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SHORT RESEARCH AND DISCUSSION ARTICLE

First report of heavy metal presence in muscular tissue of loggerhead turtles *Caretta caretta* (Linnaeus, 1758) from the Balearic Sea (Balearic Islands, Spain)

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Abstract

The concentrations of cadmium (Cd), mercury (Hg) and lead (Pb) were determined in muscular tissue of eleven loggerhead turtles (*Caretta caretta*) from the Balearic Islands (Spain, Western Mediterranean). The metal levels found in the present study were similar or lower than concentrations detected in Andalusia (mainland Spain), Italy, Canary Islands (Spain) or Japan. As the main source of metals in the loggerhead turtle is the diet, low metal burdens could be explained by its opportunistic feeding way. No significant differences were found in metal concentrations between juveniles and subadults in any of the heavy metals analysed. Furthermore, no significant correlation was detected between heavy metal concentrations and straight carapace length (SCL) of the studied individuals. These results could derive from the homogeneity in age and size of the turtles sampled, so further studies including adults are needed in order to assess the heavy metal accumulation with turtle growth.

Keywords *Caretta caretta* · Heavy metals · Cadmium · Mercury · Lead · Western Mediterranean

Three species of marine turtle are known to occur regularly in the Mediterranean Sea. These species are the loggerhead turtle (*Caretta caretta*, L.), the green turtle (*Chelonia mydas* L.) and the leatherback turtle (*Dermochelys coriacea*; Vandelli, 1761) (Groombridge 1990). Among them, *C. caretta* is the most common species in the Mediterranean (Franzellitti et al. 2004). However, loggerhead turtles inhabiting this basin are threatened as a result of human activity being bycatch, vessel strikes and environmental pollution (including debris and chemical pollution) the most common threats (Lutcavage 1997; Bolten et al. 2011; Pagano et al. 2019).

Since the Industrialization began, large quantities of a wide variety of chemicals have been released into the environment, modifying the natural amount of these compounds (Haynes and Johnson 2000; Guzzetti et al. 2018; Prokić et al. 2019; Strungaru et al. 2019). Xenobiotics and also microplastics have been found in several marine species from different areas (Faggio et al. 2018; Savoca et al. 2019a, b). Among all these compounds, heavy metals are of great relevance because of their toxic potential to living organisms, their high persistence in the environment and the potential to accumulate in long living species (Clark 1992; Caurant et al. 1999; Storelli et al. 2005). Nevertheless, data concerning heavy metal determination and quantification in tissues of *C. caretta* are insufficient and the effects of the continuous exposure to these contaminants on marine turtles are still unknown (Storelli et al. 1998a, 1998b; Godley et al. 1999; Storelli and Marcotrigiano 2003;

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Torrent et al. 2004). Therefore, more information is needed in order to assess the possible harmful effects of these toxic chemicals and to develop efficient manage measures for the protection and conservation of marine turtles.

In this context, the aim of this study was to determine the concentrations of cadmium (Cd), mercury (Hg) and lead (Pb) in muscular tissue of eleven specimens of *C. caretta* and to compare the data with those reported from other marine areas. Furthermore, possible growth-related variations in heavy metal concentration were discussed.

