesting green turtles in Taiwan (Kuo, 2015). Bioaccumulation of trace elements and POPs in sea turtles pose potential risks to their health, in particular physiological and immune function, development and growth, and reproductive success (Grillitsch and Schiesari, 2010; Jakimska et al., 2011). Despite the threats posed by these pollutants, most previous studies generally have quantified pollutant loading in sea turtles (Cortés-Gómez et al., 2017), with less consideration given to assessing potential impacts (Hopkins et al., 2001; Weir et al., 2010).

The objectives of this study are to determine the recent baseline levels of trace elements, methylmercury (MeHg) and PBDEs in

green turtles in the South China region and to compare these concentrations to data reported 10 years ago as part of the information needed for management and conservation of this species. Risk assessment of selected trace elements and MeHg measured in green turtle livers using a hazard quotient approach was also conducted.

2. Materials and methods

2.1. Sample collection

Samples were collected from foraging green turtles collected as by-catch or stranded individuals in the South China Region, including Hong Kong, Guangdong and Taiwan, from 2005 to 2013. Necessary CITES import and export licences were granted by the Endangered Species Import and Export Management Office of the People's Republic of China, the Bureau of Foreign Trade Ministry of Economic Affairs and the Council of Agriculture of Taiwan, the U.S. Fish and Wildlife Service Division of Management Authority and the Agriculture, Fisheries and Conservation Department of the Government of the Hong Kong Special Administrative Region of the People's Republic of China.

Scute scrapings, liver and pectoral muscle tissues of green turtles were sampled. Approximately 1–2 g of scute scrapings were obtained by scratching ~1 mm depth of the scutes at the posterior end of the carapace (Day et al., 2005; Sakai et al., 2000a) using a ceramic knife, and the scrapings were stored at –20 °C in polyethylene bags for analysis of trace elements and MeHg. 10 g of liver and muscle tissue were dissected from freshly dead turtles using a hexane-rinsed scalpel (Keller et al., 2004; Lazar et al., 2011) during post-mortem examinations and placed in hexane-rinsed aluminum foil and then in polyethylene bags and stored at –20 °C for PBDE analysis. An additional 10 g of liver was dissected using a ceramic knife and stored in only a polyethylene bag at –20 °C for trace element analysis. Curved carapace length (CCL) of each green turtle sampled was recorded and is presented as mean ± standard deviation and range. Life stage was defined using carapace length intervals described for the best-available and geographically closest green turtle population, that in Hawaii described by Balazs (1980). The following age classes were used: a juvenile is a post-hatchling to an individual of 65 cm straight carapace length (SCL); a sub-adult is an individual of SCL from 65 cm to 81 cm; and an adult is an individual of SCL > 81 cm and reproductively mature. According to the conversion between SCL and CCL where $CCL = -0.414 + 1.039 SCL$ (Bjorndal and Bolten, 1989), the life stage of green turtles based on CCL was defined as: juvenile, a post-hatchling to an individual of 67 cm CCL; sub-adult, CCL from 67 cm to 84 cm; and adult, CCL > 84 cm.

2.2. Laboratory analysis

Laboratory analysis of trace elements generally followed the methods reported in Connell et al. (2002) and Lam et al. (2004) with modifications. Detailed methods can be found in the Supplementary Material. Briefly, scute scrapings and liver samples were freeze-dried and homogenized before microwave digestion in duplicate in 5 ml of concentrated nitric acid (Aldrich Sigma, trace metal grade, ≥ 65%). Levels of As, Ag, Ba, Cd, Cu, Cr, Co, Cs, Fe, Mn, Pb, Ni, Se, Sr, Tl, V and Zn were measured with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (PerkinElmer 2100DV, PerkinElmer, Waltham, MA, USA).

Reagent blanks and sample matrix spikes were included in every digestion batch. Matrix recoveries ranged from 75% to 125%. A certified reference material (DOLT-2 dogfish liver tissue; National Research Council, Ottawa, Canada) was analysed for method

validation. Recoveries of 70%–120% of the certified values were achieved. Detection limits (DLs) were determined as three times the standard deviation of the signals of the blanks. The DLs for trace element analysis ranged from 0.010 to 0.060 mg/kg.

For measurement of MeHg levels, 0.05 g freeze-fried sample was digested with 4 ml 25% KOH/methanol and heated at 80 °C for 6 h. In order to obtain full digestion, some samples were heated twice. 10 µl of the digested samples was analysed for MeHg level based on USEPA Method 1630 (2001) using a MERX Automated Methyl Mercury System (Brooks Rand Labs, Seattle, WA, USA). The DL was 0.0001 mg/kg for MeHg analysis. Standard reference material DORM-4 (National Research Council, Canada) was used to validate the method, and recoveries of 82–94% of the certified values were obtained.

