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Distribution of Heavy Metal Residues in Some Tissues of *Caretta caretta* (Linnaeus) Specimen Beached Along the Adriatic Sea (Italy)

M. M. Storelli, E. Ceci, G. O. Marcotrigiano

Chemistry Institute, Veterinary Medicine Faculty, University of Bari, Italy strada prov. le per Casamassima Km 3, 70010 Valenzano (BA), Italy

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At the present time there are seven marine turtle species all over the world. Among these only three are present in the Mediterranean Sea: the Loggerhead (*Caretta caretta*), Green (*Chelonia mydas*) and Leatherback (*Dermochelys coriacea*) (Arnold and Burton, 1985). *Caretta caretta* is regarded as threatened, a term applied to species somewhat further from extinction (Waldichuk, 1987), and is therefore protected by the authorities in all countries in which this species is present. In Italy, it has been protected since February 24, 1976 (G.U., 1993). Different factors are causing the decrease in the number of this turtle:

i) coastal anthropization, that makes places unsuitable for nidification (in the Mediterranean Sea this reptile nidifies above all on the island of Zakinthos in the Ionian Sea and, along the South-East coastal area of Turkey) (Jones, 1990);

ii) the use of eggs as food in several countries (Nishimura and Nakahigashi, 1990; Hutchinson and Simmonds, 1991);

iii) the casual capture of young and adult specimen during the swordfish and tuna fishing campaign (Venizelos, 1991; Groombridge, 1989) and finally iv) the remarkable problem of marine pollution.

Most of the studies carried out on marine turtles concern the presence of plastic, glass and paper. As a matter of fact fragments of plastic, glass and pieces of paper were found in the intestine of some *Caretta caretta* specimens, caught along coasts of the Azores, and of *Dermochelys coriacea* specimens, caught along North-West European coasts (Bourne, 1985; Gramentz, 1988; Wehle and Coleman, 1983; Bjorndal et al., 1994). As far as the presence of chemical pollutants, such as heavy metals is concerned, not much has been published so far in the literature (Davenport and Wrench, 1990; Witkowski and Frazier, 1982; Aguirre et al., 1994). Most of the available papers concern pollutant residues in eggs (Stoneburner et al., 1980; Blumton et al., 1990; Clark and Krynitsky, 1985; Vazquez et al., 1997; Sakai et al., 1995; Bishop et al., 1991). The aim of this work is to assess the presence of heavy metals (Hg, Pb, Cd, Cr, As, Se) in different tissues of *Caretta caretta* specimens beached along Italian coasts (S. Adriatic Sea).

MATERIALS AND METHODS

Twelve *Caretta caretta* specimens, found beached along Apulian coasts (South

Correspondence to: G. O. Marcotrigiano

Adriatic Sea) between August 1990 and February 1991 were analysed (the weight and the sex of these samples are reported in Table 1). Liver, lung, kidney and muscle were taken from samples and stored in plastic bag in accordance with FAO methods (FAO, 1983). The tissues were dissected with plastic materials that were washed with HNO_3 and rinsed with distilled and deionized water, in order to avoid metal contamination. The samples were preserved at - 25 °C until their analysis after thawing and subsequent homogenisation in a blender (Ultra-turrax). The dry weight was determined from samples, kept at 60 °C, until constant weight. The mean values of the ratio fresh/dry weight was 3.39±0.35 for liver, 3.72±0.52 for lung, 3.22±0.60 for kidney and 3.99±0.39 for muscle. Samples (0.5 - 0.7 g of dried tissue) for quantitative analysis of heavy metals by atomic absorption spectrophotometry (Perkin Elmer 5000) were digested into the reaction flask with 11 ml of the mixture HNO_3-$HClO_4$ (8:3) for Pb, Cr, Cd (Ciusa and Giaccio, 1984), and with 10 ml of the mixture H_2SO_4-HNO_3 (1:1) for Hg, As and Se (G.U., 1990). For Pb, Cr and Cd determination, a graphite furnace (HGA-500 Perkin Elmer) was used. Hg was determined by the cold vapour technique after reduction by $SnCl_2$ (A.V.A. Thermo Jarrel Ash Corp.), while As and Se were measured as volatile hydrides after reduction by $NaBH_4$ (MHS-10 Perkin Elmer). Acid washed glassware, analytical grade reagents and double distilled deionized water were used in the tissue analysis. In order to check on the purity of the chemical used, a number of chemicals blanks were run; there was no evidence of any contamination in these blanks. Analytical quality control was achieved using TORT-1 Lobster Hepatopancreas (National Research Council of Canada) (Table 2). All data were computed on a mg/kg dry weight basis.

