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Heavy metals in tissues from loggerhead turtles (*Caretta caretta*) from the southwestern Mediterranean (Spain)☆

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ABSTRACT

Cadmium, lead, copper, and zinc were measured in tissues of 21 loggerhead turtles (*Caretta caretta*) from the southwestern Mediterranean coastline. Mean concentrations (dry weight) of essential elements (Zn and Cu) were 107 and 21.6 μg/g in liver, 27.9 and 3.8 μg/g in kidney, 65.4 and 5.0 μg/g in pectoral muscle, 11.1 and 3.45 μg/g in brain, and finally 19.2 μg/g and undetected in bone, respectively. Mean concentrations of heavy metals (Cd and Pb) were 23.4 and 2.8 μg/g in liver, 31.5 and 0.5 μg/g in kidney, 0.2 and 0.3 μg/g in pectoral muscle, 0.2 and 0.7 μg/g in brain, and undetected and 1.2 μg/g in bone, respectively. Metal concentrations were similar to other studies conducted on Mediterranean turtles. However, cadmium concentrations varied widely among individuals, which has been associated with potential sources of cadmium in Mediterranean Sea. This is the first study into metal accumulation in tissues of loggerhead turtle from Spanish Mediterranean coastline.

1. Introduction

The study of the accumulation of metals in marine fauna is of great interest due to the potentially toxic effects these elements can cause, especially in predators (Cardenallicchio et al., 2000; Das et al., 2003). Such studies allow us to estimate the type and degree of exposure to which these species are subjected and moreover, estimate the potential risks for their conservation or survival (Das et al., 2003). The toxic effects of metals on reproductive, immune and nervous systems, behavior, and carcinogenesis have not only been described in terrestrial species but also in a range of marine species (De Guise et al., 2003; Franson, 1996; Law, 1996; Peakall, 1996; Reijnders, 2003). Sublethal exposure to certain heavy metals during the developing stages can alter gonadal steroid hormones, as well as adrenal and thyroid hormones (Colborn, 2002; Dawson, 2000), which could be the cause of potentially serious effects on the survival of certain particularly sensitive or vulnerable populations. It is not surprising therefore that similar effects are induced in marine turtles. Finally, environmental contaminants have been described as one of the factors which contribute to the development of viral infections causing fibropapilloma in marine turtles as a result of reduced immune capacity (Balazs and Pooley, 1991; Herbst and Klein, 1995), above all in coastal areas with intense human activity and low water-renewal rates (Limpus and Miller, 1994).

The loggerhead (*Caretta caretta*) is the most abundant marine turtle species in the Mediterranean Sea and in other parts of the world (Day et al., 2005). Young turtles migrate from the North Atlantic to Gibraltar, and from here, taken by the currents, they enter the Mediterranean Sea where they mature and reproduce (Witham, 1980; Groombridge, 1990). The Mediterranean, being a closed sea surrounded by highly industrialized countries, constitutes a high-risk marine environment due to contamination by toxic compounds (Bacci, 1989; Borrell et al., 1997; Kuetting, 1994; Meadows, 1992). According to Groombridge (1990), the populational status of all nesting species has been classified as poor to very poor, with the loggerhead turtle being classified as "endangered" (IUCN, 2001). Some authors maintain that it is the increased contamination of marine environments, together with the loss of ideal nesting sites that are the main threats to the survival of sea turtles worldwide (Storelli et al., 2005). Several authors suggest the main source of metal deposits in the loggerhead turtle is via their diet (Anan et al., 2001; Caurant et al., 1999) there being interspecific differences in the contaminant load (Caurant et al., 1999; Godley et al., 1999; Sakai et al., 2000b). According to Storelli et al. (2005), when large differences exist in the concentration of metals in members of one particular species, it would then be the geographical location of the feeding and

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overwintering grounds of each population and the size and age of the animals which would explain such differences.

A range of studies have been carried out on metals in the loggerhead turtle in different parts of the world, such as Australia (Gordon et al., 1998), Florida (Homer et al., 2000), Hawaii (Aguirre et al., 1994), Japan (Saeki et al., 2000; Sakai et al., 2000b), Mexico (Gardner et al., 2006), the UK (Godley et al., 1998), and the Central and Eastern Mediterranean (Franzellitti et al., 2004; Godley et al., 1999; Kaska et al., 2004; Maffucci et al., 2005; Storelli and Marcotrigiano, 2003; Storelli et al., 1998, 2005). In Spain, only one published study is available on marine loggerhead turtles from the Atlantic waters of the Canary Islands (Torrent et al., 2004). Therefore, there are currently no available studies regarding marine turtles from the southwestern Mediterranean Sea. In the present study, the primary objective was to evaluate the accumulation of two essential elements (zinc and copper) and non-essential heavy metals (cadmium and lead) in tissues from loggerhead turtles from the southwestern Mediterranean Sea and to compare the data with those reported from other locations. Secondly, cadmium and lead concentrations and correlations between all elements were discussed in order to investigate the risk these contaminants may pose to the loggerhead turtles's survival.

2. Material and methods

2.1. Sample collection

Twenty-one specimens of stranded loggerhead turtles were collected between 2001 and 2004 (six specimens per year, excepting three in 2003) along the southwestern Mediterranean coastline (Andalusia) (Fig. 1). Only recently perished specimens were chosen for this study. A few stranded turtles were still alive when they were found; nevertheless, they died during the movement to the rehabilitation center (CREMA—Threatened Marine Species Rehabilitation Centre, Malaga, Spain) or after the surgical treatment. Since no method for age determination in sea turtles has yet been established, the straight carapace length (SCL) was used to determine size as a relative indicator of age. The SCL of each turtle was recorded, taken from the anterior nuchal notch to the furthest rear point of the carapace. A total of 60 samples from liver ($n = 16$), kidney ($n = 19$), pectoral muscle ($n = 20$), brain ($n = 3$), and bone ($n = 2$) were collected via necropsy by the staff from CREMA. After collection, the samples were packed in plastic bags and placed on ice for transport to CREMA where they were frozen at −40 °C until analysis.