PBDE content in muscle and liver tissues of green turtles was measured using the methods with some modifications as stipulated in previous studies (Lam et al., 2009; Zhu et al., 2014). Detailed methods can be found in the [Supplementary Material](#). Briefly, approximately 1 g of freeze-dried homogenized sample was analysed. 5 ng of ¹³C-labeled BDE surrogate standard was added to each sample and then the sample was subject to accelerated solvent extraction (Dionex ASE[®] 200, Dionex, Sunnyvale, CA, USA) in dichloromethane:hexane (4:1 v/v, 40 mL) followed by gel permeation chromatography and column cleanup using sodium sulfate and activated silica gel. ¹³C-labeled BDE139 was used as a recovery spike before the instrumental analysis.

PBDEs were identified and quantified using a liquid chromatography–tandem mass spectrometer (LC–MS/MS) system. Details on instrumental parameters can be found in the [Supplementary Material](#). The DLs ranged from 0.010 to 0.26 ng/g lipid weight (lw). Recoveries of ¹³C-labeled PBDE congeners in the samples were between 101 ± 9% to 109 ± 13% and 99% ± 9% to 108 ± 12% for tri-BDEs to hepta-BDEs and octa-BDEs to deca-BDE, respectively. The 12 major individual PBDE congeners (BDE-28, -47, -99, -100, -153, -154, -183, -196, -197, -206, -207, -209) quantified were summed to derive total PBDE concentrations (ΣPBDEs). PBDE concentrations are presented in ng/g lipid weight, wet weight and dry weight for comparison with the results of previous studies.

2.3. Data analysis

Measured levels of trace elements and PBDEs in different tissues are presented as mean ± standard deviation and range. The measured contaminant levels in this study were compiled along with the levels measured previously in green turtle tissues in other areas to compare the spatial distribution of these contaminants in green turtles. The ratio of dry-to-wet weights of the liver samples of green turtles was determined by dividing each liver sample's dry weight by its corresponding wet weight. The average ratio for green turtle livers was found to be 0.268 and used to convert contaminant concentrations in wet weight to dry weight reported in previous studies for comparison.

Data normality was assessed using Levene's test for equality of variances and Kolmogorov-Smirnov one-sample tests (IBM SPSS Statistics 20). Significant differences in measured levels of each trace element and MeHg in green turtle scutes between stranded and by-catch individuals and between adult and immature (juvenile and sub-adult) individuals were investigated by two-way ANOVA. Two-sample *t*-tests were used to determine any significant differences in the measured levels of trace elements and PBDEs in liver and muscle samples between stranded adult and immature individuals. Regression analyses were performed between levels of selected trace elements measured in scutes and livers of green turtles to investigate the utility of non-invasive sampling (i.e. scute scrapings). Statistical significance was determined at $p < 0.05$.

Risk assessment of selected trace elements and MeHg to green turtles was conducted using the more readily available toxicological data from birds and freshwater turtles based on liver concentrations. Hazard quotients (HQs) were determined by dividing the measured concentrations (MEC) of the specific trace element in green turtles with predicted no-effect concentrations (PNECs), i.e. the threshold level below which no observable adverse effect is anticipated ($HQ = MEC/PNEC$; values are discussed below and are summarized in [Table 5 in the Supplementary Material](#)). PNECs specific for green turtles were calculated by dividing the no-observed-adverse-effect level (NOAEL) derived for a bird or freshwater turtle species by a standard assessment factor, 1000, to account for uncertainties due to intra- (factor = 10) and inter-specific variability (10) and chronic exposure conditions (10) (European Commission, 2003) ($PNEC \text{ for green turtle} = NOAEL \text{ for bird or freshwater turtle}/1000$). HQs on a best-case scenario were determined using the minimum MEC and maximum PNEC ($HQ_{\text{best}} = MEC_{\text{min}}/PNEC_{\text{max}}$), while HQs on a worst-case scenario were determined using the maximum MEC and minimum PNEC ($HQ_{\text{worst}} = MEC_{\text{max}}/PNEC_{\text{min}}$). According to [Hernando et al. \(2006\)](#), $HQ < 0.01$ refers to “unlikely to pose risk”; $0.01 \leq HQ < 0.1$: “Low risk”; $0.1 \leq HQ < 1$: “Medium risk”; and $HQ \geq 1$: “High risk”.

3. Results

3.1. Trace element and MeHg levels in scutes and livers

Eighty-six green turtles (CCL: mean 58 cm ± S.D. 24 cm; range: 13–197 cm) from the South China region, including 20 adults and 66 sub-adults and juveniles, were analysed for trace element and MeHg levels ([Table 1a](#)). Detailed data for [Table 1a](#) can be found in the [Supplementary Material](#). The measured levels of trace elements in the scutes were not significantly different between stranded and by-catch individuals for adult and immature stages (two-way ANOVA, $p < 0.05$). While most of the trace elements between adult and immature green turtles were not significantly different, levels of Cd (Two-way ANOVA, $F_{0.05(1), 1, 1} = 21.937$ and $p < 0.05$) in the scutes of adult green turtles were significantly higher than those of immature individuals and the opposite was observed for Tl levels ($F_{0.05(1), 1, 1} = 5.188$ and $p < 0.05$).

