

Distribution of heavy metals in tissues of stranded Loggerhead Turtles (*Caretta caretta*) on Kazanlı Beach, Turkey

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Abstract. The mean concentrations of metals ($\mu\text{g g}^{-1}$ dry wt) measured in the tissue of six dead-stranded female loggerhead turtles from Kazanlı beach, Mersin, Eastern Mediterranean, Turkey followed these patterns: Zn>As>Cu>Pb>Mn>Cd>Cr in the muscle tissue and Zn>As>Cu>Mn>Cd>Pb>Cr in the liver tissue. The mean concentrations of Zn and As were higher in the muscle tissue than in the liver tissue. However, the remaining elements were higher in the liver than in the muscle. A significant difference was found between the muscle and liver tissues only for Pb ($p<0.05$). There was a highly negative linear relationship between turtle size and As in the muscle tissue ($p<0.05$). The average levels of As in both sampled tissues may be more likely to affect the health of turtles. The concentrations of heavy metals in turtle tissues may depend mainly on their feeding habits and may be indicative of chronic exposure in the areas where they feed. The six turtles, found on the same day, had likely become entangled in trawl nets and died by drowning.

Key words: Heavy metals, *Caretta caretta*, strandings, Kazanlı beach, Turkey.

Introduction

In the Eastern Mediterranean, nesting loggerhead (*Caretta caretta*) and green turtles (*Chelonia mydas*) are reported regularly on the beaches, and leatherbacks (*Dermochelys coriacea*), considered migrants from the wider Atlantic, have also been observed (Godley et al. 1998, Casale et al. 2003, Margaritoulis 2003). On the beaches of Turkey, surveys have identified 21 important nesting areas (Türkozan & Kaska 2010), including the Davultepe 100. Yıl beach in Mersin (Ergene et al. 2016). Stranded loggerhead, green, and leatherback turtles have been regularly reported on the Turkish Mediterranean coasts and Marmara Sea coasts (Türkozan & Durmuş 2000, Türkozan et al. 2013, Tonay & Oruç 2016, Candan & Canbolat 2018, Yalçın Özdilek et al. 2018). A study by Türkozan et al. (2013) presented for the first time a comprehensive dataset of sea turtle strandings on the Eastern Mediterranean coast of Turkey. Subsequently, stranded turtles on the coasts of Turkey have been studied by other researchers (Başkale et al. 2018, Sönmez 2018, Yalçın Özdilek et al. 2018). During a monitoring program along the Turkish Mediterranean coast, Kaska et al. (2004) documented the possible cause of mortalities of stranded sea turtles, as well as the heavy metal (Cd, Pb, Fe, Cr, Ni, Se, Sb, As, and Cu) concentrations which were measured and statistically compared in the tissues of dead-stranded loggerhead and green sea turtles. In addition to the research on the concentrations of heavy metals measured in stranded sea turtles in Turkey, a few other studies have presented heavy metal concentrations in the nesting environment and nest contents of sea turtles, including eggshells, remaining yolk, and the embryonic livers of hatchling samples (Kaska & Furness 2001, Çelik et al. 2006, Yalçın Özdilek et al. 2011).

In Northern Cyprus in the Eastern Mediterranean, Godley et al. (1999) determined the concentrations of heavy metals (Hg, Cd, and Pb) in internal organs and the nest contents of loggerhead and green turtles.

The studies in the Italian Mediterranean (Storelli et al. 1998, Storelli & Marcotrigiano 2003, Franzellitti et al. (2004),

Maffucci et al. 2005, Storelli et al. 2005, Andreani et al. 2008), the Spanish Mediterranean (García-Fernández et al. 2009, Jerez et al. 2010), the Atlantic coasts of France (Caurant et al. 1999), the Baja California Peninsula in northwestern Mexico (Gardner et al. 2006), from Japanese waters (Sakai et al. 1995, Sakai et al. 2000), from the North Pacific (Saeki et al. 2000), and from southeastern Queensland, Australia (Gordon et al. 1998) presented heavy metal concentrations in loggerhead turtles and discussed the toxic effects of heavy metals in this species. From the Mediterranean Sea and the Atlantic and Pacific Oceans, D'Illo et al. (2011) presented an overview of available literature on the occurrence of trace elements and Persistent organic pollutants (POPs) in different tissues, organs, and fluids of loggerhead sea turtles. The definition of "heavy metal" in this study is in accordance with the review by Engwa et al. (2019), i.e. metals with specific density above 5 g/cm^3 and atomic weight greater than 40.04.