2.2. Preparation of samples for metal analyses

Prior to anodic stripping voltammetry (ASV), all organic impurities which might interfere with the results must be eliminated (Oehme and Lund, 1979). Organic matter was digested using a high-temperature digestion with a mixture of nitric, perchloric, and sulphuric acids (8:8:1), following the method described by García-Fernández et al. (1995). In order to avoid the risk of external contamination of samples, all reagents used were Suprapur® quality (Merck, Darmstadt, Germany). The quartz tubes used (10 × 100 mm) for wet digestion were previously washed with 2% nitric acid for 48 h and then rinsed twice with tetradistilled water and dried in an oven at 100 °C. Approximately 1 g of wet tissue of each sample was dried in an oven at 80 °C until a constant dry weight was obtained. A volume of 0.05 g of this dry tissue was placed in the quartz digestion tube, to which 0.5 ml of the acid mixture was added. Samples were digested with a thermostated apparatus equipped for controlling temperatures from 10 to 450 °C and an aluminum-heating block. Thermal digestion was performed by raising the temperature from 100 to 200 °C at a rate of 5 °C/min, followed by digestion at 200 °C for 2 h, and a further temperature increase to 370 °C at a rate of 20 °C/5 min; this temperature was maintained until complete dryness was obtained.

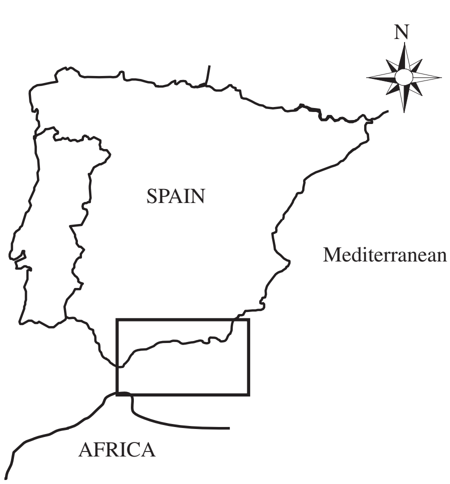

Fig. 1. Map of southern Spain showing the sampling locations (rectangle). Scale $A = 1{:}16{,}800{,}000$.

2.3. Analysis of metals

The digested sample was allowed to cool, and 100 μl of hydrochloric acid was added as a support electrolyte, being the pH of the final solution between 1 and 2 as required in the analytical procedure. Finally, the sample was transferred to the electrolytic cell using 10 ml of tetradistilled, purified water. The ASV (VA-646 processor and VA-647 workstation, Methrom, Switzerland) was equipped with three standard electrodes: a working electrode (hanging mercury drop), reference electrode (Ag/AgCl, KCl 3 mol/L) and auxiliary electrode (platinum). We used the differential pulse normal technique with an electrolysis time of 180 s and modulation amplitude of 50 mV. The concentration of each metal in digested samples was calculated with two standard solution additions (Sigma, St. Louis, MO) per analysis in a constant volume of 100 μl, at a concentration that yielded 1.5- and 3-fold higher peaks than those given by the unadulterated samples. The detection limits were 2.0, 1.0, 3.5, and 4 μg/L for zinc, cadmium, lead, and copper, respectively. To calculate recovery percentages, we processed five samples of each tissue, which had been spiked with known amounts of the metals. Mean recoveries approached 97% for all metals; recovery was lowest for cadmium in bone tissue (80%). The reproducibility of the method (mean ± SE), which was determined by analyzing 10 identical samples of the standard reference material (SRM) 1577b (National Institute of Standards and Technology), was 96.5 ± 5.2 for zinc, 97.9 ± 4.7 for cadmium, 98.2 ± 2.2 for lead, and 90.5 ± 3.2 for copper.

2.4. Statistical analysis

The statistical analysis of the data was performed using SPSS v.11.5 statistical software (SPSS Inc., 1989–1999). Reported statistics were arithmetic and geometric means, standard deviation and ranges in μg/g on a dry weight basis. To perform mean comparison tests, samples with non-detected values were assigned 1/2 the detection limit. The non-parametric Kruskal–Wallis test was used in order to detect differences between sampling years. Analyses were conducted for each metal separately. Spearman's rank non-parametric correlation test was applied to check for relationships between metal concentrations in each tissue and turtle SCL. This test was also applied to observe correlations between tissues for each metal and between metals for each tissue. In all cases, the level of significance was set at $\alpha = 0.05$. For the purposes of comparison with other studies, our values obtained as dry weight were converted to wet weight using the mean humidity obtained for each tissue.

3. Results

3.1. Metal concentrations

Since there were no significant differences in trace element concentrations between sampling years, the results were presented together. Zn, Cd, Pb, and Cu concentrations in wet and dry weights for tissues taken from loggerhead turtles from the Alboran Sea (southwestern Mediterranean) are reported in Tables 1–4.

Mean concentrations (dry weight) of essential elements (Zn and Cu) were 107 and 21.6 μg/g in liver, 27.9 and 3.8 μg/g in kidney, 65.4 and 5.0 μg/g in pectoral muscle, 11.1 and 3.45 μg/g in brain, and finally 19.2 μg/g and below the detection limit in bone, respectively. Mean concentrations of heavy metals (Cd and Pb) were 23.4 and 2.8 μg/g in liver, 31.5 and 0.5 μg/g in kidney, 0.2 and 0.3 μg/g in pectoral muscle, 0.2 and 0.7 μg/g in brain, and undetected and 1.2 μg/g in bone, respectively.