Trace element and MeHg levels were quantified in liver samples obtained from 14 stranded green turtles (CCL: 57 cm ± 20 cm; 32–96 cm), including 3 adults and 11 sub-adults and juveniles ([Table 1b](#)). Detailed data of [Table 1b](#) can be found in the [Supplementary Material](#). There were no significant differences in trace element and MeHg levels in adult and immature stranded green turtle livers (two-sample *t*-test, $p > 0.05$).

Cu concentrations in scutes were significantly and positively regressed with those in liver ($R^2 = 0.761$, $p < 0.05$, $n = 7$), while levels of Zn in scutes showed a significant negative regression with those measured in liver ($R^2 = 0.404$, $p < 0.05$, $n = 11$). The remaining elements showed no significant relationships: levels of Se, Cd, Mn and Sr in scutes increased with decreasing levels in liver, while levels of Pb, Ni, Tl and MeHg in scute increased with those in liver (see [Table 2 in the Supplementary Material](#)).

3.2. PBDEs levels and congener profiles in liver and muscle of green turtles

Liver ($n = 13$), including 3 adult and 10 immature individuals, and muscle ($n = 11$), including 3 adult and 9 immature individuals, samples of stranded green turtles were analysed for PBDE levels ([Table 2](#) and b). There were no significant differences in total PBDE concentrations between adult and immature green turtles in both liver and muscle (two-sample *t*-test, $p > 0.05$). BDE-47, -100

Table 1

Levels of trace elements and MeHg (mg/kg, dry weight) in (a) scute (n = 86) and (b) liver (n = 14) tissues of green turtles. Matrix recoveries of Fe and Ag in liver tissues (<50%) were unacceptable and therefore their levels are not reported. "n > DL" represents the number of samples above the detection limits (DLs). DLs for trace element ranged from 0.010 to 0.060 mg/kg.

| (a) Scute | | | | | | | |
|-----------|--------|--------|------|--------|------|------|------|
| Element | DL | n > DL | Mean | Median | SD | Max | Min |
| Se | 0.05 | 85 | 30 | 29 | 8.1 | 56 | 16 |
| Zn | 0.01 | 86 | 180 | 160 | 120 | 560 | 18 |
| Pb | 0.02 | 77 | 3.3 | 3 | 2.1 | 11 | 0.54 |
| Co | 0.01 | 2 | 2 | n/a | n/a | 2.4 | 1.6 |
| Cd | 0.01 | 15 | 0.66 | 0.51 | 0.42 | 1.8 | 0.32 |
| Ni | 0.01 | 83 | 3.1 | 1.7 | 4.2 | 24 | 0.12 |
| Ba | 0.01 | 10 | 8 | 3 | 10 | 30 | 0.83 |
| Fe | 0.01 | 85 | 100 | 28 | 270 | 2400 | 2.6 |
| Mn | 0.01 | 47 | 22 | 4.4 | 65 | 370 | 0.18 |
| Cr | 0.01 | 7 | 1.9 | 1.7 | 1.5 | 4.9 | 0.3 |
| V | 0.01 | 12 | 1.1 | 0.91 | 0.37 | 1.7 | 0.68 |
| Cu | 0.01 | 45 | 9.6 | 1.7 | 20 | 95 | 0.39 |
| Ag | 0.01 | 6 | 5 | 3.6 | 4.1 | 13 | 2.1 |
| Sr | 0.01 | 69 | 18 | 7.1 | 40 | 350 | 0.82 |
| Cs | 0.10 | 15 | 11 | 5.9 | 11 | 36 | 0.17 |
| Tl | 0.06 | 65 | 18 | 18 | 4.5 | 30 | 11 |
| MeHg | 0.0001 | 86 | 0.09 | 0.06 | 0.1 | 0.57 | 0.01 |
| (b) Liver | | | | | | | |
| Element | DL | n > DL | Mean | Median | SD | Max | Min |
| Se | 0.05 | 12 | 39 | 34 | 22 | 91 | 12 |
| Zn | 0.01 | 14 | 190 | 170 | 81 | 340 | 58 |
| Pb | 0.02 | 13 | 8.6 | 5.8 | 8 | 25 | 2.1 |
| Co | 0.01 | 3 | 3.5 | 2.6 | 2.7 | 6.6 | 1.5 |
| Cd | 0.01 | 14 | 42 | 41 | 35 | 120 | 1.8 |
| Ni | 0.01 | 12 | 1.7 | 1.5 | 0.96 | 3.6 | 0.18 |
| Ba | 0.01 | 5 | 11 | 2 | 14 | 32 | 0.75 |
| Mn | 0.01 | 11 | 7.5 | 8.3 | 4 | 14 | 3.2 |
| Cr | 0.01 | 11 | 3 | 1 | 3.4 | 11 | 0.3 |
| V | 0.01 | 10 | 7.2 | 1.6 | 18 | 58 | 0.62 |
| Cu | 0.01 | 14 | 200 | 160 | 160 | 620 | 26 |
| Sr | 0.01 | 13 | 8.3 | 6 | 6.6 | 22 | 0.94 |
| Tl | 0.06 | 10 | 19 | 20 | 5.8 | 25 | 9.5 |
| MeHg | 0.0001 | 14 | 0.15 | 0.08 | 0.15 | 0.52 | 0.02 |