Skeletal muscle samples were collected from the right flipper of eleven loggerhead turtles found in 2017 along Balearic Islands coastline (Western Mediterranean, Spain) for Cd, Hg and Pb analyses. Nine specimens were found dead and two of them died during the recovery period in the Rehabilitation Centre (Palma Aquarium Foundation). For this reason, muscle was the only collected tissue, since other tissues such as kidney and liver were in poor state of conservation. In terms of heavy metals, Cd, Hg and Pb were the only metals analysed because of the wide availability of similar published studies. The aim was to compare the results obtained in the present work with other similar studies analysing these three metals in muscular tissue of *C. caretta* individuals in many areas of the world. Causes of stranding were ingestion of hooks in three individuals, entanglement in two cases and undetermined causes in the remaining turtles. Since a validated method for age determination in marine turtles is not yet available (Bjorndal et al. 1998), body length of the specimens was used as an indicator of age like in previous studies (Bjorndal et al. 2000; Sakai et al. 2000a; Franzelliti et al., 2004; García-Fernández et al. 2009). Specimens were classified into three age classes determined by straight carapace length (SCL): pelagic juveniles (SCL < 42 cm), subadults (42 cm ≤ SCL ≤ 70 cm) and adults (SCL > 70 cm) according to Bjorndal et al. (2000), Seminoff et al. (2004) and Casale et al. (2005). Mean ± standard deviation (S.D.) of SCL was 43.95 ± 14.46 cm (21.5–65.00 cm) ($n = 10$). Four specimens were identified as juveniles and six as subadults, while SCL of the remaining turtle could not be recorded. Body length was used to assess a potential relationship between growth and heavy metal concentrations in the analysed individuals. Sex could be determined in only four individuals, obtaining one juvenile male, one subadult male and two subadult females. Sex of the remaining turtles could not be obtained because of the poor condition of the specimens.

The method used in the present work for metal analysis followed the methodology carried out in previous studies (Costas et al. 2011; Arechavala-Lopez et al. 2019). Muscle samples for heavy metal determinations were frozen at − 20 °C until chemical analysis. For the preparation of samples, aliquots of 0.4 g of wet muscular tissue of each turtle were dried for 3 days at 60 °C until a constant weight was obtained, following the procedures indicated by the technical specialists in this methodology of the scientific-technical services of the University. Dry tissues were digested with 8 ml of 65% nitric acid (HNO_3) and 2 ml hydrogen peroxide (H_2O_2). Samples were kept covered with an opaque material to avoid the penetration of light during this process. After digestion the samples were filtered with 0.45-μm filters to avoid impurities and then, analysed for Cd, Hg and Pb. Quantitative determinations of heavy metals were made by inductively coupled plasma—mass spectrometry (ICP-MS) (Agilent technologies model 7700x, Santa Clara, CA, USA), using scandium, germanium, rhodium and rhenium as internal standards at 500 ppb. The sample introduction system used with this instrument was the high-temperature torch-integrated sample introduction system (hTISIS) and a high-efficiency nebulizer (HEN, Meinhard Glass Products, Golden, CO, USA). Calibration standards at different concentrations (0, 0.5, 1, 5, 10, 50, 100 and 500 ppb) were elaborated using certified reference material (Multielement Std, CP33MS, SCP Science, Canada). Each injection was read three times, and the calibration curve had at least $r^2 > 0.995$. Blanks with Millipore water and blank spikes were also performed along with all samples for quality assurance. The limit of detection was considered as 3 times the standard deviation of the blanks and was less than 0.001 ppb for the analysed elements. The chemical blanks for the experimental procedure were fewer than 2% of the sample signal and were used to correct the sample results. For quality control, Certified Reference Material (CRM) DORM 4 Fish protein from the National Research Council of Canada (NRCC) was used.

The statistical analysis was performed using R Studio v. 1.1.453. Mean ± S.D. range of heavy metal concentrations were calculated in microgram per gram on a wet weight basis. The non-parametric Wilcoxon–Mann–Whitney test was used in order to detect significant differences in heavy metal concentration between age classes. Finally, correlation among SCL of specimens and heavy metal concentration was analysed with Spearman correlation coefficient. Differences were considered statistically significant at $p < 0.05$.

Cd, Hg and Pb concentrations in wet weight from muscular tissue of *C. caretta* specimens are reported in Table 1. Metal concentrations in muscular tissue of turtles from different locations are also presented in Table 1. In general, metal concentrations were quite similar to those found in other regions, both the Mediterranean Sea and other areas (Table 1).