The current paper aims to evaluate the concentrations of heavy metals (Cu, Zn, Cr, Mn, Pb, As, and Cd) measured with an inductively coupled plasma-mass spectrometer (ICP-MS) in the liver and muscle tissues of samples collected from six dead-stranded female loggerheads and to compare the data with those reported from other locations. Besides, the paper aims to explain the possible cause of mortality.

Material and Methods

Sample collection and study area

All six dead-stranded female loggerhead turtles were found washed ashore on February 14, 2009, in the K1 section of the Kazanlı nesting beach in Mersin Bay, approximately 12 km away from Mersin city center. The D-7 drainage channel (Çomak) ($36^{\circ}48.262' \text{ N}$, $034^{\circ}47.289' \text{ E}$) is located at the easternmost end of the beach, and Soda Sanayii A.Ş. and Kromsan Factory ($36^{\circ}48.677' \text{ N}$, $034^{\circ}43.430' \text{ E}$) are at the western end of the beach. The beach has a total length of 6,1 km, with the part that is suitable for sea turtle nesting being 4.7 km long. Kazanlı beach, designated as a Nature Site area, is one of the most important nesting sites for *C. mydas* in Turkey, and a few *C. caretta* also nest in Mersin along the southeast coast of Turkey (Türkozan & Kaska 2010).

The sex of the turtles was determined on the basis of morphological characteristics following Marquez (1990). The following measurements were taken to the nearest 0.5 cm using a wooden mechanical caliper and flexible tape: curved carapace length (CCL), curved carapace width (CCW), straight carapace width (SCW), and minimum straight carapace length (MSCL) (Bolten 1999). However, age was not determined. In order to determine the cause(s) of death, a thorough post-mortem evaluation was performed within 24 h after the carcasses were found. During the necropsy, no hooks, plastic bags etc. were found in the digestive tracts and no holes were observed on the heads or carapaces. Advanced stages of decomposition and decay were observed in all specimens. The liver and muscle tissue samples were taken during the necropsies, and stored in plastic bags to avoid any contamination. All tissue samples were stored frozen at -70°C until further chemical analysis could be conducted.

Chemical Processing

The muscle and liver tissues were kept in the deep freezer until the chemical analysis, and the tissue samples were dried completely by keeping them in an incubator for 3 days at 105°C . The samples were transferred into 20 ml glass tubes after weighing on an analytical balance scale. A 65% nitric acid solution was added to the tubes to fill them to 10 ml. Acid added glass tubes were placed in a beaker containing water and kept on the hotplate at 200°C for 4 hours. Then, 1 ml hydrogen peroxide was added to each tube, and the burning process was applied to the tubes for another 4 h. For every 10 samples, one blind sample was prepared. The samples were filled to 11 ml with 2% nitric acid in the acid-resistant tubes. The dilution factors of all samples were found from their dry weight. Next, the samples were measured on an AGILENT 7500ce ICP-MS.

Statistical Analysis

The statistical analysis of the data was performed using SPSS for Windows version 11.5 statistical software (SPSS Inc. 2002), and the data are presented as mean \pm standard deviation (SD) in $\mu\text{g g}^{-1}$ on a dry weight basis (see Table 1 and 2). To test for normality, a Shapiro-Wilk test was carried out. Data were normally distributed and the statistical analyses were performed with a paired samples *t*-test in order to detect differences in the accumulation of elements between the muscle and liver tissues. A Pearson correlation test was applied to check for relationships between the metal concentrations in each tissue and turtle size (CCL, CCW, MSCL, and SCW). The selected threshold for statistical significance was $p < 0.05$.