and -153 contributed the most to ΣPBDEs measured in liver, accounting on average for 22%, 27% and 23%, respectively. BDE-28, -47 and -209, which accounted for 28%, 30% and 44% of ΣPBDEs, respectively, predominated in the muscle of green turtles (Fig. 1). The small sample size did not permit the calculation of BDE congener ratios in the samples.

4. Discussion

4.1. Trace element and MeHg levels in green turtles in South China and other areas

Measured levels of trace elements in scute and liver tissues of green turtles in this study were compared with the best available information reported in previous studies (Tables 3 and 4 in Supplementary Material). Publications on trace element levels in scutes of sea turtles are limited. As shown in Table 3 in Supplementary Material, mean concentrations of Zn, Pb, Cd, Mn and Cu in green turtle scutes measured in this study were similar to those in green turtles from other parts of the Pacific Ocean, such as Japan and San Diego Bay in California, USA. Mean levels of Se, Ni, Fe and Ag were 30 times, 3 times, 10 times and 5 times higher in the present study, respectively, than those reported in other studies. On the other hand, mean levels of Sr in this study were half of those reported in green turtles in San Diego Bay. To the best of our knowledge, no published data about MeHg levels in scute tissues of

green turtles is available for other areas. In foraging green turtles in Baja California, Mexico, MeHg comprised 18–22% of total Hg in muscle tissues, and 9–19% of total Hg in liver tissues (Kampalath et al., 2006). Taking these values into account, a range of 9–22% MeHg as part of total Hg in tissues of green turtles, the total Hg levels of 0.5–1 mg/kg measured in loggerhead turtle scutes from South Carolina to Florida in the southeastern United States (Day et al., 2005) indicate estimated MeHg levels of 0.05–0.22 mg/kg; the average MeHg level of 0.09 mg/kg in scutes in the present study was within this range.

Regarding trace element levels in liver tissues of green turtles (Table 4 in Supplementary Material), mean levels of Se, Zn, Co, Ni, Mn, Cr, Cu and Cd in the present study were comparable to those reported in green turtles from other parts of the world, namely Japan, Australia, the Hawaiian Islands and Caribbean coasts of Mexico and Costa Rica. Mean concentrations of Cd in livers of green turtles in this study were almost 2-fold lower than those determined in turtles sampled in the industrialized port estuary of Gladstone, Australia (Gaus et al., 2012). Of particular concern are the approximately 10-fold higher mean levels of Pb, Ba, V and Tl observed in the liver tissues of green turtles in this study in relation to previous studies conducted over 10 years ago in Hong Kong (Lam et al., 2004) and Japan (Sakai et al., 2000a, 2000b; Anan et al., 2001). In addition, while liver Cd levels in the current study were similar to those reported in Japan and 2-fold lower than those reported for Australia, the levels in the present study were 40-fold greater than those detected by Lam et al. (2004), which measured trace element levels in liver tissues of 1 adult and 2 juvenile green turtles stranded in Hong Kong in 2001 and 2003. These differences in measured Cd levels may be due to different sample sizes and/or temporal increases in environmental Cd concentrations in the foraging grounds of green turtles in the China region (Zhang and Shan, 2008). The higher levels of Pb, Ba, V, Tl and Cd measured in the present study warrant further investigation of the potential risks of these elements to green turtles found in South China, which may in turn affect major source rookeries in Malaysia, Micronesia, Indonesia, Marshall Islands, Japan and Taiwan identified by mixed stock analysis of the genetic composition (Ng et al., 2017). Based on the previous report that 9–19% of total Hg level was MeHg in liver tissues of green turtles (Kampalath et al., 2006), the total Hg levels of 0.55 mg/kg measured in livers from green turtles from the Mediterranean Sea (Godley et al., 1999) indicate estimated MeHg levels of 0.05–0.10 mg/kg. The MeHg levels in liver in the present study were 6–750 times higher than the range of MeHg levels observed in green turtles in Baja California, Mexico and similar to those estimated in green turtles in the Mediterranean Sea, which is a historically industrialized area (Table 4 in Supplementary Material).