Table 1
Concentrations (μg/g) of metals in liver of loggerhead turtle

Liver	Zinc	Cadmium	Lead	Copper	Area
Present study (*n* = 16) dry weight	107.3±82.51 (80.2) 19.09–324.8	23.38±53.66 (7.1) 0.41–219.84	2.75±1.64 (2.5) 1.19–8.09	21.60±8.03 (18.8) 11.19–35.42	Andalusia (Spain)
Maffucci et al. (2005) (*n* = 14) dry weight	66.00±42.70 23.8–178	19.30±34.20 1.6–114	NA[a]	37.3±8.70 9.4–41.8	Italy
Gardner et al. (2006) (*n* = 5) dry weight	69.14 42.0–91.88	1.75 ND–30.60	ND[b]	33.90 16.60–58.98	Mexico
Gordon et al. (1998) (*n* = 8) dry weight	71.2±9.4 42.8–102	51.2±10.3 22.8–110	NA	NA	Australia
Godley et al. (1999) (*n* = 4) dry weight	NA	8.64 5.1–13.0	ND	NA	Cyprus
Kaska et al. (2004) (*n* = 32) dry weight	NA	10.8±3.9 3.4–19.0	3.55±1.31 1.18–5.38	2.98±0.90 0.27–4.18	Turkey
Present study (*n* = 16) wet weight	26.82±20.63 (20.05) 4.77–81.20	5.85±13.42 (1.78) 0.10–54.96	0.69±0.41 (0.63) 0.30–2.02	5.40±2.01 (4.7) 2.80–8.86	Andalusia (Spain)
Torrent et al. (2004) (*n* = 78) wet weight	13.48±1.70 0.09–91.38	2.53±0.45 0.04–21.98	2.94±0.59 0.05–33.09	15.02±2.07 0.01–65.57	Canary Is. (Spain)
Caurant et al. (1999) (*n* = 7) wet weight	25.00±9.50 14.5–38.4	2.58±4.12 0.30–11.8	NA	8.25±6.59 2.32–20.9	France
Franzellitti et al. (2004) (*n* = 30) wet weight	27.90±6.50	2.84±0.72	NA	7.40±3.90	Italy
Storelli et al. (2005) (*n* = 19) wet weight	29.30±7.71 18.8–46.5	3.36±1.94 1.10–6.55	0.16±0.05 ND–0.29	7.69±4.63 1.43–17.8	Italy
Storelli et al. (1998) (*n* = 12) wet weight	NA	2.24±1.78 0.90–5.97	NA	NA	Italy
Sakai et al. (1995) (*n* = 7) wet weight	27.90±10.40	9.30±3.30	NA	17.90±8.17	Japan
Sakai et al. (2000b) (*n* = 6) wet weight	28.10±4.70	9.74±3.37	0.08±0.03	17.70±8.90	Japan

The data are presented as mean±1 SD. The median is provided in parentheses, followed by the range. Concentrations on wet weight were estimated taking into account the humidity in this study of approximately 75% for liver samples.
[a] NA: not analyzed.
[b] ND: not detected.

Table 2
Concentrations (μg/g) of metals in kidney of loggerhead turtle

Kidney	Zinc	Cadmium	Lead	Copper	Area
Present study (*n* = 19) dry weight	27.88±26.77 (13.6) 1.59–111.97	31.47±70.75 (13.8) 2.45–319.98	0.52±0.49 (0.5) ND–1.93	3.77±3.50 (2.7) 0.12–11.77	Andalusia (Spain)
Maffucci et al. (2005) (*n* = 19) dry weight	97.00±31.70 62.4–206	57.20±34.60 10.9–158	NA[a]	2.60±0.70 1.7–4.7	Italy
Gardner et al. (2006) (*n* = 5) dry weight	32.47 2.70–130	73.10 14.0–140	0.03 ND–69.90	4.35 1.40–8.20	Mexico
Gordon et al. (1998) (*n* = 3) dry weight	76.3±3.75 69.6–88.7	117.9±23.70 47.5–164.2	NA	ND[b]	Australia
Godley et al. (1999) (*n* = 2) dry weight	NA	30.5 18.8–42.2	ND	NA	Cyprus
Kaska et al. (2004) (*n* = 20) dry weight	NA	16.96±9.81 3.14–33.32	3.99±2.11 0.89–11.05	2.08±0.60 1.18–3.08	Turkey
Present study (*n* = 19) wet weight	9.29±8.92 (4.53) 0.53–37.32	10.49±23.58 (4.60) 0.82–106.66	0.17±0.16 (0.17) ND–0.64	1.26±1.17 (0.9) 0.04–3.92	Andalusia (Spain)
Torrent et al. (2004) (*n* = 78) wet weight	9.09±1.07 0.07–38.53	5.01±1.02 0.01–61.08	2.44±0.48 0.02–17.29	4.60±0.97 0.13–49.06	Canary Is. (Spain)
Caurant et al. (1999) (*n* = 5) wet weight	23.60±6.19	13.3±13.6	NA	2.21±0.46	France
Storelli et al. (1998) (*n* = 12) wet weight	NA	7.52 0.12–19.9	0.21 ND–0.42	NA	Italy
Storelli et al. (2005) (*n* = 19) wet weight	23.10±4.53 16.6–27.9	8.35±4.83 1.26–16.4	0.12±0.07 ND–0.21	1.21±0.54 0.36–2.12	Italy
Sakai et al. (1995) (*n* = 7) wet weight	25.8 19.2–30.4	39.4 18.1–56.5	NA	1.30 0.99–1.56	Japan
Sakai et al. (2000b) (*n* = 6) wet weight	25.4±4.39	38.3±17.5	0.16±0.05	1.30±0.21	Japan

The data are presented as mean±1 SD. The median is provided in parentheses, followed by the range. Concentrations on wet weight were estimated taking into account the humidity in this study of approximately 66% for kidney samples.
[a] NA: not analyzed.
[b] ND: not detected