Cd concentrations in muscle were very similar to those reported in Andalusia (Spain, Western Mediterranean; García-Fernández et al. 2009), Italy (Adriatic and Ionian Sea, Eastern Mediterranean; Storelli et al. 2005), France (Atlantic coast; Caurant et al. 1999) and Japan (Sakai et al. 1995, 2000b). However, Cd concentrations showed lower values than those reported from other studies carried out in Italy (Adriatic Sea; Storelli et al. 1998a; Franzellitti et al. 2004), Cyprus (Eastern Mediterranean; Godley et al. 1999)

Table 1 Cadmium, mercury and lead concentration in muscular tissue of specimens of *C. caretta* from different locations. Values are reported as microgram per gram of wet weight and are expressed as mean ± S.D. (range), except values indicated with asterisk, which are expressed as median (range). Range of SCL (cm) or range of weight (kg) and age classes (if possible) are reported for each study (J: juveniles, S: subadults, A: adults). Only in one case body size range is indicated as curved carapace length (CCL). Finally, the area of each study is reported

Cadmium (Cd)	Mercury (Hg)	Lead (Pb)	Range SCL (cm)/weight (kg)	Area	References
0,053 ± 0,049 (0.012–0.188)	0.078 ± 0.064 (0.033–0.262)	0.020 ± 0.014 (0.008–0.046)	21.5–65.0 cm (J, S)	Balearic Is. (Spain)	Present study (*n* = 11)
0.140 ± 0.160 (0.023–0.550)	-	0.130 (N. D.–0.180)	1.8–100.0 kg (J, A)	Adriatic Sea (Italy)	Storelli et al. (1998a) (*n* = 12)
-	0.210 (0.070–0.430)	-	6.7–18.0 kg	Italy	Storelli et al. (1998b)
0.360 ± 0.110	-	-	24.5–74.0 cm (J, S, A)	Italy	Franzellitti et al. (2004) (*n* = 17)
0.070 ± 0.030 (N. D.–0.130)	-	0.040 ± 0.030 (N. D. –0.090)	21–71 cm (J, S, A)	Italy	Storelli et al. (2005) (*n* = 19)
0.120* (0.060–0.300)	0.100* (N. D.–0.370)	0.520* (N. D.–1.160)	56–79 cm (CCL) (S, A)	Cyprus	Godley et al. (1999)
0.040 ± 0.030 (0.004–0.100)	-	1.010 ± 0.390 (0.410–1.880)	17–65 cm (J, S)	Andalusia (Spain)	García-Fernández et al. (2009) (*n* = 20)
1.140 ± 0.280 (0.150–12.480)	-	2.260 ± 0.510 (0.220–21.070)	15–65 cm (J, S)	Canary Is. (Spain)	Torrent et al. (2004) (*n* = 78)
0.080 ± 0.050 (0.004–0.180)	-	-	21.3–34.5 cm (J, S)	France (Atlantic coast)	Caurant et al. (1999) (*n* = 21)
0.062 ± 0.026 (0.041–0.117)	0.108 (0.053–0.190)	-	76–92 cm (A)	Japan	Sakai et al. (1995) (*n* = 7)
0.064 ± 0.030	0.094	0.020 ± 0.030	F: 83 ± 6 cm M: 85 cm (A)	Japan	Sakai et al. (2000b) (*n* = 6)

