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Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in loggerhead turtles (*Caretta caretta*) from the Western Mediterranean Sea (southern Italy)

F. Maffucci [a,*], F. Caurant [b], P. Bustamante [b], F. Bentivegna [a]

[a] *Stazione Zoologica "Anton Dohrn", Villa Comunale I, 80121 Naples, Italy*
[b] *Laboratoire de Biologie et Environnement Marins, FRE 2727 du CNRS, Université de La Rochelle,*
22, Avenue Michel Crépeau, 17042 La Rochelle Cedex, France

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Abstract

Cadmium (Cd), copper (Cu), mercury (Hg), selenium (Se) and zinc (Zn) were determined in the liver, kidney and muscle of 29 loggerhead turtles, *Caretta caretta*, from the South Tyrrhenian Sea (Western Mediterranean). No significant differences ($p > 0.05$) were detected between males and females. Trace element concentrations were not influenced by the size of the specimen except Se in the liver, which was negatively correlated with the curved carapace length ($p < 0.001$). Muscles generally displayed the lowest trace element burdens, with the exception of Zn which contained concentrations as high as 176 $\mu g\,g^{-1}$ dwt. Kidneys displayed the highest Cd and Se mean concentrations (57.2 ± 34.6 and 15.5 ± 9.1 $\mu g\,g^{-1}$ dwt, respectively), while liver exhibited the highest Cu and Hg levels (37.3 ± 8.7 and 1.1 ± 1.7 $\mu g\,g^{-1}$ dwt, respectively). Whichever tissue is considered, the toxic elements had elevated coefficients of variation (i.e. from 60% to 177%) compared to those of the essential ones (i.e. from 14% to 65%), which is a consequence of homeostatic processes for Cu, Se and Zn. Globally, the concentrations of Hg remained low in all the considered tissues, possibly the result of low trophic level in sea turtles. In contrast, the diet of loggerhead turtles would result in a significant exposure to Cd. Highly significant correlations between Cd and Cu and Zn in the liver and kidney suggest that efficient detoxification processes involving MT occur which prevent Cd toxicity in loggerhead turtles.

Keywords: Bioaccumulation; Trophic transfer; Heavy metals; Marine vertebrates; Sea turtles; Tyrrhenian Sea

1. Introduction

Trace elements are natural components of rocks and soil and enter the marine environment as a consequence of weathering and erosion (Garrett, 2000). Many of them are biologically essential but all have the potential to be toxic to biota above a threshold concentration (O'Shea and Geraci, 1999). Following industrialization, unnatural quantities of such elements have been released, and continue to be released into the sea altering the natural biological equilibrium (Haynes and Johnson, 2000). Therefore some concern has arisen regarding their possible adverse effects on marine wildlife, particularly on those long living species such as marine

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[*] Corresponding author. Tel.: +39 81 5323 400/5833 400; fax: +39 81 5833 294.
E-mail address: maffucci@szn.it (F. Maffucci).

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mammals, seabirds and sea turtles, which have the potential to accumulate these contaminants (Caurant et al., 1999; Anan et al., 2001). Although the first two taxa have been thoroughly studied, only a limited number of papers on trace element accumulation in sea turtles have been published to date (see the review of Storelli and Marcotrigiano, 2003). Because of their solitary life style, duration of the pelagic phase and long lasting apnoeas, sea turtles are in fact among the most difficult marine animals to assess (Witherington, 2003).

Two of the seven species of sea turtles are known to reproduce regularly in the Mediterranean Sea, the loggerhead turtle, *Caretta caretta*, and the green turtle, *Chelonia mydas* (Groombridge, 1990). The former is the most abundant and extends its distribution to the whole basin with Western Mediterranean waters providing some of the most important feeding grounds (Margaritoulis et al., 2003). The neritic juvenile stage and adult foraging stage occur in the neritic zone (Bolten, 2003). Turtles feed primarily on sessile or slowly moving benthic prey (Mortimer, 1995; Bentivegna et al., 2001) although they do capture organisms throughout the water column (Bolten, 2003). The loggerhead turtle is currently classified as "endangered" by the IUCN (International Union for the Conservation of Nature and Natural Resources).