Table 3
Concentrations (μg/g) of metals in muscle of loggerhead turtle

Muscle	Zinc	Cadmium	Lead	Copper	Area
Present study ($n = 20$) dry weight	65.39±28.3	0.20±0.14	0.26±0.23	5.04±1.93	Andalusia (Spain)
	(56.9)	(0.2)	(0.2)	(4.6)	
	31.34–129.2	0.02–0.51	Nd-0.81	2.06–9.39	
Maffucci et al. (2005) ($n = 26$) dry weight	107.0±26.1	0.20±0.20	NA[a]	2.7±1.4	Italy
	76.4–177	0.06–0.78		0.8–7.0	
Gardner et al. (2006) ($n = 5$) dry weight	31.1	0.10	0.01	0.41	Mexico
	0.6–100	ND–1.45	ND–1.6	ND–3.4	
Godley et al. (1999) ($n = 4$) dry weight	NA	0.57	ND[b]	NA	Cyprus
		0.30–1.43			
Kaska et al. (2004) ($n = 32$) dry weight	NA	3.57±5.86	2.42±3.24	1.55±0.82	Turkey
		0.58–14.96	0.76–19.78	0.86–5.45	
Present study ($n = 20$) wet weight	13.08±5.66	0.04±0.03	0.05±0.05	1.01±0.39	Andalusia (Spain)
	(11.38)	(0.04)	(0.04)	(0.92)	
	6.27–25.84	0.004–0.10	Nd-0.16	0.41–1.88	
Torrent et al. (2004) ($n = 78$) wet weight	6.70±0.96	1.14±0.28	2.26±0.51	2.85±0.52	Canary Is. (Spain)
	0.05–32.37	0.15–12.48	0.22–21.07	0.01–27.25	
Caurant et al. (1999) ($n = 21$) wet weight	19.60±5.70	0.08 ± 0.05	NA	0.73±0.45	France
Franzellitti et al. (2004) ($n = 17$) wet weight	30.90±8.00	0.36±0.11	NA	1.50±0.40	Italy
Storelli et al. (2005) ($n = 19$) wet weight	27.90±4.85	0.07±0.03	0.04±0.03	0.59±0.41	Italy
	19.8–35.1	ND–0.13	ND–0.09	0.19–1.35	
Storelli et al. (1998) ($n = 12$) wet weight	NA	0.14±0.16	0.13	NA	Italy
		0.02–0.55	ND–0.18		
Sakai et al. (1995) ($n = 7$) wet weight	24.20±3.80	0.06±0.03	NA	0.83±0.26	Japan
	19.5–31.0	0.04–0.12		0.53–1.28	
Sakai et al. (2000b) ($n = 6$) wet weight	25.0±3.50	0.06±0.03	0.02±0.03	0.81±0.28	Japan

The data are presented as mean±1 SD. The median is provided in parentheses, followed by the range. Concentrations on wet weight were estimated taking into account the humidity in this study of approximately 80% for muscle samples.
[a] NA: not analyzed.
[b] ND: not detected.

Table 4
Concentrations (μg/g) of metals in bone and brain of loggerhead turtle

	Zinc	Cadmium	Lead	Copper	Area
Bone					
Present study ($n = 2$) dry weight	19.17±26.94	ND[a]	1.24±1.75	ND	Andalusia (Spain)
	(19.2)		(1.2)		
	0.12–38.22		ND–2.48		
Present study ($n = 2$) wet weight	15.34±21.55	ND	0.99±1.40	ND	Andalusia (Spain)
	(15.34)		(0.96)		
	0.1–30.58		ND–1.98		
Torrent et al. (2004) ($n = 78$) wet weight	6.70±0.96	1.36±0.35	2.36±0.50	3.81±0.66	Canary Is. (Spain)
	0.05–32.37	0.15–22.79	0.08–19.92	0.09–24.49	
Sakai et al. (2000b) ($n = 6$) wet weight	197±26.5	0.13±0.03	3.53±1.62	0.20±0.06	Japan
Brain					
Present study ($n = 3$) dry weight	11.05±11.23	0.22±0.22	0.69±0.48	3.45±0.88	Andalusia (Spain)
	(6.9)	(0.1)	(0.6)	(3.1)	
	2.50–23.77	0.09–0.47	0.23–1.20	2.77–4.44	
Present study ($n = 3$) wet weight	2.76±2.81	0.06±0.06	0.17±0.12	0.86±0.22	Andalusia (Spain)
	(1.73)	(0.03)	(0.15)	(0.78)	
	0.63–5.94	0.02–0.12	0.06–0.30	0.69–1.11	
Sakai et al. (2000b) ($n = 6$) wet weight	8.78±1.72	0.27±0.07	$<$ 0.03	2.05±1.11	Japan

The data are presented as mean±1 SD. The median is provided in parentheses, followed by the range. Concentrations on wet weight were estimated taking into account the humidity in this study of approximately 20% for samples of bone and 75% for brain.
[a] ND: not detected.

In general, trace metal concentrations were low in muscle, bone, and brain samples; however, the number of samples of bone and brain was inadequate for the results to be considered meaningful. On the other hand, the highest concentrations were found in liver, except for cadmium which was highest in kidney. Cd and Pb were similar in pectoral muscle (Table 3); however, lead concentrations were slightly higher in brain and much higher in bone (Table 4).

3.2. Metal correlations

Relationships between SCL and metal concentrations were only positive for zinc in muscle ($p = 0.017$, $\rho = 0.59$). When Spearman's test was applied to metal levels between different tissues a few positive correlations were found, such as between hepatic and renal cadmium ($p = 0.03$, $\rho = 0.718$), hepatic and renal zinc ($p = 0.06$, $\rho = 0.673$). Also positive correlations were found when this test was applied between metals in each tissue, such as between Cd and Zn ($p = 0.000$, $\rho = 0.750$) and between Cd and Cu ($p = 0.001$, $\rho = 0.684$) in kidneys.

4. Discussion

4.1. Zinc and copper

In general, copper and zinc concentrations were very similar to those found in other areas (Tables 1–4), both in the Mediterranean and other parts of the world. Nonetheless, copper concentrations were lower than those described by Torrent et al. (2004) for Atlantic turtles from the Canary Islands. In the case of zinc, our results were slightly higher in liver and muscle and lower ones in kidney than did those from the Canary Islands. Despite the slight differences mentioned, the data would seem to indicate that copper and zinc are regulated through homeostatic processes, maintaining a balance between metabolic requirements and prevention against toxic effects, which has been suggested recently by Maffucci et al. (2005). In this sense, one could consider the copper and zinc concentrations detected to be physiological; and presuming so, these elements are actually not a problem on the health status of these loggerhead turtles.