4.2. Risk assessment of trace elements and MeHg in green turtles

Pb, Hg and Cd can be extremely toxic and are considered endocrine disruptors that can affect endocrine function and reproductive success in animals (Grillitsch and Schiesari, 2010). The determination of HQs on a best-case and a worst-case scenario based on the measured levels of Pb, Cd and MeHg in green turtle liver in this study and PNECs estimated in bird and freshwater turtle liver (Franson, 1996; Furness, 1996; Kim et al., 1996; Yu et al., 2011) is presented in Table 5 in the Supplementary Material. With HQs ≥ 1, exposure to the measured Pb and Cd levels in green turtles likely poses high risk of toxic physiological effects and reproductive success to green turtles in the present study. With the HQ_{best} < 0.1 and HQ_{worst} < 1, exposure to the measured MeHg levels in green turtle livers likely poses low-to-medium risk to green turtles in terms of reproductive success in

Table 2

Levels of PBDEs (ng/g, lipid weight, wet weight and dry weight) in (a) liver (n = 13) and (b) muscle (n = 11) tissues of green turtles. n > DL represents the number of samples above the detection limits (DLs). DLs ranged from 0.010 to 0.26 ng/g lipid weight for BDE congeners.

| (a) Liver | | | Liver (ng/g, lipid weight) | | | | | | Liver (ng/g, wet weight) | | | | | Liver (ng/g, dry weight) | | | | |
|---------------|-------------------------|----------|----------------------------|--------|------|------|-------|-------|--------------------------|-------|-------|--------|-------|--------------------------|-------|-------|-------|--|
| PBDE Congener | DL (ng/g, lipid weight) | n > DL | Mean | Median | SD | Max | Min | Mean | Median | SD | Max | Min | Mean | Median | SD | Max | Min | |
| BDE28 | 0.02 | 6 | 9.0 | 7.4 | 7.7 | 22 | 1.4 | 0.49 | 0.51 | 0.40 | 1.0 | 0.050 | 1.7 | 2.0 | 1.2 | 3.0 | 0.25 | |
| BDE47 | 0.26 | 9 | 21 | 15 | 19 | 56 | 2.0 | 1.0 | 0.57 | 1.1 | 3.4 | 0.070 | 3.8 | 2.40 | 3.9 | 13 | 0.36 | |
| BDE100 | 0.02 | 10 | 26 | 23 | 15 | 49 | 0.67 | 1.3 | 1.1 | 1.1 | 3.6 | 0.080 | 5.0 | 5.00 | 3.2 | 9.0 | 0.24 | |
| BDE99 | 0.05 | 12 | 13 | 13 | 11 | 41 | 0.34 | 0.58 | 0.45 | 0.47 | 1.4 | <0.010 | 2.4 | 2.10 | 2.1 | 7.4 | 0.04 | |
| BDE154 | 0.05 | 9 | 9.1 | 8.2 | 6.9 | 23 | 1.6 | 0.44 | 0.27 | 0.39 | 1.1 | 0.050 | 1.8 | 1.90 | 1.4 | 4.1 | 0.20 | |
| BDE153 | 0.05 | 12 | 22 | 11 | 23 | 69 | 0.69 | 1.3 | 0.51 | 1.8 | 6.4 | 0.020 | 4.5 | 2.00 | 4.9 | 14 | 0.10 | |
| BDE183 | 0.05 | 12 | 6.6 | 2.6 | 7.7 | 22 | 0.18 | 0.48 | 0.14 | 0.96 | 3.4 | <0.010 | 1.5 | 0.50 | 2.2 | 7.7 | 0.02 | |
| BDE197 | 0.05 | 11 | 11 | 9.9 | 8.5 | 30 | 1.7 | 0.51 | 0.28 | 0.51 | 1.8 | 0.15 | 2.1 | 1.40 | 1.7 | 5.4 | 0.55 | |
| BDE196 | 0.05 | 10 | 2.4 | 1.9 | 2.1 | 6.2 | 0.22 | 0.18 | 0.05 | 0.34 | 1.1 | <0.010 | 0.57 | 0.28 | 0.79 | 2.5 | 0.03 | |
| BDE207 | 0.02 | 6 | 0.57 | 0.27 | 0.91 | 2.4 | 0.020 | 0.020 | 0.010 | 0.030 | 0.090 | <0.010 | 0.10 | 0.04 | 0.16 | 0.42 | 0.01 | |
| BDE206 | 0.01 | 6 | 0.16 | 0.12 | 0.17 | 0.41 | 0.010 | 0.010 | 0.010 | 0.010 | 0.020 | 0.010 | 0.050 | 0.050 | 0.010 | 0.060 | 0.040 | |
| BDE209 | 0.1 | 4 | 3.8 | 3.8 | 3.0 | 7.3 | 0.16 | 0.13 | 0.12 | 0.11 | 0.27 | 0.010 | 0.59 | 0.53 | 0.50 | 1.3 | 0.04 | |
| ΣPBDE | | | 96 | 88 | 75 | 230 | 2.3 | 5.0 | 3.5 | 5.9 | 21 | 0.060 | 19 | 14.00 | 17 | 48 | 0.31 | |
| | | adult | 35 | 13 | 41 | 82 | 10 | 1.1 | 0.22 | 1.5 | 2.7 | 0.18 | 4.3 | 1.60 | 4.8 | 9.9 | 1.6 | |
| | | immature | 110 | 120 | 75 | 230 | 2.3 | 6.2 | 4.1 | 6.3 | 21 | 0.060 | 23 | 17.00 | 17 | 48 | 0.31 | |