N. D. not determined

and Canary Islands (Spain, Atlantic Ocean; Torrent et al. 2004) (Table 1). According to some authors, Cd tends to accumulate in marine vertebrates with age (Stewart et al. 1994; Dietz et al. 1996; Caurant et al. 1999) and the main source of Cd for marine turtles is food intake (Caurant et al. 1999; Maffucci et al. 2005; Storelli et al. 2005). *C. caretta* is a generalist predator (Tomas et al. 2001) that feeds mainly on low trophic organisms as molluscs and crustaceans, although it also feeds on jellyfish and sponges (Sakai et al. 2000b; Torrent et al. 2004). Cephalopods and jellyfish are well known as Cd accumulators and important vectors of this element to top marine predators (Martin and Flegal 1975; Bustamante et al. 1998; Caurant et al. 1999), while crustaceans and filtering benthic molluscs accumulate lower concentrations of metals since they occupy low trophic levels (Sakai et al. 2000b; Torre et al. 2013; Pagano et al. 2017; Capillo et al. 2018). This opportunistic feeding way could explain lower Cd concentrations in *C. caretta* in comparison with carnivorous species such as *D. coriacea*, and higher concentrations with respect to *C. mydas*, a largely herbivorous species (Caurant et al. 1999)

Hg levels detected in Italy (Adriatic Sea; Storelli et al. 1998b), Cyprus (Godley et al. 1999) and Japan (Sakai et al. 1995, 2000b) were slightly higher than those reported in the present study (Table 1). Hg is known to biomagnify in high trophic levels (Honda et al. 1987; Gray 2002). However, the biomagnification of this metal in *C. caretta* is relatively low since its preys occupy low trophic levels and, therefore, are low exposed to Hg (Sakai et al. 2000b; Maffucci et al. 2005; Storelli et al. 2005). Moreover, marine turtles do not bioaccumulate Hg to such greater levels as other marine vertebrates with a long-life span (Caurant et al. 1994; Maffucci et al. 2005; Storelli et al. 2005). It could be mainly attributed to the nature of their diet (Sakai et al. 2000b; Maffucci et al. 2005; Storelli et al. 2005), as Hg uptake by marine turtles is mainly through food intake (Storelli et al. 2005) and it is known that their preys occupy low trophic levels.

Pb concentrations in the present study were similar to those reported in Italy (Adriatic and Ionian Sea; Storelli et al. 2005) and Japan (Sakai et al. 2000b). However, levels detected in 1998 in Italy (Adriatic Sea; Storelli et al. 1998a), Cyprus (Godley et al. 1999), Andalusia (García-Fernández et al. 2009) and Canary Islands (Torrent et al. 2004) were slightly higher than those described in the current work (Table 1). Storelli et al. (2005) reported a reduction in Pb concentrations in *C. caretta* from the eastern Mediterranean with respect to a previous study carried out in the same area 10 years earlier (Storelli et al. 1998a). According to these authors, this fact could be attributed to the regulation of the consumption of leaded petrol in many European countries since the 1970s, leading to a Pb decrease in the Mediterranean Sea as a consequence of this policy (Nicolas et al. 1994). In the present study, Pb concentrations in muscle were quite like those

Table 2 Heavy metal concentration in muscular tissue of juvenile and subadult specimens of *C. caretta* from the present study. Values are reported as microgram per gram of wet weight and are expressed as mean ± S.D. (range)

	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)
Juveniles (n = 4)	0.068 ± 0.081 (0.018–0.188)	0.074 ± 0.016 (0.064–0.098)	0.013 ± 004 (0.008–0.018)
Subadults (n = 6)	0.042 ± 0.026 (0.012–0.087)	0.088 ± 0.086 (0.041–0.262)	0.026 ± 0.017 (0.008–0.046)
Total (n = 11)	0.053 ± 0.049 (0.012–0.188)	0.078 ± 0.064 (0.033—0.262)	0.020 ± 0.014 (0.008–0.046)

reported by Storelli et al. (2005), but the lack of previous data does not allow to know the trend that Pb has followed in the Balearic waters.