4.2. Cadmium

In the case of cadmium, the results offer a very different perspective when compared with published data (Tables 1–4). It is probable that the body burden of this contaminant in marine turtles was conditioned by the geographical zone inhabited by these specimens (Storelli et al., 2005). Maffucci et al. (2005) upholds the same hypothesis, placing special emphasis on the liver and kidney (Tables 1 and 2) due to their accumulatory role in long-term exposures. Our data on cadmium in liver, kidney, and muscle are similar to those offered by Maffucci et al. (2005) and Storelli et al. (2005) (Tables 1–3). The scarce references available on cadmium in bone and brain, as well as the low number of samples analyzed in the present study, impede us from establishing this effect as an influence in these tissues. The high deviation in cadmium concentrations implies that levels of this metal may not be regulated internally by the turtles and, therefore, cadmium concentrations would reflect the level of exposure (Maffucci et al., 2005), both in terms of intensity and frequency.

The cadmium concentrations in the present study were similar to those recorded for loggerheads from the central and western Mediterranean (Caurant et al., 1999; Franzellitti et al., 2004; Storelli et al., 1998, 2005) and from the Atlantic waters of the Canary Islands (Torrent et al., 2004), but are lower than those found in other locations such as Australia or Japan (Gordon et al., 1998; Sakai et al., 1995, 2000b). According to Storelli et al. (2005), there are two reasons which would explain the differences between geographic zones: firstly, the environmental contamination specific to each area, which influences the contaminant load of feeding zones; and secondly, the age of the specimens sampled. Although cadmium is not prone to biomagnification (Gray, 2002), high concentrations have been reported in long-lived vertebrates, such as loggerhead turtles (Caurant et al., 1994; Dietz et al., 1996; Stewart et al., 1994). High cadmium concentrations have been detected in marine mammals and marine birds that feed primarily on cephalopods which are considered an important vector of this element for marine predators situated at the top of the food chain (Bustamante et al., 1998; Caurant and Amiard-Triquet, 1995). In a previous study on stranded common dolphins (*Delphinus delphis*) on southwestern Mediterranean coastlines, the average liver and kidney cadmium concentrations (1.3 and 4.3 μg/g, d.w., respectively) were several times lower than those of the present study (Tables 1 and 2) (García-Fernández et al., 2000). According to Maffucci et al. (2005), marine turtles receive cadmium mainly via their diet; and Storelli et al. (2005) upholds that for a generalistic predator (Tomas et al., 2002), it is difficult to predict the effects of exposure due to diet.

The low variability observed for all metal concentrations and tissues, except for hepatic and renal cadmium and renal zinc (Tables 1 and 2) is noteworthy. In the case of cadmium, this was due to two specimens which showed the highest concentrations. These high standard deviations suggest the need for gaining knowledge on the range of cadmium concentrations defining toxicity thresholds for this metal. According to most authors, the lack of information available on marine turtles means such data cannot currently be known. Storelli et al. (2005) attempted to estimate these threshold concentrations using the available data from experimental studies on the freshwater painted turtle (*Chrysemys picta*) exposed to cadmium chloride. These authors suggested that these experimental data provided evidence cadmium concentrations of 3.36 μg/g (w.w.) in liver and 8.35 μg/g (w.w.) in kidney were high enough to affect the health of threatened or endangered marine species. Assuming the hypothesis by Storelli et al. (2005) holds, the specimens from the present study would also be in a delicate situation, since the means obtained herein were 5.85 and 10.49 μg/g (w.w.) in liver and kidney, respectively. It is true that these means were influenced by the two specimens highlighted previously which experienced extremely high concentrations of cadmium in liver and kidney; so much so that these were the highest yet described in literature for common turtles.

The loggerhead turtle which presented the highest renal cadmium concentration (320 μg/g, d.w.) perished following a period of sickness due to a massive infestation of parasites. Balazs and Pooley (1991) suggested that the viral infection produced by fibropapilloma in marine turtles was, to a great extent, due to the decrease in immune system capacity; while Limpus and Miller (1994) maintain that this illness was seen in areas close to the coast, adjacent to areas of intense human activity and areas of low renovation of waters. It is possible that the cadmium accumulated over quite some time by this loggerhead turtle were the cause of chronic problems in the immune system which would have depleted its capacity to defend itself against parasites. The liver cadmium concentration of this specimen was also very high (49 μg/g, d.w.) but lower than the renal concentration. According to Bernard and Lauwerys (1984), renal cadmium concentrations in humans greater than 200 μg/g are enough to induce renal lesions capable of causing death. Environmental exposure to cadmium determines a distribution pattern in which the ratio between hepatic cadmium and renal cadmium increases with the intensity of exposure (Friberg et al., 1974). It is considered that in cases of chronic exposure, the cadmium renal concentration would be higher than that for liver (hepatic Cd:renal Cd$<$1) (Scheuhammer, 1987). In this sense, most specimens in this study suffered chronic exposure (ratio$<$1), including the aforementioned highly contaminated turtle. On the contrary, one specimen displayed a much higher hepatic concentration (220 μg/g, d.w.) than in kidney (54.7 μg/g, d.w.), which would suggest a case of acute, severe, and recent exposure. In light of such data, it may be suggested that sources of cadmium exist in this part of the Mediterranean which pose a threat for this species; however, this hypothesis should be confirmed by future studies.