| (b) Muscle | | | Muscle (ng/g, lipid weight) | | | | | Muscle (ng/g, wet weight) | | | | | Muscle (ng/g, dry weight) | | | | |
|---------------|-------------------------|----------|-----------------------------|--------|------|-----|-------|---------------------------|--------|-------|-------|--------|---------------------------|--------|-------|------|--------|
| PBDE Congener | DL (ng/g, lipid weight) | n > DL | Mean | Median | SD | Max | Min | Mean | Median | SD | Max | Min | Mean | Median | SD | Max | Min |
| BDE28 | 0.02 | 10 | 45 | 38 | 28 | 99 | 9.2 | 0.54 | 0.37 | 0.50 | 1.7 | 0.080 | 2.4 | 1.8 | 1.7 | 5.3 | 0.70 |
| BDE47 | 0.26 | 10 | 48 | 48 | 21 | 76 | 12 | 0.67 | 0.44 | 0.80 | 2.8 | 0.14 | 2.9 | 2.3 | 2.4 | 8.9 | 0.68 |
| BDE100 | 0.02 | 11 | 12 | 9.1 | 10 | 38 | 1.4 | 0.37 | 0.070 | 0.96 | 3.3 | 0.010 | 1.3 | 0.42 | 3.0 | 10 | 0.050 |
| BDE99 | 0.05 | 11 | 11 | 7.6 | 9.8 | 34 | 1.8 | 0.23 | 0.070 | 0.51 | 1.8 | 0.010 | 0.89 | 0.28 | 1.6 | 5.6 | 0.090 |
| BDE154 | 0.05 | 7 | 5.4 | 2.8 | 6.4 | 15 | 0.15 | 0.20 | 0.020 | 0.45 | 1.2 | <0.010 | 0.68 | 0.16 | 1.4 | 3.9 | <0.010 |
| BDE153 | 0.05 | 10 | 6.6 | 4.3 | 6.8 | 17 | 0.060 | 0.15 | 0.030 | 0.37 | 1.3 | <0.010 | 0.58 | 0.23 | 1.2 | 4.0 | <0.010 |
| BDE183 | 0.05 | 8 | 3.7 | 2.9 | 4.0 | 11 | 0.050 | 0.10 | 0.020 | 0.24 | 0.69 | <0.010 | 0.37 | 0.12 | 0.75 | 2.2 | <0.010 |
| BDE197 | 0.05 | 7 | 9.7 | 8.3 | 7.2 | 23 | 0.58 | 0.17 | 0.10 | 0.25 | 0.72 | <0.010 | 0.63 | 0.47 | 0.76 | 2.3 | 0.020 |
| BDE196 | 0.05 | 9 | 2.1 | 2.7 | 1.5 | 4.4 | 0.070 | 0.040 | 0.010 | 0.080 | 0.24 | <0.010 | 0.15 | 0.080 | 0.23 | 0.76 | <0.010 |
| BDE207 | 0.02 | 7 | 1.2 | 0.59 | 1.3 | 3.8 | 0.050 | 0.010 | <0.010 | 0.020 | 0.050 | <0.010 | 0.050 | 0.020 | 0.070 | 0.21 | <0.010 |
| BDE206 | 0.01 | 7 | 1.3 | 1.1 | 0.85 | 2.3 | 0.33 | 0.010 | 0.010 | 0.010 | 0.020 | <0.010 | 0.050 | 0.040 | 0.040 | 0.10 | <0.010 |
| BDE209 | 0.1 | 7 | 70 | 33 | 100 | 290 | 7.1 | 0.71 | 0.100 | 1.4 | 3.9 | 0.040 | 3.4 | 1.2 | 5.7 | 16 | 0.36 |
| ΣPBDE | | | 160 | 160 | 110 | 450 | 34 | 2.4 | 1.0 | 3.9 | 14 | 0.27 | 10 | 5.5 | 12 | 43 | 2.2 |
| | | adult | 120 | 120 | 45 | 160 | 69 | 4.9 | 0.66 | 7.6 | 14 | 0.43 | 18 | 6.0 | 22 | 43 | 4.7 |
| | | immature | 170 | 160 | 120 | 450 | 34 | 1.6 | 1.1 | 1.8 | 6.0 | 0.27 | 7.5 | 5.4 | 7.1 | 25 | 2.2 |