Because of the slow renewal of its waters, the large concentrations of human populations along its coasts, the intense industrial development and the presence of natural geochemical anomalies, levels of contaminants in the Mediterranean sea could became locally elevated (Bacci, 1989; Gabrielides, 1995; Turley, 1999). For example, the Tyrrhenian Sea (Western Mediterranean), is subject to a Hg enrichment due to cinnabar reserves of Monte Amiata (Tuscany) and the related extraction activities carried out there for many centuries (Bacci, 1989). Several studies revealed an enhancement of Hg in cephalopods, fish or dolphins from the Tyrrhenian Sea compared to other areas (Renzoni et al., 1973; Bernhard, 1988; André et al., 1991). However the paucity of data regarding trace element burdens in loggerhead turtles from the Mediterranean Sea (Storelli et al., 1998a,b; Godley et al., 1999) makes it difficult to evaluate accumulated levels and compare concentrations among different sites.

The primary objective of this study was therefore to determine the concentrations of five trace elements, Cd, Cu, Hg, Se, and Zn, in the tissues of loggerhead turtles from the Western Mediterranean Sea and to compare these data with those reported from other locations. Secondly, the influence of sex and size on trace element concentrations was tested. Correlations between the elements were determined in order to investigate physiological disorders and the hazard that these contaminants may pose to loggerhead turtles' survival were discussed.

2. Materials and methods

2.1. Sampling and sample preparation

Tissue samples were taken during necropsies of 29 loggerhead turtles, which stranded (dead) along the South Tyrrhenian coasts (Western Mediterranean) between March 2000 and May 2001 (Fig. 1). The curved carapace length (CCL), measured to the nearest 1 cm using a flexible meter tape, ranged from 37 to 82 cm. The necropsies were undertaken within 24 h after the detection of the carcass. Liver, kidney, and pectoral muscle were sampled when possible in a minimum quantity of 20 g and stored in plastic bags to avoid any contamination. The samples were frozen at −20 °C until the chemical analysis was carried out. Sex was determined via visual examination of the gonads. The CCL, sex, sampled tissue and the sampling date of each turtle are shown in Table 1. Tissue samples were freeze dried at −60 °C and 6 mbar. Fresh and dry weights were determined and the water content, expressed as a percentage of fresh weight, was calculated.

2.2. Analytical procedures

Approximately 200–300 mg of each homogenized dry sample was heated with supra-pure nitric acid at 60 °C until the solution was clear. Then, the residues were brought to 10 ml with ultrapure Milli-Q quality water. All the elements except Hg were determined by flame

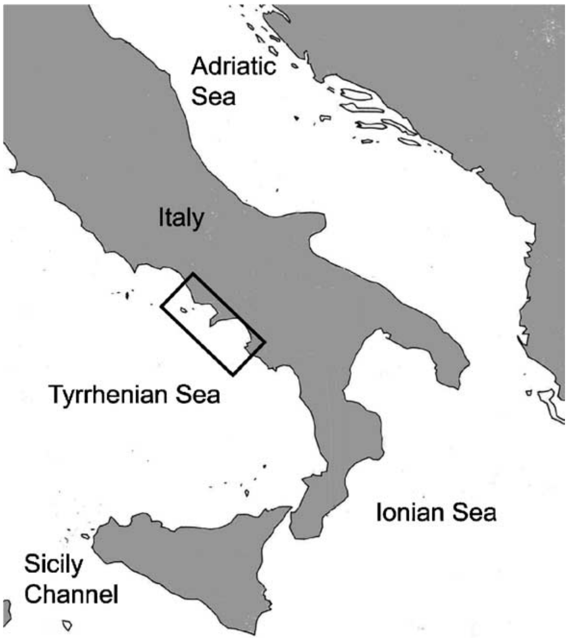

Fig. 1. The rectangle indicates the sampling location.