Results

Size distribution of loggerhead turtles

The carapace measurements of the six dead-stranded female loggerhead turtles were as follows: CCL 63.17 ± 4.17 cm (range: 58-69), CCW 58.83 ± 3.92 cm (range: 55-63), MSCL 60.00 ± 3.90 cm (range: 55-65), and SCW 46.67 ± 2.50 cm (range: 43-50).

Heavy metal concentrations in tissues

The Cu, Zn, Cr, Mn, Pb, As, and Cd concentrations in the tissues of our specimens are reported in Tables 1 and 2. Zn showed the highest levels in both muscle (91.09 ± 33.01 , range: 59.29–135.78 $\mu\text{g g}^{-1}$ dry wt) and liver (83.21 ± 52.17 , range: 47.92–186.95 $\mu\text{g g}^{-1}$ tissues), and Cr showed the lowest values in both muscle (0.39 ± 0.49 , range: below detection limit (BDL)–1.10 $\mu\text{g g}^{-1}$ dry wt) and liver (0.47 ± 0.36 , range: 0.11–1.01 $\mu\text{g g}^{-1}$ dry wt) tissues. The Cr concentration in the muscle tissue from one sample and the Cd concentration in the liver tissue from another sample were not measured because the two elements were below the detection limit. The mean

concentrations of metals followed these patterns: Zn>As>Cu>Pb>Mn>Cd>Cr in the muscle and Zn>As>Cu>Mn>Cd>Pb>Cr in the liver (Tables 1 and 2).

Heavy metal correlations between tissues

For the concentration of Pb ($P=0.029$), A significant difference ($p < 0.05$) in concentration between the muscle and liver tissues was detected only for Pb ($P=0.029$). For all other elements, no significant differences were found between the two tissues.

The correlations of heavy metal concentrations in tissues and turtle size

According to the Pearson correlation test, a highly negative linear relationship was found between the concentration of As in muscle and turtle size (CCL, CCW, SCW, MSCL) ($p < 0.05$). However, no statistically significant correlations were registered between the size of the individuals and the concentrations of other metals ($p > 0.05$).

Discussion

Size distribution of loggerhead turtles

All specimens were classified into categories depending on the CCL. Individuals with $\text{CCL} \leq 30$ cm were assumed to be in the oceanic stage, and the sub-adult stage was assumed to be between 30 and 70 cm. Individuals with $\text{CCL} \geq 70$ cm were considered adults (Casale et al. 2005, Casale et al. 2010, Türkozan et al. 2013). In our study, we could not determine sexual maturity, but considering the measurements presented in the Results section, some individuals were exactly sub-adults and some were probably sub-adults or adults.

Alternatively, in Franzellitti et al. (2004), the dead loggerhead turtles collected along the Adriatic Sea coast from the Po Delta to the Reno mouth (Italy) were classified into four size categories according to their minimum straight-line carapace length (MSCL), following a dimensional classification modified from Dodd (1988). The MSCL of our specimens ranged between 55–65 cm, so they were distributed into only one size class, namely, benthonic sub-adults and adults.

Heavy metal concentrations in tissues

The mean concentrations of As and Cu in our study were higher than the levels established in Kaska et al. (2004), but concentrations of Cd, Pb, and Cr were lower (see Tables 1 and 2).