4.3. Lead

In contrast to cadmium, lead is easily detected in all samples of tissues collected from terrestrial living beings (García-Fernández et al., 1995). However, in the present study, the geometric mean for cadmium was three and 25 times higher in liver and kidney, respectively, than that of lead (Tables 1 and 2). Moreover, lead was not detected in various samples of kidney, brain, and muscle. For this reason, it is probable that studies from other areas have in general not analyzed lead in the loggerhead turtle, while various authors who have, did not find values above the detection limit (Gardner et al., 2006; Godley et al., 1999). Storelli et al. (2005) were of the opinion that lead concentrations below 0.5 μg/g (w.w.) should be considered low. In the present study, 50% of the livers analyzed ($n = 8$) showed concentrations above this; however, only one kidney was above but close to this threshold (0.64 μg/g). Storelli et al. (2005) described a significant decrease in lead-tissue concentrations with respect to a previous study conducted 10 years earlier in the same area (the Adriatic Sea) (Storelli et al., 1998). According to these authors, this drop was mainly due to the reduction of leaded petrol in many European countries since the 1970s (Storelli et al., 2005). In the present study, lead concentrations in all tissues were much higher than those described by Storelli et al. (1998, 2005); however, given that we have no data prior to the abolishment of leaded petrol in Spain, we cannot know if the same tendency towards a decrease has also taken place. Despite this, in previous studies in the South of the Iberian Peninsula on common kestrels (*Falco tinnunculus*) and Murciano-Granadina goats, this tendency has been observed (García-Fernández et al., 2003, 2005) and as such it is probable that this has also occurred in turtles from the southwestern Mediterranean. In any case, although the concentrations are high in comparison to other studies, the highest concentrations in all tissues were below the thresholds of toxicity for birds (Franson, 1996). Likewise, these concentrations were very similar to those found in a previous study on the common dolphin (*D. delphis*) from the same area (García-Fernández et al., 2000).

4.4. Metal correlations

Since no definitively ideal method exists for determining the age of marine turtles (Bjorndal et al., 1998), we used the SCL in order to assess variations in the trace element concentrations in terms of growth. Positive relationships ($p = 0.017$, $\rho = 0.59$) were found for zinc in muscle alone, whereby one would expect lead, and mainly cadmium, to accumulate with age. Maffucci et al. (2005) found no clear trends for cadmium or mercury either; while in other species, such as the green turtle and Carey's turtle, this trend has been observed (Anan et al., 2001; Gordon et al., 1998; Sakai et al., 2000a). According to Maffucci et al. (2005), the size effect in the sample is concealed by the unequal distribution of specimens across size classes, and by the absence of very young and very old turtles (SCL < 35 cm and SCL > 82 cm). In the present study, all but one turtle, with an SCL of 17 cm, were from 33 to 65 cm in length. According to Bjorndal (1997), the youngest turtles feed in pelagic habitats in the open sea, for which they can be subject to different degrees of exposure to trace metals than older animals which mainly feed on benthic prey (Maffucci et al., 2005).

The positive correlations between hepatic and renal cadmium, and hepatic and renal zinc suggest the importance of these tissues in the kinetics and the accumulation of these metals in marine turtles. Moreover, both Zn and Cu, and to a greater extent Cd, are inductors of metallothionein (MT) in terrestrial and marine animals (García-Fernández et al., 1996; Das et al., 2003). These low molecular weight proteins are characterized by a high affinity for binding divalent cations, and therefore are able to bind non-essential metals such as cadmium and transport it to the kidney where it accumulates as Cd-MT for years without any toxic effects (Das et al., 2003). On the other hand, the significant positive correlations found between Cd and Zn, and between Cd and Cu in kidneys suggest the implication of these MTs in the transport of these metals and in the prevention of the toxic effects of cadmium (Tohyama et al., 1986; Vogiatzis and Loumbourdis, 1998; ATSDR, 1999; Maffucci et al., 2005). Anan et al. (2002) verified the presence of these proteins in marine turtles and their possible tolerance to cadmium via MT induction as a consequence of chronic exposure to concentrations below the toxicity threshold.

5. Conclusions

The loggerhead turtle from the southwestern Mediterranean had similar concentrations of zinc and copper in its tissues as those from other parts of the Mediterranean and indeed the world, and as such these could be within the physiological range for concentrations of such elements. The significant positive correlations between concentrations of these oligoelements and renal cadmium suggest the implication of an efficient detoxificant mechanism involving MTs with the aim of preventing the toxic effects of cadmium in the loggerhead turtle. Regarding cadmium, our results suggest that regional differences in exposure determine differences in cadmium concentration and marine turtle diet is a stronger determinant of cadmium concentrations in tissues rather than accumulation with age since cadmium and lead concentration did not correlate with the turtle size. Lastly, lead concentrations do not appear to be high and, possibly, lead exposure is not a problem for individuals from this area; however, cadmium concentrations are high, and in some cases very high. A greater number of samples as well as a greater amount of information on these animals are needed to determine if a threshold for toxic effects exists in order to better predict the health effects of such high concentrations.

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References

Aguirre, A.A., Balazs, G.H., Zimmerman, B., Gales, F., 1994. Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) affected with fibropapillomas in the Hawaiian Island. Mar. Pollut. Bull. 28, 109–114.

Anan, Y., Kunito, T., Watanabe, I., Sakai, H., Tanabe, S., 2001. Trace element accumulation in hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Yaeyama Islands, Japan. Environ. Toxicol. Chem. 20, 2802–2814.

Anan, Y., Kunito, T., Sakai, H., Tanabe, S., 2002. Subcellular distribution of trace elements in the liver of sea turtles. Mar. Pollut. Bull. 45, 224–229.

ATSDR, 1999. Toxicological profile for cadmium. Prepared for US Department Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Bacci, E., 1989. Mercury in the Mediterranean. Mar. Pollut. Bull. 20, 317–338.

Balazs, G.H., Pooley, S.G., 1991. Research plan for marine turtle fibropapilloma. NOAA Tech Mem, NMFS-SWFSC-156.

Bernard, A., Lauwerys, R., 1984. Cadmium, in human population. Experientia 40, 143–152.

Bjorndal, K.A., 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), The Biology of Sea Turtles. CRC Press, Florida, pp. 199–231.