Table 1
Size (curved carapace length), sex and tissue sampled of 29 loggerhead turtles found dead on the South Tyrrhenian coast of Italy between 2000 and 2001

Specimen	Sex	Size (cm)	Tissue	Date of sampling
1	♀	75	K, L, M	24-05-00
2	♂	45	K, L, M	26-05-00
3	ND	ND	M	05-06-00
4	ND	71	K, L, M	11-06-00
5	♀	58	K, L, M	12-06-00
6	♂	54	L, M	18-06-00
7	♀	44	K	23-06-00
8	♂	43	L, M	27-07-00
9	ND	63	L, M	07-08-00
10	♀	71	L, M	07-08-00
11	♂	64	K, L, M	10-08-00
12	♂	57	K, M	11-08-00
13	♂	62	K, L, M	16-08-00
14	♀	62	K, L	21-08-00
15	♀	71	M	26-08-00
16	♂	53	L, M	01-09-00
17	ND	ND	K, L, M	16-09-00
18	♀	67	K, L, M	22-09-00
19	♀	58	L, M	03-10-00
20	♂	ND	K, L, M	04-10-00
21	♀	37	K, L, M	20-10-00
22	ND	ND	M	03-11-00
23	♂	59	K, L, M	20-11-00
24	♀	70	K, L	20-11-00
25	♀	77	K, L, M	29-11-00
26	ND	ND	K, M	03-01-01
27	♂	82	K, L, M	04-01-01
28	♀	51	K, M	16-01-01
29	♀	73	K, L, M	19-03-01

K = kidney, L = liver, M = muscle, ND = not determined.

or graphite furnace atomic absorption spectrophotometry (AAS) with a Varian spectrophotometer Spectra 250 Plus. Hg analysis was carried out with an Advanced Mercury Analyser spectrophotometer, ALTEC AMA 254, which does not require an acid-digestion of the samples. Aliquots ranging from 10 to 50 mg of dried samples were directly analysed after they had been inserted into the oven of the apparatus. After drying, the samples were heated under oxygen atmosphere for 3 min and Hg was subsequently amalgamated on a gold-net. Then the net was heated after a 45 s waiting time to liberate the collected Hg, which was measured by AAS.

Standards of dogfish liver DOLT-2 of the NRCC (National Research Council of Canada) were analysed as analytical quality control using the same procedure as the samples. Recoveries of all elements ranged from 93% to 110%. Trace element concentrations are presented as $\mu g\,g^{-1}$ of tissue on dry weight (dwt) basis.

2.3. Data analyses

Because the data were not normally distributed, statistical analyses of the data were performed by non-parametric tests with Minitab 11.2 (Minitab Inc., Pennsylvania). The coefficients of variation (CV) were calculated. The Wilcoxon's test for matched pairs was used to detect differences in the accumulation of trace elements between sexes and the Kruskall–Wallis test was used to evaluate the organotropism of each element. Spearman rank coefficients were calculated between pairs of elements in each tissue and between trace element concentrations and CCL. A p value of less than 0.05 was considered to indicate statistical significance (Fowler et al., 1998).

3. Results

3.1. Tissue concentrations

Cd, Cu, Hg, Se and Zn concentrations in the tissues of loggerhead turtles from the Tyrrhenian Sea are reported in Table 2. As no significant differences in trace element concentrations have been found between sexes and size (with the exception of hepatic Se which was negatively correlated with the CCL, Table 3), results in Table 2 were presented together. Trace element concentrations were generally low in muscle, except for Zn which exhibited the highest mean concentration in this tissue. Kidney displayed the highest Cd and Se concentrations, reaching 158 and 41.8 $\mu g\,g^{-1}$ dwt, respectively.

Table 2
Trace element concentrations ($\mu g\,g^{-1}$ dwt) in the tissues of loggerhead turtles from the South Tyrrhenean coast of Italy

Elements	Liver			Kidney			Muscle		
	n	Mean ± SD	Range (CV)	*n*	Mean ± SD	Range (CV)	*n*	Mean ± SD	Range (CV)
Cd	14	19.3 ± 34.2	1.6–114 (177)	19	57.2 ± 34.6	10.9–158 (60)	26	0.20 ± 0.20	0.06–0.78 (84)
Cu	14	37.3 ± 8.7	9.4–41.8 (23)	19	2.6 ± 0.7	1.7–4.7 (26)	26	2.7 ± 1.4	0.8–7.0 (50)
Hg	22	1.10 ± 1.70	0.42–8.76 (153)	20	0.90 ± 0.70	0.37–3.41 (71)	26	0.40 ± 0.30	0.14–1.92 (77)
Se	22	9.8 ± 5.3	1.0–24.9 (55)	21	15.5 ± 9.1	4.5–41.8 (59)	26	11.2 ± 4.9	4.0–24.1 (44)
Zn	14	66.0 ± 42.7	23.8–178 (65)	21	97.0 ± 31.7	62.4–206 (37)	24	107.0 ± 26.1	76.4–177 (24)

n: number of samples, CV: coefficient of variation (%).