RESULTS AND DISCUSSION

The concentration of the different metals in tissues of *C. caretta* are presented in Table 3. The large standard deviations of the means reflect the wide range in weight of the animals. Among the metals analysed, the highest concentrations of mercury and lead were found in the liver. The mercury concentration was between 0.35 and 3.72 mg/kg (aver. 1.68 mg/kg), while the lead concentration was between N.D. and 3.38 mg/kg (aver. 1.23 mg/kg). In the other tissues examined, the average mercury and lead concentrations were between 0.42 and 0.68 mg/kg respectively and between 0.54 and 0.70 mg/kg. Very low concentrations of mercury and lead (Hg liver: 0.39 mg/kg; Hg muscle: 0.12 mg/kg; Pb liver: 0.12 mg/kg; Pb muscle: 0.31 mg/kg) were reported by Davenport and Wrench (1990) in a male *Dermochelys coriacea*, having a weight of 916 kg, found in Cardigan Bay. The highest cadmium levels were found in the kidney (aver. 24.23 mg/kg), according to the trend observed in other marine mammals (Honda et al., 1983), followed by the liver (aver. 7.60 mg/kg), lung (aver. 2.15 mg/kg) and finally in the muscle (aver. 0.55 mg/kg). Considerably low cadmium concentrations were reported in both the liver (aver. 0.22 mg/kg) and in the muscle (aver. 0.06 mg/kg) of *Dermochelys coriacea* (Davenport and Wrench, 1990). Chromium was present in all tissues examined with comparable concentrations between 1.05 and 1.57 mg/kg. Only the lung presented higher mean values (2.29 mg/kg). Higher arsenic concentrations were observed in the

muscle (68.94 mg/kg) than in the other organs that had levels between 21.67 and 29.91 mg/kg. However, it is probable that most of the arsenic was present in organometallic form, such as arsenocoline and arsenobetaine, which are very stable and physiologically inactive complexes. Only a small percentage (2-10%) of this metal is present in the potentially toxic inorganic form, as reported by other researchers (Ozretic et al., 1990). The highest selenium concentrations were found in the liver (aver. 15.88 mg/kg) while in other organs the values were lower (lung: aver. 10.77 mg/kg; kidney: aver. 10.33 mg/kg; muscle: aver. 10.81 mg/kg). The concentrations reported from Davenport and Wrench (1990) for this element were considerably lower (liver: aver. 1.41 mg/kg; muscle: aver. 3.61 mg/kg). The same authors underlined that the concentrations of heavy metals found in all examined tissues of *Dermochelys coriacea* appeared to be particularly low (Davenport and Wrench, 1990).

A comparison between concentrations (Table 4) in young specimens (1.8-2.8 kg) and those found in adult (50-100 kg) (Table 5) underlines the fact that in the different organs examined and for all metals, except for arsenic, the highest levels are peculiar to young. In particular significant ($P < 0.05$) differences in cadmium concentrations between various-sized individuals have been observed in the kidney and muscle. Kidney and muscle of young have cadmium mean concentrations equal to 37.81 mg/kg and 0.96 mg/kg respectively, while adult turtles show considerably lower mean levels (kidney: 10.65 mg/kg; muscle: 0.21 mg/kg).

Moreover, young organisms unlike adult have shown good correlations between Hg and Cd concentrations and weight in all examined tissues (Table 6).