Bjorndal, K.A., Bolten, A., Bennet, R., Jacobson, E.R., Wronski, T.J., Valeski, J.J., Eleazar, P.J., 1998. Age and growth in sea turtles: limitations of skeletochronology for demographic studies. Copeia 1, 23–30.

Borrell, A., Aguilar, A., Pastor, T., 1997. Organochlorine pollutant levels in Mediterranean monk seals from the western Mediterranean and the Sahara coast. Mar. Pollut. Bull. 34, 505–510.

Bustamante, P., Caurant, F., Fowler, S.W., Miramand, P., 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in north-east Atlantic Ocean. Sci. Total Environ. 220, 71–80.

Cardenallicchio, N., Giandomenico, S., Ragone, P., Leo, D., 2000. Tissue distribution of metals in striped dolphins (*Stenella coeruleoalba*) from the Apulian coasts, Southern Italy. Mar. Environ. Res. 49, 55–66.

Caurant, F., Amiard-Triquet, C., 1995. Cadmium contamination in pilot whales Globicephala melas: source and potential hazard to the species. Mar. Pollut. Bull. 30, 207–210.

Caurant, F., Amiard, J.C., Amiard-Triquet, C., Sauriau, P.G., 1994. Ecological and biological factors controlling the concentrations of trace-elements (As, Cd, Cu, Hg, Se, Zn) in delphinids Globicephala melas from the North-Atlantic Ocean. Mar. Ecol. Prog. Ser. 103, 207–210.

Caurant, F., Bustamante, P., Bordes, M., Miramand, P., 1999. Bioaccumulation of cadmium, copper and zinc in some tissues of three species of marine turtles stranded along the French Atlantic coasts. Mar. Pollut. Bull. 38, 1085–1091.

Colborn, T., 2002. Clues from wildlife to create an assay for thyroid system disruption. Environ. Health Perspect. 110 (Suppl. 3), 363–367.

Das, K., Debacker, V., Pillet, S., Bouquegneau, J.M., 2003. Heavy metals in marine mammals. In: Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J. (Eds.), Toxicology of Marine Mammals. Taylor and Francis, London, pp. 135–167.

Dawson, A., 2000. Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife: a review. Ecotoxicology 9, 59–69.

Day, R.D., Christopher, S.J., Becker, P.R., Whitaker, D.W., 2005. Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. Environ. Sci. Technol. 39, 437–446.

De Guise, S., Beckmen, K.B., Holladay, S.D., 2003. Contaminants and marine mammals immunotoxicology and pathology. In: Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J. (Eds.), Toxicology of Marine Mammals. Taylor and Francis, London, pp. 38–54.

Dietz, R., Riget, F., Johansen, P., 1996. Lead, cadmium, mercury and selenium in Greenland marine animals. Sci. Total Environ. 186, 67–93.

Franson, J.C., 1996. Interpretation of tissue lead residues in birds other than waterfowl. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife. Interpreting Tissue Concentrations. CRC, Lewis Publishers, Boca Raton, pp. 265–279.

Franzellitti, S., Locatelli, C., Gerosa, G., Vallini, C., Fabbri, E., 2004. Heavy metals in tissues of loggerhead turtles (*Caretta caretta*) from northwestern Adriatic Sea. Comp. Biochem. Physiol. Part C 138, 187–194.

Friberg, L., Piscator, M., Nordberg, G.F., Kjellström, T., 1974. Cadmium in the Environment, second ed. CRC Press, Cleveland, OH.

García-Fernández, A.J., Sánchez-Garcia, J.A., Jiménez, P., Luna, A., 1995. Lead and cadmium in wild birds in southeastern Spain. Environ. Toxicol. Chem. 14, 2049–2058.

García-Fernández, A.J., Sánchez-Garcia, J.A., Gomez, M., Luna, A., 1996. Distribution of cadmium in blood and tissues of wild birds. Arch. Environ. Contam. Toxicol. 30, 252–258.

García-Fernández, A.J., Motas, M., María-Mojica, P., Romero, D., Fernández, E., 2000. Lead and cadmium in internal tissues of common dolphins (*Delphinus delphi*) stranded on the coast of Andalusia (SW Mediaterranean): influence of biological factors. In: Evans, P.G.H., Cruz, J., Raga, J.A. (Eds.), European Research on Cetaceans 13, 421–426.

García-Fernández, A.J., Navas, I., Romero, D., Gómez-Zapata, M., Luna, A., 2003. Influence of leaded-gasoline regulations on the blood lead concentrations in Murciano-Granadina goats from Murcia Region (SE Spain). Bull. Environ. Contam. Toxicol. 70, 1178–1183.

García-Fernández, A.J., Romero, D., Martínez-López, E., Navas, I., Pulido, M., María-Mojica, P., 2005. Environmental lead exposure in the European kestrel (*Falco tinnunculus*) from southeastern Spain: the influence of leaded gasoline regulations. Bull. Environ. Contam. Toxicol. 74, 314–319.

Gardner, S.C., Fitzgerald, S.L., Acosta-Vargas, B., Méndez-Rodríguez, L., 2006. Heavy metals accumulation in four species of sea turtles from the Baja California peninsula, Mexico. Biometals 19, 91–99.

Godley, B.J., Gaywood, M.J., Law, R.J., McCarthy, C.J., McKenzie, C., Patterson, I.A.P., Penrose, R.S., Reid, R.J., Ross, H.M., 1998. Patterns of marine turtle mortality in Britain water (1992–1998) with reference to tissue contaminant levels. J. Mar. Biol. Assess. 78, 973–984.

Godley, B.J., Thompson, D.R., Furness, R.W., 1999. Do heavy metal concentrations pose a threat to marine turtles from the Mediterranean Sea? Mar. Pollut. Bull. 38, 497–502.

Gordon, A.N., Pople, A.R., Ng, J., 1998. Trace metal concentrations in livers and kidneys of sea turtles from southeastern Queensland, Australia. Mar. Freshwater Res. 49, 409–414.