Table 3
Spearman correlations between trace elements within the tissues of the loggerhead turtles *Caretta caretta* from the South Tyrrhenian coast of Italy

Elements	Liver	Kidney	Muscle
Cd	$\underline{\text{+Zn}}$, +Hg	$\underline{\text{+Zn}}$, +Hg	+Se
Cu		+Hg	
Hg	+Cd	+Cd, +Cu, $\underline{\text{+Zn}}$	
Se	$\underline{\text{−CCL}}$	+Zn	+Cd
Zn	$\underline{\text{+Cd}}$	$\underline{\text{+Cd}}$, $\underline{\text{+Hg}}$, +Se	

Not underlined: $p < 0.05$; underlined: $p < 0.001$. CCL: curve carapace length.

Finally, the liver exhibited the highest Cu and Hg loads, containing 41.8 and 8.76 $\mu g\,g^{-1}$ dwt, respectively. Toxic elements (i.e. Cd and Hg) were separated from those considered essential (i.e. Cu, Se, and Zn) according to their coefficients of variation of the concentrations (CV). Indeed, CV ranged from 60% to 177% for Cd and Hg while they remained lower than 65% for Cu, Se and Zn (Table 2). The mean water content was 68%, 76%, 79% for liver, kidney and muscle, respectively.

3.2. Trace element correlations

Table 3 shows the correlations between trace elements within the three tissues. In kidney, Zn was correlated with Cd ($p < 0.001$), Hg ($p < 0.001$) and Se ($p < 0.05$) (Table 3). Renal Hg concentrations were also positively correlated with Cu ($p < 0.05$) and Cd ($p < 0.05$) (Table 3). In liver, Cd concentrations were correlated with Hg ($p < 0.05$) and Zn ($p < 0.001$) (Table 3). Only hepatic Se was correlated with the CCL ($p < 0.001$) (Table 3).

4. Discussion

Sea turtles can achieve life span greater than 50 years, and have a potential to bioaccumulate heavy metals and pesticides (Lutcavage et al., 1997). In the costal zone, chronic pollution from industrial, agricultural wastes, and urban runoff constitutes a threat to sea turtles (National Research Council, 1990). Unfortunately, there is little information about this problem. The present results showed that although certain costal areas in the Western Mediterranean are heavily polluted either because of natural sources (i.e. Hg) or because of industrial activities (Kuetting, 1994; Gabrielides, 1995; Turley, 1999), trace element concentrations in loggerhead turtles from the Tyrrhenian Sea are comparable or even lower than those determined in the same species from other areas (Table 4). For comparative purposes, data which were originally presented on a wet weight basis were converted to approximate dry weights using the mean water content of each tissue determined in this study (see Section 3). In general Cu, Se, Zn concentrations did not vary much among sites (Table 4), consistently, the CV of these elements were the lowest found in all the analysed tissues (Table 1). Therefore it appears that loggerhead turtles can regulate Cu, Se, and Zn concentrations through homeostatic processes in a balance between metabolic requirements and prevention against toxic effects. On the contrary geographical area influences Cd and Hg concentrations especially in those organs like liver and kidney, which are known to play a main role in their long term accumulation (Table 4). The high CV found (Table 2) implies that Cd and Hg levels are not actively controlled by turtles and should change according to the level of exposure. In this context it is noteworthy that Hg concentrations in all specimens analysed remained very low for an area supposed to have natural mercury enrichment (Renzoni et al., 1973; Bacci, 1989; André et al., 1991; Capelli et al., 2000). More generally, loggerhead turtles do not accumulate Hg to such a great extent (Table 4) as other long-lived marine vertebrates (Caurant et al., 1994; Cardelicchio et al., 2002). Since this metal is known to biomagnify in the marine food web, the absence of long term Hg accumulation suggests that this species, which is an opportunistic predator (Tomas et al., 2002), feeds generally on prey of low trophic level. This is confirmed by investigations of the stomach and intestine contents of loggerhead turtles in the study area, which revealed that their diet is composed mainly of bivalves, gastropods and crabs (Bentivegna et al., 2001). Such a diet involves only few bioaccumulative steps and generally displays low organo-Hg contents compared to a fish-based diet (Cappon and Smith, 1982).