It is difficult to interpret the significance of these findings because so little is known about baseline levels and physiological effects of environmental contaminants on marine turtle populations (Witkowski and Frazier, 1982; Bishop et al., 1991). Nevertheless the levels of the examined metals was generally higher in the liver than in other tissues. The highest cadmium levels, however, were found in the kidney where the mean concentration (24.23 mg/kg) was approximately three times higher than that found in the liver (7.60 mg/kg). Cadmium concentrations in the kidney approximately four times higher than those found in the liver were reported by Sakai (1995) in loggerhead turtles (kidney: 39.4 mg/kg; liver: 9.29 mg/kg). The characteristic accumulation of mercury in the liver and cadmium in the kidney has been reported by many researchers for other marine organisms like dolphins, seals and porpoises (Honda et al., 1983; Law et al., 1991). In fact, in marine mammals, the liver is the organ where mercury is preferentially accumulated. This possibly relates to a combination of factors such as different distribution of specific proteins, transport of mercury on a differential basis to particular organs and peculiarity of transport through certain cellular barriers (Gaskin, 1982). Also the retention of cadmium in the kidneys of marine mammals is related to its selective storage or sequestration in the protein metallothionein (Law et al., 1992).

The significant positive correlations between mercury and cadmium concentrations in the tissues of the young and their weight suggest that these metals tend to accumulate in tissues, although no correlation among adults have

been found. This may be related to the onset of sexual maturity. *C. caretta* is expected to reach sexual maturity at about 80 kg or even before (Bruno, 1986; Bustard, 1972) at which time increasing hormonal activity and consequent biochemical changes may alter the metabolic processes responsible for the uptake and distribution of metals by various tissues. The study of Sakai et al. (1995) on the presence of metal residues occurring in loggerhead turtle organs and eggs, underline that essential metals such as iron, manganese, zinc and copper are easily transferred from mother to eggs, but also that toxic metals such as cadmium and mercury, although only in limited amounts, are transferred and mercury is more easily tranferred than cadmium. In the opinion of Walker (1976), the fact that mercury concentrations in mature selechian females proved to be lower than those in the young, is to be ascribed to the release of metal by deposition in the developing ova.

Owing to the shortage of studies on the physiological effects of environmental contaminants on marine turtle populations, analytical data cannot be placed in proper context and hence there is clearly a need for a central source of data that may be used to assess the scope and magnitude of the problem. Our work in progress may help to fill this void and stimulate similar studies.

Table 1. Weight and sex of C. caretta specimen.

SAMPLES	WEIGHT (kg)	SEX
C. caretta 1	1.8	M
C. caretta 2	2.0	F
C. caretta 3	2.2	M
C. caretta 4	2.5	F
C. caretta 5	2.8	F
C. caretta 6	54	F
C. caretta 7	57	F
C. caretta 8	59.2	F
C. caretta 9	69.4	F
C. caretta 10	50	M
C. caretta 11	55	F
C. caretta 12	100	F

Table 2. Metal concentrations in reference material (TORT-1), coefficient of variation (CV), recovery and detection limit (D.L.).

	Pb	Cr	Cd	Hg	As	Se
TORT-1 (mg/kg)	10.4±2.0	2.4±0.6	26.3±2.1	0.33±0.06	24.6±2.2	6.88±0.47
Determined values (mg/kg)	9.9±0.83 n = 10	2.1±0.12 n = 10	26.4±0.45 n = 10	0.32±0.02 n = 10	24.4±0.3 n = 10	6.37±0.18 n = 10
CV %	8.35	9.76	1.70	13.62	1.25	2.82
Recovery %	95	88	100	97	99	93
D.L. (ng/g)	20	3	2	50	50	50

Table 3. Pb, Cr, Cd, Hg, As and Se min., max., mean and standard deviations values expressed in mg/kg dry wt. of all samples investigated (n = 12).

METALS	LIVER	LUNG	KIDNEY	MUSCLE
Pb	N.D.-3.38	N.D.-1.10	N.D.-1.35	N.D.-0.74
	1.23±1.01	0.60±0.35	0.70±0.35	0.54±0.17
Cr	0.20-2.07	0.38-5.41	0.20-6.80	0.30-2.89
	1.05±0.58	2.29±1.73	1.57±2.05	1.43±0.87
Cd	3.06-20.23	0.32-10.50	0.39-64.00	0.09-2.21
	7.60±6.05	2.15±2.80	24.23±21.40	0.55±0.63
Hg	0.35-3.72	0.12-0.97	0.30-1.53	0.17-1.81
	1.68±1.04	0.45±0.28	0.65±0.34	0.69±0.46
As	0.83-56.55	10.62-44.93	6.09-139.60	11.21-139.60
	21.67±17.22	24.00±10.70	29.91±39.48	68.94±45.80
Se	2.12-27.44	4.12-30.52	5.73-15.57	6.51-15.45
	15.88±7.40	10.77±7.01	10.33±3.25	10.81±2.89

Table 4. Pb, Cr, Cd, Hg, As and Se min., max., mean and standard deviations values expressed in mg/kg dry wt. of young samples (1.8-2.8 kg) (n = 5).