In our study, the mean concentrations of Cd, Cu, Pb, Mn, and Cr showed higher levels in the liver tissue than in the muscle tissue, but the highest Zn and As levels were found in the muscle tissue, as previously reported in studies by Andreani et al. (2008) for Zn in Italy (Adriatic and Mediterranean coasts of Italy), Maffucci et al. (2005) for Zn in western Italy (the southern Tyrrhenian coast of Italy), Storelli et al. (1998) for As in eastern Italy (South Adriatic Sea), Saeki et al. (2000) for As from the North Pacific, Jerez et al. (2010) for Zn and As in eastern Spain, Caurant et al. (1999) for Zn in western France (Atlantic coast of France), Sakai et al. (1995) for Zn in Japan, and Sakai et al. (2000) for Zn in Japan. The last three studies were originally presented on a wet weight

Table 1. Heavy metal (Cu, Zn, Cr, Mn, Pb, As, and Cd) concentrations ($\mu\text{g g}^{-1}$ dry wt) in the muscle tissue of loggerhead turtles from different locations; data are expressed as mean values \pm SD; N: number of samples; L: line; BDL: below detection limit; ^a geometric mean

Location	N	Cd		Cu		Pb		Zn		As		Mn		Cr		References
		Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD		
Kazanlı-Turkey	6	0.48 \pm 0.91	6.50 \pm 8.22	2.00 \pm 1.29	91.09 \pm 33.01	72.85 \pm 39.32	1.53 \pm 0.81	0.39 \pm 0.49	1.51 \pm 0.78						Present study	
Mediterranean coast-Turkey	32	3.57 \pm 5.86	1.55 \pm 0.82	2.42 \pm 3.24		20.80 \pm 9.97									Kaska et al. (2004)	
Northern Cyprus	4	0.57		2.46											Godley et al. (1999)	
Italy	10	0.81 \pm 0.04	2.4 \pm 0.24	BDL	105 \pm 14		1.35 \pm 0.24								Andreani et al. (2008)	
West Italy	26,26,24	0.20 \pm 0.20	2.7 \pm 1.4		107.0 \pm 26.1										Maffucci et al. (2005)	
East Italy	12	0.55 \pm 0.63		0.54 \pm 0.17		68.94 \pm 45.80		1.43 \pm 0.87							Storelli et al. (1998)	
West France	21	0.38 \pm 0.23	3.47 \pm 2.14		93.3 \pm 27.1										Caurant et al. (1999)*	
East Spain	13	0.08 \pm 0.06		0.2 \pm 0.25	113.29 \pm 267.33	40.95 \pm 37.32									Jerez et al. (2010)	
Andalusia (Spain)	20	0.20 \pm 0.14	5.04 \pm 1.93	0.26 \pm 0.23	65.39 \pm 28.3										García-Fernández et al. (2009)	
Mexico	5	0.1	0.41	0.01	31.11 ^a		0.84								Gardner et al. (2006)	
Japan	7	0.29 \pm 0.12	3.95 \pm 1.23		115.2 \pm 18.1										Sakai et al. (1995)*	
Japan	7	0.31 \pm 0.13	3.87 \pm 1.31		119.0 \pm 16.61										Sakai et al. (2000)*	
North Pacific	4					20.6 \pm 13.1									Saeki et al. (2000)	

* In these studies, the values reported in wet weight were already converted to dry weight using the mean water content as determined by Maffucci et al. (2005). So, these values of dry weight were used in Table 1.

Table 2. Heavy metal (Cu, Zn, Cr, Mn, Pb, As, and Cd) concentrations ($\mu\text{g g}^{-1}$ dry wt) in the liver tissue of loggerhead turtles from different locations; data are expressed as mean values \pm SD; N: number of samples; L: line; BDL: below detection limit; ^a geometric mean