Gray, J.S., 2002. Biomagnification in marine systems: the perspective of an ecologist. Mar. Pollut. Bull. 45, 46–52.

Groombridge, B., 1990. Marine turtles in the Mediterranean: distribution, population status, conservation. Nat. Environ. Ser. 48, 1–98.

Herbst, L.H., Klein, P.A., 1995. Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofactors. Environ. Health Perspect. 103 (Suppl. 4), 27–30.

Homer, B.L., Foley, A., Fick, K.J., Lores, M.C., Redlow, A.E., Jacobson, E.R., 2000. Lesions, pathogens and toxins identified in 13 stranded marine turtles in Florida. In: Proceedings of the 18th International Symposium on Sea Turtle Biology and Conservation, NOAA Technical Memorandum, NMFS-SEFSC-436, Mazatlan, Sinaloa, Mexico, pp. 117–118.

IUCN, 2001. IUCN Red List of Threatened Animals. IUCN, Gland, Switzerland/Cambridge, UK.

Kaska, Y., Çelik, A., Bag, H., Aureggi, M., Özel, K., Elçi, A., Kaska, A., Elçi, L., 2004. Heavy metal monitoring in stranded sea turtles along the Mediterranean coast of Turkey. Fresenius Environ. Bull. 13, 769–776.

Kuetting, G.A.F., 1994. Mediterranean pollution. Mar. Policy 18, 233–247.

Law, R.J., 1996. Metals in marine mammals. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife. Interpreting Tissue Concentrations. CRC, Lewis Publishers, Boca Raton, pp. 357–376.

Limpus, C.J., Miller, J.D., 1994. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. In: James R. (Ed.) Proceedings of the Australian Marine Turtle Conservation Workshop, Gold Coast 14–17 November 1990, Queensland Department of Environment and Heritage and The Australian Nature Conservation Agency, Brisbane, pp. 186–188.

Maffucci, F., Caurant, F., Bustamante, P., Bentivegna, F., 2005. Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in loggerhead turtles (*Caretta caretta*) from the western Mediterranean Sea (southern Italy). Chemosphere 58, 535–542.

Meadows, P.S., 1992. Pollution, conservation and the Mediterranean ecosystem. A perspective view. Bull. Mar. Biol. Res. Centre of Tajura 9B, 269–298.

Oehme, M., Lund, W., 1979. Comparison of digestion procedures for the determination of heavy metals (Cd, Cu, Pb) in blood by anodic stripping voltammetry. Fresenius Z. Anal. Chem. 298, 260–268.

Peakall, D.B., 1996. Disrupted patterns of behaviour in natual populations as index of ecotoxicity. Environ. Health Perspect. 104 (Suppl. 2), 331–335.

Reijnders, P.J.H., 2003. Reproductive and developmental effects of environmental organochlorines on marine mammals. In: Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J. (Eds.), Toxicology of Marine Mammals. Taylor and Francis, London, pp. 55–66.

Saeki, K., Sakakibara, H., Sakai, H., Kunito, T., Tanabe, S., 2000. Arsenic accumulation in three species of sea turtle. Biometals 13, 241–250.

Sakai, H., Ichihashi, H., Suganuma, H., Tatsukawa, R., 1995. Heavy metal monitoring in sea turtle using eggs. Mar. Pollut. Bull. 30, 347–353.

Sakai, H., Saeki, K., Ichihashi, H., Kamezaki, N., Tanabe, S., Tatsukawa, R., 2000a. Growth-related changes in heavy metal accumulation in green turtle (*Chelonia mydas*) from Yaeyama Islands, Okinawa, Japan. Arch. Environ. Contam. Toxicol. 39, 378–385.

Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., Tatsukawa, R., 2000b. Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar. Pollut. Bull. 40, 701–709.

Scheuhammer, A.M., 1987. The chronic toxicity of aluminium, cadmium, mercury and lead in birds: a review. Environ. Pollut. 46, 263–295.

Stewart, F.M., Thompson, D.R., Furness, R.W., Harrison, N., 1994. Seasonal variation in heavy metals in tissues of common guillemots, *Uria algae*, from northwest Scotland. Arch. Environ. Contam. Toxicol. 27, 168–175.

Storelli, M.M., Marcotrigiano, G.O., 2003. Heavy metals residues in tissues of marine turtles. Mar. Pollut. Bull. 46, 397–400.

Storelli, M.M., Ceci, E., Marcotrigiano, G.O., 1998. Distribution of heavy metal residues in some tissues of *Caretta caretta* (Linnaeus) specimens beached along the Adriatic Sea (Italy). Bull. Environ. Contam. Toxicol. 60, 546–552.

Storelli, M.M., Storelli, A., D'Addabbo, R., Marano, C., Bruno, R., Marcotrigiano, G.O., 2005. Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation. Environ. Pollut. 135, 163–170.

Tohyama, C., Himeno, S.I., Watanabe, C., Suzuki, T., Morita, M., 1986. The relationship of the increased level of metallothionein with heavy metal levels in the tissue of the harbor seal (*Phoca vitulina*). Ecotoxicol. Environ. Saf. 12, 85–94.

Tomas, J., Guitart, R., Mateo, R., Raga, J.A., 2002. Marine debris ingestion in loggerhead sea turtles, *Caretta caretta*, from the Western Mediterranean. Mar. Pollut. Bull. 44, 211–216.

Torrent, A., González-Díaz, O.M., Monagas, P., Orós, J., 2004. Tissue distribution of metals in loggerhead turtles (*Caretta caretta*) stranded in the Canary Islands, Spain. Mar. Pollut. Bull. 49, 854–860.

Vogiatzis, A.K., Loumbourdis, S., 1998. Cadmium accumulation in live rand kidney and hepatic metallothionein and glutathione levels in Rana ridibunda, after exposure to $CdCl_2$. Arch. Environ. Contam. Toxicol. 34, 64–68.

Witham, P.R., 1980. The "lost year" question in young sea turtles. Am. Zool. 20, 525–530.