Cd mean concentrations were comparable within the Mediterranean Sea but lower than those found in other ocean locations (Table 4). However this element, which contrary to mercury does not biomagnify (Gray, 2002), accumulates at levels comparable to those reported for other long living marine vertebrates (Caurant et al., 1994). In marine mammals or sea birds, such concentrations (and also higher) are often encountered in animals feeding mainly on squid which are considered to be an important vector of this element to top marine predators (Bustamante et al., 1998). Also loggerhead turtles are most likely to take up Cd via their diet, but the paucity of data on the total Cd content and its physico-chemical form within the prey tissues precludes drawing any final conclusion on this question.

Because no reliable age determination method exists for sea turtles (Bjorndal et al., 1998), we used the curved carapace length (CCL) to evaluate growth-related variations in trace element concentrations. Although we analysed the largest sample of loggerhead turtles to date, we did not detect any clear trends, the only exception being

Table 4
Reported trace element concentrations (μg g^{-1} dwt) in the tissues of loggerhead turtles from different locations

Location	Cd			Cu			Hg			Se			Zn			Reference
	n	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	
Liver																
Australia	8	51.2 ± 10.3	22.8–110.0		–		6	0.05 ± 0.02	0.0–0.10	6	6.9 ± 0.6	4.44–8.44	5	71.2 ± 9.4	42.8–102	Gordon et al. (1998)
Cyprus[a]	4	8.6	5.1–13.0		–		5	2.41	0.82–7.50		–			–		Godley et al. (1999)
Japan	7	29.0 ± 10.3	17.7–45.6	7	55.9 ± 25.5	20.2–105.9	7	4.44 ± 9.15	0.79–25.5		–		7	87.2 ± 13.6	72.5–109.7	Sakai et al. (1995)
Japan	7	30.4 ± 10.5		7	55.3 ± 27.9		7	?					7	87.8 ± 14.6		Sakai et al. (2000a)
West Italy	14	19.3 ± 34.2	1.6–113.0	14	37.3 ± 8.7	9.4–41.8	22	1.1 ± 1.7	0.42–8.76	22	9.8 ± 5.3	1.0–24.9	14	66.0 ± 42.7	23.8–178.0	Present study
East Italy	12	7.60 ± 6.05	3.06–20.23		–		12	1.68 ± 1.04	0.35–3.72	12	15.88 ± 7.40	2.12–27.44		–		Storelli et al. (1998a)
West France	7	8.1 ± 12.9	0.9–36.9	7	25.8 ± 20.6	7.2–65.3		–					7	78.1 ± 29.7	45.3–120.0	Caurant et al. (1999)
Kidney																
Australia	3	117.9 ± 23.7	47.5–164.2		–		3	0.19 ± 0.05	0.14–0.28	3	6.3 ± 0.6	5.32–7.41	5	76.3 ± 3.75	69.6–88.7	Gordon et al. (1998)
Cyprus[a]	2	30.50	18.80–42.20		–		2	0.47	0.13–0.80		–			–		Godley et al. (1999)
Japan	7	164.2 ± 67.5	75.4–235.4	4	5.42 ± 0.80	4.12–6.49	7	1.02 ± 0.54	0.16–1.84		–		7	107.5 ± 17.4	80.1–126.7	Sakai et al. (1995)
Japan	7	159.6 ± 72.91		7	5.42 ± 0.90		7	?								Sakai et al. (2000a)
West Italy	19	57.2 ± 34.6	10.9–158	19	2.6 ± 0.7	1.7–4.7	20	0.9 ± 0.7	0.37–3.41	21	15.5 ± 9.1	4.5–41.8	21	97.0 ± 31.7	62.4–206.0	Present study
East Italy	12	24.23 ± 21.407	0.39–64.00		–		12	0.65 ± 0.34	0.30–1.53	12	10.33 ± 3.25	5.73– 15.57		–		Storelli et al. (1998a)
West France	5	55.4 ± 56.6	6.81–148.75	5	9.20 ± 1.92	7.32–11.79		–			–		5	98.3 ± 28.7	68.7–140.8	Caurant et al. (1999)
Muscle																
Cyprus[a]	4	0.57	0.30–1.43		–		7	0.48	Bdl–1.78		–			–		Godley et al. (1999)
Japan	7	0.29 ± 0.12	0.19–0.56	7	3.95 ± 1.23	2.52–6.02	7	0.51 ± 0.23	0.26–0.90		–		7	115.2 ± 18.1	92.9–147.6	Sakai et al. (1995)
Japan	7	0.31 ± 0.13		7	3.87 ± 1.31		7	?					7	119.0 ± 16.61		Sakai et al. (2000a)
West Italy	26	0.2 ± 0.2	0.06–0.78	26	2.7 ± 1.4	0.8–7.0	26	0.4 ± 0.3	0.14–1.92	26	11.2 ± 4.9	4.0–24.1	24	107.0 ± 26.1	76.4–177.0	Present study
East Italy	12	0.55 ± 0.63	0.09–2.21		–		12	0.69 ± 0.46	0.17–1.81	12	10.81 ± 3.25	6.51–15.45		–		Storelli et al. (1998a)
West France	21	0.38 ± 0.23	0.02–0.87	21	3.47 ± 2.14	1.62–10.61		–			–		21	93.3 ± 27.1	58.1–172.8	Caurant et al. (1999)