Location	N	Cd		Cu		Pb		Zn		As		Mn		Cr		References
		Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD	Mean-SD		
Kazanlı-Turkey	6	4.59 \pm 2.98	27.7 \pm 15.83	2.73 \pm 0.93	83.21 \pm 52.17	37.60 \pm 37.61	5.08 \pm 4.06	0.47 \pm 0.36							Present study	
Mediterranean coasts - Turkey	32	10.84 \pm 3.89	2.98 \pm 0.90	3.55 \pm 1.31		14.23 \pm 7.60		2.77 \pm 0.93							Kaska et al. (2004)	
Northern Cyprus	4	8.64		BDL											Godley et al. (1999)	
Italy	11	2.4 \pm 0.4	17.5 \pm 2.44	0.1 \pm 0.08	103 \pm 14		7.48 \pm 1.04								Andreani et al. (2008)	
West Italy	14	19.3 \pm 34.2	37.3 \pm 8.7		66.00 \pm 42.70										Maffucci et al. (2005)	
East Italy	12	7.60 \pm 6.05		1.23 \pm 1.01		21.67 \pm 17.22		1.05 \pm 0.58							Storelli et al. (1998)	
West France	7	8.1 \pm 12.9	25.8 \pm 20.6		78.1 \pm 29.7										Caurant et al. (1999)*	
East Spain	13	0.81 \pm 0.48		0.2 \pm 0.11	30.23 \pm 12.24	12.73 \pm 13.03									Jerez et al. (2010)	
Andalusia (Spain)	16	23.38 \pm 53.66	21.6 \pm 8.03	2.75 \pm 1.64	107.3 \pm 82.51		1.29								García-Fernández et al. (2009)	
Mexico	5	1.75	33.94 ^a	BDL	69.14										Gardner et al. (2006)	
Japan	7	29.0 \pm 10.3	55.9 \pm 25.5		87.2 \pm 13.6										Sakai et al. (1995)*	
Japan	7	30.4 \pm 10.5	55.3 \pm 27.9		87.8 \pm 14.6										Sakai et al. (2000)*	
North Pacific	4					6.32 \pm 1.56									Saeki et al. (2000)	
Australia	8,5,6	51.2 \pm 10.3			71.2 \pm 9.4	1.44 \pm 0.75									Gordon et al. (1998)*	
															Jerez et al. (2010)**	

* In these studies, the values reported in wet weight were already converted to dry weight using the mean water content as determined by Maffucci et al. (2005). ** Only. As value for liver reported in wet weight were converted to dry weight by Jerez et al. (2010) adapting the mean water reported by Maffucci et al. (2005). So, these converted values were used in the Table 2 in our study.

basis and were converted to approximate dry weights using the mean water content of each tissue determined by Maffucci et al. (2005) (see Tables 1 and 2). However, the highest Zn values were found in liver tissue by García-Fernández et al. (2009) in Andalusia (Spain) and Gardner et al. (2006) in Mexico (see Tables 1 and 2).

The average levels of heavy metals found in our specimens were similar to those previously reported from other Mediterranean areas for Cd and Zn in muscle tissue. The mean concentrations of Pb in muscle tissue in Kaska et al. (2004) ($2.42 \pm 3.24 \mu\text{g g}^{-1}$ dry wt) and in our study ($2.00 \pm 1.29 \mu\text{g g}^{-1}$ dry wt) (both in Turkey) were higher than in eastern Italy (South Adriatic Sea) ($0.54 \pm 0.17 \mu\text{g g}^{-1}$ dry wt [Storelli et al. 1998]), eastern Spain ($0.2 \pm 0.25 \mu\text{g g}^{-1}$ dry wt [Jerez et al. 2010]), and Andalusia (Spain) ($0.26 \pm 0.23 \mu\text{g g}^{-1}$ dry wt [García-Fernández et al. 2009]). The average level of Cr ($0.39 \pm 0.49 \mu\text{g g}^{-1}$ dry wt) in the muscle tissue from Kazanlı was in the third rank in three studies, which include the current study, the study by Kaska et al. (2004) along the Turkish Mediterranean coast, and the study of eastern Italy (South Adriatic Sea) by Storelli et al. (1998) (see Table 1). However, the mean concentrations of As ($72.85 \pm 39.32 \mu\text{g g}^{-1}$ dry wt), Cu ($6.50 \pm 8.22 \mu\text{g g}^{-1}$ dry wt), and Mn ($1.53 \pm 0.81 \mu\text{g g}^{-1}$ dry wt) in the muscle tissue were higher than those detected in the other areas (see Table 1).