METALS	LIVER	LUNG	KIDNEY	MUSCLE
Pb	ND - 1.41	ND - 1.10	ND - 1.35	ND - 0.74
	0.95 ± 0.39	0.98 ± 0.15	1.08 ± 0.25	0.66 ± 0.07
Cr	0.55 - 1.76	1.79 - 4.39	0.77 - 6.80	1.58 - 2.89
	1.13 ± 0.48	2.92 ± 1.12	2.83 ± 2.71	2.13 ± 0.63
Cd	3.53 - 20.23	1.38 - 10.50	6.98 - 64.00	0.30 - 2.21
	8.45 ± 7.04	3.88 ± 3.84	37.81 ± 24.46	0.96 ± 0.78
Hg	0.35 - 3.72	0.12 - 0.97	0.32 - 1.53	0.17 - 0.57
	1.77 ± 1.25	0.58 ± 0.35	0.75 ± 0.46	0.43 ± 0.16
As	8.62 - 47.31	10.62 - 30.70	11.39 - 38.88	11.21 - 61.01
	23.83 ± 14.29	18.98 ± 7.30	20.31 ± 10.90	42.18 ± 21.42
Se	14.94 - 27.44	7.04 - 30.52	10.58 - 15.57	6.74 - 15.45
	19.29 ± 5.31	15.63 ± 8.89	12.42 ± 1.96	11.41 ± 3.67

Table 5. Pb, Cr, Cd, Hg, As and Se min., max., mean and standard deviations values expressed in mg/kg dry wt. of adult samples (50-100 kg) (n = 7).

METALS	LIVER	LUNG	KIDNEY	MUSCLE
Pb	ND - 3.38	ND - 0.62	0.34 - 0.59	ND - 0.65
	1.44 ± 1.34	0.37 ± 0.17	0.47 ± 0.09	0.47 ± 0.17
Cr	0.20 - 2.07	0.38 - 5.41	0.20 - 0.90	0.30 - 1.94
	0.99 ± 0.70	1.84 ± 2.02	0.57 ± 0.30	0.85 ± 0.57
Cd	3.06 - 18.30	0.32 - 1.48	0.39 - 16.45	0.09 - 0.39
	6.90 ± 5.69	0.92 ± 0.50	10.65 ± 6.58	0.21 ± 0.11
Hg	0.85 - 3.28	0.17 - 0.65	0.30 - 0.68	0.30 - 1.81
	1.60 ± 0.93	0.35 ± 0.19	0.54 ± 0.15	0.89 ± 0.54
As	0.83 - 56.55	12.59 - 44.93	6.09 - 139.60	17.07 - 139.60
	19.88 ± 20.53	27.58 ± 11.76	39.51 ± 56.20	91.25 ± 50.16
Se	2.12 - 27.11	4.12 - 9.82	5.73 - 13.15	6.51 - 13.15
	13.04 ± 8.09	7.30 ± 1.92	8.24 ± 2.99	10.32 ± 2.30

Table 6. Correlation coefficients (r) for mercury and cadmium concentrations in tissues of young (a) and adult (b) C. caretta specimen.

a	Liver	Lung	Kidney	Muscle
Hg	r = 0.95; P < 0.01	r = 0.98; P < 0.005	r = 0.92; P < 0.03	r = 0.87; P < 0.05
Cd	r = 0.91; P < 0.03	r = 0.88; P < 0.04	r = 0.89; P < 0.04	r = 0.95; P < 0.01
b	Liver	Lung	Kidney	
Hg	r = 0.41; P < 0.003	r =-0.33; P < 0.001	r =-0.89; P < 0.004	r =-0.34; P < 0.002
Cd	r = 0.44; P < 0.03	r = 0.37; P < 0.001	r =-0.83; P < 0.004	r =0.27; P < 0.002

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