Values reported in wet weight were converted to dry weight using the mean water content as determined in present study.
n = number of samples.
[a] Concentrations of metals reported as median.

Se (Table 3). This was surprising since elements such as Cd and Hg are expected to accumulate with age because of their long biological half-life (Gray, 2002). However such trends were previously reported in both green (*C. mydas*) and hawksbill turtles (*Eretmochelys imbricata*) (Gordon et al., 1998; Sakai et al., 2000b; Anan et al., 2001). Consequently we believe that the size effect in our sample was concealed by the unequal distribution of specimens throughout the size classes, and by the absence of young and very old turtles (CCL < 35 cm and CCL > 82 cm). The early juvenile loggerhead turtles foraging in open ocean pelagic habitats (Bjorndal, 1997) may in fact experience different trace element exposure than older animals which feed prevalently on benthic prey.

The results of this study show that sex has no significant influence on trace element concentrations in loggerhead turtles. Although no other study has analysed such dependence in this species, no gender differences in trace element concentrations were also observed by Anan et al. (2001) in both green and hawksbill turtles. It might be probable that sexual differences in feeding rates, metabolism or growth rates are reduced in these species.

Overall, no particularly high concentrations of trace element in loggerhead turtles have been found in this study. This included Hg, one of the most dangerous marine contaminants, which has been found at very low levels compared to those reported in other long living marine organisms. The only exception may be the very toxic Cd, which accumulates to relatively high concentrations compared to those reported for other marine vertebrates (Nicholson and Osborn, 1983; Caurant and Amiard-Triquet, 1995; Elinder and Jarup, 1996; Storelli and Marcotrigiano, 2003). However the significant relationship between Cd and Zn in both liver and kidney (Table 3) suggests the involvement of metallothionein proteins (MTs) in the prevention of toxic effects of Cd (WHO, 1992; Vogiatzis and Loumbourdis, 1998; ASTDR, 1999). The presence of these molecules has been recently verified in loggerhead turtle (Anan et al., 2002). Further studies are necessary to evaluate physiological effects of Cd on loggerhead turtles and the potential tolerance to this toxic element via MT induction because disturbances due to a chronic exposure happen at concentrations lower than the toxic threshold.

5. Conclusion

The loggerhead turtles from the Tyrrhenian Sea displayed concentrations of trace elements similar to those reported from other areas. The low Hg concentrations in their tissues, even in the largest individuals (up to 82 cm of length), suggest that loggerhead turtles feed mainly on prey of low trophic level, as confirmed by stomach content analysis. However, these prey probably accounted for a significant exposure to Cd, which is accumulated in relatively high amounts in the kidney and liver. Highly significant correlations between Cd and Cu and Zn in the liver and kidney suggest that efficient detoxification processes involving MT occur which helps prevent Cd toxicity in loggerhead turtles.

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