In Kazanlı nesting beach, the average levels of heavy metals found in the loggerhead sea turtles were similar to those previously reported from other Mediterranean areas for Cd, Cu, Pb, and Zn in liver tissue. The average level of Cr ($0.47 \pm 0.36 \mu\text{g g}^{-1}$ dry wt) in the liver tissue from Kazanlı was in the third rank in the three studies, which include the current study, Kaska et al. (2004), and Storelli et al. (1998) (see Table 2). The second highest level of Mn ($5.08 \pm 4.06 \mu\text{g g}^{-1}$ dry wt) in liver tissue was reported in our study from (see Table 2). However, the mean concentration of As ($37.60 \pm 37.61 \mu\text{g g}^{-1}$ dry wt) in the liver tissue was higher than the levels detected from the other areas (see Table 2).

It is possible that the small sample in our study (six turtles) could mask the results and the studied material could not be enough for interpretations and statistics even for small samples. In addition, the standard deviations of the mean concentrations of some metals (Cu and Cd in muscle, Cr, Mn and As in liver) are equal or even higher than the mean concentrations. However, when the available literature is reviewed, there are a number of studies with similarly small samples (4, 5, 6, 7 and 8 specimens), in which the standard deviations of the mean concentrations of these metals are equal or even higher than their mean concentrations, as can be clearly seen in Tables 1 and 2. The underlying reasons for such results could be discussed further, but at present they are generally accepted and used for comparative analyses in other studies - e.g., Franzellitti et al. (2004), Maffucci et al. (2005), Jerez et al. (2010), and D'Ilio et al. (2011).

In our study, in both muscle and liver tissues, the patterns of the mean concentrations of Zn, As and Cu have been ranked similarly as $\text{Zn} > \text{As} > \text{Cu}$ and another similarity is that Cu is at the end of this pattern in these two tissues but patterns for Pb, Mn and Cd differed between tissues. The patterns of the mean concentrations of heavy metals in muscle and liver tissues have been reported in previous studies, and

the results of our study and these other studies are compared in Table 3.

The range of mean values for elements in $\mu\text{g g}^{-1}$ on a dry weight basis in the liver and muscle tissues of loggerhead sea turtles from the Mediterranean Sea that was reported by D'Ilio et al. (2011) in an overview of data on international literature was compared to our study for Cu, Zn, Cr, Mn, Pb, As, and Cd. In the muscle tissue, the average levels of the heavy metals Zn, Pb, and Cd were within the range, but they were lower in the case of Cr and higher for As, Cu, and Mn. In the liver tissue, the average levels of the heavy metals Cu, Zn, Cd, and Pb were within the range, but they were lower in the case of Cr and Mn and higher for As, compared to the range of mean values in Mediterranean areas by D'Ilio et al. (2011) (see Table 4).

In general, heavy metal concentrations in liver and kidney tissue are higher than in the muscle tissue of loggerhead and green turtles (Sakai et al. 1995, Sakai et al. 2000). Additionally, the levels of the studied metals are generally higher in the liver than in other tissues (Storelli et al. 1998); however, As is present mainly in muscle tissue (Storelli et al. 1998, Storelli & Marcotrigiano 2000). D'Ilio et al. (2011) states that generally, the concentration of elements tends to be low in muscle tissue and Cu, Mn, and Pb have also been measured at very low levels in this tissue. On the other hand, Zn showed the highest average value in muscle tissue in an overview of literature on loggerhead sea turtles from the Mediterranean Sea, the Atlantic and the Pacific Oceans (D'Ilio et al. 2011). Saeki et al. (2000) reported that in all three species of turtles (green turtles, loggerhead turtles, and hawksbill turtles), the general order of As concentration in the tissues was muscle > kidney > liver. Jerez et al. (2010) observed the same pattern of distribution among the tissues of loggerhead turtles. Similarly, in our study, the order of As concentration in the two tissues was muscle > liver. According to Saeki et al. (2000) the high concentration of As in muscle tissue might indicate a specific metabolic mechanism of this element.