Cd, Hg and Pb concentrations in muscular tissue of juvenile and subadult specimens of *C. caretta* from the Balearic waters are shown in Table 2. Mean values of Cd were higher in juveniles, but mean values of Hg and Pb were higher in subadults (Table 2). However, no significant differences were observed between age classes in any of the heavy metals analysed (Wilcoxon–Mann–Whitney U test; $W = 11$, $p > 0.05$ for Cd; $W = 20$, $p > 0.05$ for Hg; $W = 8.5$, $p > 0.05$ for Pb). Although metals such as Cd are expected to accumulate with age (Stewart et al. 1994; Dietz et al. 1996; Caurant et al. 1999; García-Fernández et al. 2009), no significant correlation was found between heavy metal concentrations and SCL of the individuals analysed in the present work (Spearman, $r_s = -0.04$, $p > 0.05$ for Cd; Spearman, $r_s = -0.58$, $p > 0{,}05$ for Hg; Spearman, $r_s = 0.13$, $p > 0.05$ for Pb). Storelli et al. (1998a) detected a positive correlation among Cd concentration and specimen weight, which could be attributed to the inclusion of large (and therefore, old) individuals in their study (Storelli et al. 2005). However, our data are in agreement with many other studies in which no correlation among metal levels and body size was found in tissues such as muscle (Franzellitti et al 2004; Maffucci et al. 2005; García-Fernández et al. 2009). This could be due to the homogeneity in age and size of the samples used in these studies (Torrent et al. 2004; García-Fernández et al. 2009), since all the individuals analysed were juveniles or subadults with similar feeding habits (Torrent et al. 2004) as in the present work.

According to Storelli et al. (2005), two reasons could explain the variability in metal concentration in *C. caretta* from different geographical areas. One of them would be the environmental pollution specific to each zone (Godley et al. 1999; Storelli et al. 2005), which influences the metal burden of organisms in their foraging range (Maffucci et al. 2005; Storelli et al. 2005). Secondly, the age of the specimens would also influence the levels of heavy metals detected (Caurant et al. 1999; Godley et al. 1999; Storelli et al. 2005), since metal oscillations with age are affected by various factors (Franzellitti et al. 2004). The accumulation of heavy metals with turtle growth (Sakai et al. 2000a; Franzellitti et al. 2004) as well as the change in habitat utilization and feeding behaviour between juveniles and adults seem factors which could modify the exposure of marine turtles to heavy metals (Sakai et al. 2000a; Franzellitti et al. 2004), since young animals differ from old ones in their feeding habits (Franzellitti et al. 2004; Torrent et al. 2004; Maffucci et al. 2005). Finally, other biological and ecological factors such as egg deposition (Godley et al. 1999), increased hormonal activity (Storelli et al. 1998a) and cause of death or sex (Franzellitti et al. 2004) could play an important role in heavy metal accumulation. All these factors could explain the differences observed between the Balearic Islands and other geographical areas.

The results obtained evidence the presence of heavy metals in juvenile and subadult loggerhead turtles from the Balearic Sea for the first time. However, heavy metal concentrations found in the present work do not seem to be high enough to be harmful to individuals, according to previous studies (Storelli and Marcotrigiano 2003; Franzellitti et al. 2004). Furthermore, the lack of information about the age of individuals and the cause of stranding and the small population sample difficult the interpretation of the results (Caurant et al. 1999; Storelli and Marcotrigiano 2003; García-Fernández et al. 2009). Multiple factors such as feeding ecology, behaviour, metabolism or susceptibility to pathogens may influence contaminant burden of marine turtles. For this reason, obtained values should be carefully interpreted, since the results could be biased by some of the already mentioned factors. Further studies including a larger population sample and a significant representation of adults are needed (Caurant et al. 1999; Storelli and Marcotrigiano 2003; García-Fernández et al. 2009), as well as more information about the stranding cause of all the analysed individuals. Furthermore, both including the analysis of other tissues such as liver or kidney and the determination of other metals in the study would help to assess heavy metal contamination in marine turtle populations and, hence, in marine food webs. Hence, it is recommended to monitor the heavy metal concentrations in those stranded marine turtles that arrive yearly to the coast. Obtaining all this information of marine turtle corpses would contribute to improve the tools for the management and conservation of their populations (Caurant et al. 1999; Storelli and Marcotrigiano 2003).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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