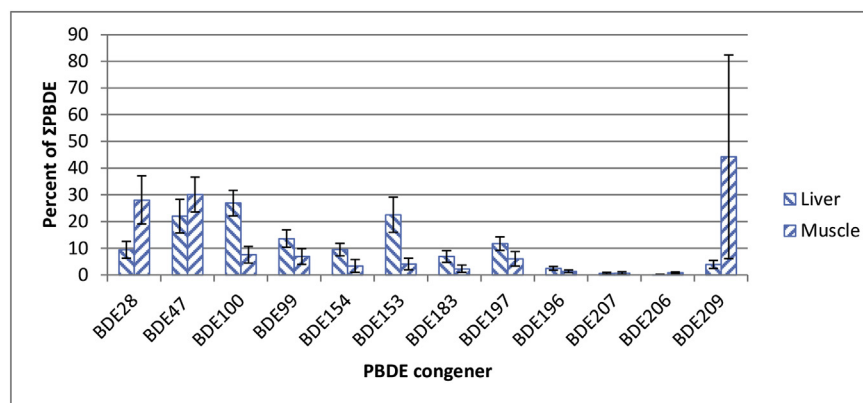


Fig. 1. Percent of ΣPBDEs comprised by each PBDE congener (ng/g, lipid weight; mean ± SE).

the present study. It should be noted that the application of PNEC values derived from birds and a freshwater turtle to assess risk of exposure in green turtles contains large uncertainties due to a number of factors, particularly intra- and inter-specific variability in sensitivity to pollutants, including metabolic capabilities, and the potential effects of chronic contaminant exposure in a long-lived species like the green turtle.

4.3. Tissue-specific distribution of trace elements in green turtles

Day et al. (2005) found that scutes were the most accurate predictor of total Hg in liver of loggerhead turtles in South Carolina, USA. Sakai et al. (2000b) also suggested that the carapace was a useful non-lethal indicator for monitoring heavy metal levels in sea turtle tissues based on correlations among Mn, Zn and Hg levels in

the carapace and internal organs of green turtles in Japan. MeHg levels in scutes varied positively but insignificantly with those in liver of green turtles in the present study. Moreover, the significant positive regression of Cu concentrations in scutes with those in liver observed in this study ($R^2 = 0.761$, $p < 0.05$) indicates that scutes may be useful in predicting Cu concentration in liver.

4.4. PBDEs in green turtles in South China and other areas

There is little published information available about tissue levels of PBDEs in green turtles; to our knowledge, this is the first study to establish baseline PBDE levels in green turtles in South China. Total PBDE mean concentrations (lipid weight) in muscle and liver of green turtles from South China in this study (Table 6 in Supplementary Material) were about 27-fold and 50-fold greater than those measured in Australia (Hermanussen et al., 2008; Van de Merwe et al., 2010) and Japan (Malarvannan et al., 2011), where PBDE inputs into the study sites were suggested by the respective authors to be low. These spatial patterns in PBDE concentrations in green turtle tissues are comparable with those reported in cetaceans collected from Asian waters (Kajiwara et al., 2006), where the highest concentrations of PBDEs were found in animals from Hong Kong, followed by Japan, and much lower levels in animals from the Philippines and India. The lack of significant differences in PBDE levels between immature and adult turtles in this study was also observed in previous studies that have reported inconsistent relationships between body size/age class and organic contaminant concentrations in sea turtles (e.g. Keller et al., 2005; Lazar et al., 2011; Malarvannan et al., 2011; Camacho et al., 2012). The high PBDE levels detected in liver tissues of green turtles in this study arouse concern and require further investigation of their potential toxicity to green turtles in South China as well as the major source rookeries; no PBDE toxicity threshold values have been determined for sea turtles.

Fifty percent of the global production of PBDEs was used in Asia according to a 2001 survey (BSEF, 2004). Qiu et al. (2010) commented that PBDE levels (0.07–4.85 ng/g dry weight) in surface sediment in Deep Bay in the northwestern part of Hong Kong were generally in the low-to-intermediate range compared with the values reported for other places. Total PBDE concentrations in sediment cores from the Pearl River Estuary in South China ranged from 0.11 to 13.03 ng/g (Zheng et al., 2004). PBDE congener levels in the sediment cores generally increased from 1948 to 2003, with the highest levels in top sediment suggesting ongoing PBDE input (Qiu et al., 2010). In contrast, considerably lower PBDE levels have been measured in Queensland, Australia sediments (124 pg/g dry weight) (Toms et al., 2008), where Hermanussen et al. (2008) found relatively lower PBDE levels in foraging green turtles (Table 6 in Supplementary Material). These results indicate that the more polluted marine environment coupled with the increasing industrialization and urbanization in South China potentially poses higher risks to the health of fauna including green turtles that usually inhabit nearshore, when compared with other regions.