Storelli & Marcotrigiano (2000) reported that As was mostly present in its organic form, with percentages varying from 90.2% to 99.7% (avg 97%) in the muscle tissue and 81.4% to 94.2% (avg 89.4%) in liver tissue, and the concentrations of inorganic As in the liver tissue were about three times higher than in the muscle tissue of loggerhead turtles. The inorganic As decreased significantly with the increase of total As concentration in muscle tissue while in liver tissue, an equally significant but opposite trend was observed. In our study, the content of total inorganic and organic As in muscle and liver tissues was not determined. We only found the higher concentrations of As in muscle tissue ($72.85 \pm 39.32 \mu\text{g g}^{-1}$ dry wt), which was approximately twice as much as that found in liver tissue ($37.60 \pm 37.61 \mu\text{g g}^{-1}$ dry wt).

Godley et al. (1999) reported that differences in diet, the prevailing environmental contamination in their foraging ranges, the age of individuals sampled, or a combination of these may affect differences in heavy metal concentrations among sea turtle populations. Some authors have stated that the main source of exposure to metal accumulation in sea turtles is their diet (Caurant et al. 1999, Franzellitti et al. 2004). McKenzie et al. (1999) showed that the differences in contaminant concentrations among species were possibly re-

lated to diet, with the varied diet of the loggerhead turtle and its higher position in the marine food web giving rise to greater exposure to contaminants than green turtles. Furthermore, they report that in green turtles, the highest contaminant burdens are found in juvenile animals, but as the individual grows and there is a decrease in contaminant intake, the initial concentrations are diluted. Gardner et al. (2006) stated that maximum exposure might occur early in the life cycle when the green turtles' food consumption is of organisms that are from a higher trophic level. However,

Table 3. The pattern of heavy metal concentrations ($\mu\text{g g}^{-1}$ dry wt) in the muscle and liver tissues of loggerhead turtles from different locations; data are expressed as mean values \pm SD; N: number of samples; BDL: below detection limit

	Location	N	The pattern of metals mean concentrations	References
Muscle	Kazanlı-Turkey	6	Zn>As>Cu>Pb>Mn>Cd>Cr	Present study
	Mediterranean coast-Turkey	32	As>Cd>Pb>Cu>Cr	Kaska et al. (2004)
	Northern Cyprus	4	Pb>Cd	Godley et al. (1999)
	Italy	10	Zn>Cu>Mn>Cd and Pb (BDL)	Andreani et al. (2008)
	West Italy	24,26,26	Zn>Cu>Cd	Maffucci et al. (2005)
	East Italy	12	As>Cr>Cd>Pb	Storelli et al. (1998)
	West France	21	Zn>Cu>Cd	<u>Caurant et al. (1999) *</u>
	East Spain	13	Zn>As>Pb>Cd	Jerez et al. (2010)
	Andalusia (Spain)	20	Zn>Cu>Pb>Cd	García-Fernández et al. (2009)
	Mexico	5	Zn>Mn>Cu>Cd>Pb	Gardner et al. (2006)
	Japan	7	Zn>Cu>Cd	<u>Sakai et al. (1995) *</u>
	Japan	7	Zn>Cu>Cd	<u>Sakai et al. (2000) *</u>
	North Pacific	4	As	Saeki et al. (2000)
	Liver	Kazanlı-Turkey	6	Zn>As>Cu>Mn>Cd>Pb>Cr
Mediterranean coast-Turkey		32	As>Cd>Pb>Cu>Cr	Kaska et al. (2004)
Northern Cyprus		4	Cd>Pb (BDL)	Godley et al. (1999)
Italy		11	Zn>Cu>Mn>Cd>Pb	Andreani et al. (2008)
West Italy		14	Zn>Cu>Cd	Maffucci et al. (2005)
East Italy		12	As>Cd>Pb>Cr	Storelli et al. (1998)
West France		7	Zn>Cu>Cd	<u>Caurant et al. (1999) *</u>
East Spain		13	Zn>As>Cd>Pb	Jerez et al. (2010)
Andalusia (Spain)		16	Zn>Cd>Cu>Pb>	García-Fernández et al. (2009)
Mexico		5	Zn>Cu>Cd>Mn and Pb BDL	Gardner et al. (2006)
Japan		7	Zn>Cu>Cd>>	<u>Sakai et al. (1995) *</u>
Japan		7	Zn>Cu>Cd	<u>Sakai et al. (2000) *</u>
North Pacific		4	As	Saeki et al (2000)
Australia		5,8,6	Zn>Cd>As	<u>Gordon et al. (1998) *</u> Jerez et al. (2010) **