4.5. PBDE congener profiles

PBDE congener profiles in biological matrices can reflect geographical variation in PBDE exposure in relation to PBDE sources, animal movement and diet. Malarvannan et al. (2011) suggested that spatial variability in PBDE concentrations in hawksbill turtles from Japan may reflect various potential sources and exposure routes for PBDEs in the coastal environment, including incidental ingestion of plastics to which the compounds including flame retardants might have adsorbed. Similarly, Bachman et al. (2014) reported that differences in another flame retardants, PCB

congener profiles among cetacean species were likely attributable to the location of their foraging grounds and the age class of the sampled individuals.

The typical pattern of predominance of BDE-28, -47, -49, -99, -100, -153, -154 among all PBDE congeners has been observed in marine biota globally, including fish, crustaceans and mammals (Hites, 2004), as well as sea turtles (Keller et al., 2005; Hermanussen et al., 2008; Swarthout et al., 2010; Malarvannan et al., 2011; Ragland et al., 2011; Stewart et al., 2011). The same typical pattern of PBDE congener predominance was observed in green turtles in the present study. Moreover, BDE-47, -100 and -153 dominated in Σ PBDEs measured in the liver of green turtles in this study as more lipophilic PBDE congeners with relatively high log K_{ow} values (i.e. BDE-47, -100 and -153) (Braekevelt et al., 2003) and high biota-sediment accumulation factors (BSAFs; Xiang et al., 2007; Qiu et al., 2010) preferentially accumulate in more lipid-rich liver tissues. BDE-47, -99 and -100 were also the most dominant congeners observed in the blubber of Indo-Pacific humpback dolphins and finless porpoises in South China (Lam et al., 2009; Zhu et al., 2014). The PBDE pattern dominated by BDE-154 and -100 in the blood plasma of transient loggerhead turtles in Florida, USA was likely due to incidental ingestion of sediment containing high levels of BDE-209, which was then de-brominated to BDE-154 during biotransformation and detected in blood plasma (Ragland et al., 2011).

Concentrations of BDE-209 are continuing to increase worldwide (Law et al., 2014) and its production and use has not yet been restricted under the Stockholm Convention, though it has been proposed for listing. BDE-209 was the major congener detected in sediment of the Pearl River Estuary and adjacent South China Sea at concentrations comparable to the highest concentrations reported in Japan, Sweden and the United Kingdom (Mai et al., 2005). The major sources of PBDEs in southern China are related to the growth of electronics manufacturing and probably from waste discharges from the cities of Guangzhou, Dongguan, and Shenzhen, the three fastest-growing urban centers in the Pearl River Delta (Mai et al., 2005; Xiang et al., 2007) and potential contamination from e-waste recycling and disposal sites (Law et al., 2014). The same predominance of BDE-209 was also reported in marine biota samples, including fish, shrimp and ducks, in the Pearl River Estuary (Xiang et al., 2007) and waterbird eggs from South China (Lam et al., 2007). Although very low BSAFs were reported for BDE-209 in the Pearl River Estuary (Xiang et al., 2007), a possible reason for the exceptionally high percentage of BDE-209 (with considerably high standard error in Fig. 1) as part of Σ PBDEs measured in muscle of a juvenile green turtle in the present study was high BDE-209 concentrations in southern China sediments and marine biota (Xiang et al., 2007) which are possible dietary items of juvenile green turtles (Morais et al., 2014; Ng, 2015; Ng et al., 2016). BDE-209 was detected in the blubber samples of Indo-Pacific humpback dolphins and finless porpoises in South China from 2003 to 2012 (Zhu et al., 2014), but was not found in specimens collected in the same region in earlier periods from 1995 to 2001 (Ramu et al., 2005).

5. Conclusion

This study reports the first baseline levels of PBDEs and MeHg in green turtles in South China. It also provides updated information on levels of 17 trace elements in green turtle tissues using a larger sample size over a broader geographical range. Relative to the previous study conducted over 10 years ago in the same region, the 10-fold higher levels of the toxic elements Pb, Ba, V and Tl, 40-fold greater Cd level and 27- and 50-fold higher PBDE levels measured in liver tissues of green turtles when compared with findings in Australia and Japan arouse concern and require further

investigation of their potential toxicity to foraging green turtles in South China and their source rookeries in Malaysia, Micronesia, Indonesia, Marshall Islands, Japan and Taiwan. Research should target pollutant monitoring of sea turtles within the West Pacific/Southeast Asia regional management unit which spans from East Asia to Southeast Asia based on biogeographical data (Wallace et al., 2011) to fill in knowledge gaps, in particular in Thailand, Vietnam, Indonesia, Malaysia and the Philippines where less or no data is available and where foraging grounds have been identified. A regional tissue bank for sea turtles (Kucklick et al., 2010) should be developed based on standardized sampling protocols to assess pollutant levels at broader temporal and spatial scales as well as potential threats to the health of sea turtle populations. Future research should focus on determination of threshold concentrations of trace elements and POPs that can cause undesirable chronic biological effects in sea turtles to enable more specific derivation of PNECs for ecological risk assessment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2017.11.100>.

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