* In these studies, the values reported in wet weight were already converted to dry weight using the mean water content as determined by Maffucci et al. (2005). ** Only, As value for liver reported in wet weight were converted to dry weight by Jerez et al. (2010) adapting the mean water reported by Maffucci et al. (2005). So, these converted values were used in the Table 3 in our study.

Table 4. The comparison of the mean (\pm SD) values of heavy metal concentrations ($\mu\text{g g}^{-1}$ dry wt) in the liver and muscle tissues of six dead loggerhead turtle collected from Kazanlı beach and the range of mean values in Mediterranean areas presented by D'Illo et al. (2011). (T: tissues; N: sample size; E: elements; a: median)

T	E	The mean values in this study	State of comparison	The range of mean values in Mediterranean areas (D'Illo et al. 2011)
Muscle	Cr	0.39 \pm 0.49	<	1.4
	Mn	1.53 \pm 0.81	>	1.4 \pm 0.24
	Cu	6.50 \pm 8.22	>	1.5–5.0
	Zn	91.09 \pm 33.01	In the range	7.1–107
	As	72.85 \pm 39.32	>	20.8–69
	Cd	0.48 \pm 0.91	In the range	0.2–3.6
	Pb	2.00 \pm 1.29	In the range	0.5–2.5 ^a
Liver	Cr	0.47 \pm 0.36	<	1.1–2.8
	Mn	5.08 \pm 4.06	<	7.5
	Cu	27.72 \pm 15.83	In the range	3.0–37.3
	Zn	83.21 \pm 52.17	In the range	66.0–107
	As	37.56 \pm 37.61	>	14.2–22
	Cd	4.59 \pm 2.98	In the range	2.4–23
Pb	2.73 \pm 0.93	In the range	0.1–3.6	

according to Tomas et al. (2001) there are three phases: the pelagic phase, the benthic-demersal phase, and an intermediate neritic phase in loggerheads' developmental shift from pelagic-oceanic to benthic-neritic foraging habitats. During the last phase, loggerheads feed upon both pelagic and benthic prey. Franzellitti et al. (2004) reported that being at the top of its food web, *C. caretta* could be exposed to high levels of heavy metals resulting from bioamplification processes across the trophic chain. Based on previous studies (Gordon et al. 1998, Saeki et al. 2000), Jerez et al. (2010) believed that the fluctuation in the results suggests differences between the base levels of As contamination in the different areas studied or in the feeding habits of the individual specimens.

In the light of the ideas reported by the above-mentioned authors, in our study, we hypothesize that the concentrations of As and other heavy metals in loggerhead turtle tissues could depend mainly on their feeding habits in different areas. These areas are not only Kazanlı beach on the Turkish Eastern Mediterranean coasts, but also in different areas other than our study area. Additionally, we believe that the higher average levels observed in our loggerhead specimens (As in both muscle and liver tissue) may be indicative of chronic exposure. Of course, a question then arises: in which areas did these six loggerhead turtles feed in their lives? Based on the numbers and spatial distribution of dead sea turtles washed onto the shores in Turkey, Kasperek & Baran (1989) report that immature green turtles stay more or less around their hatching beach and later reproduction grounds whereas loggerhead turtles apparently migrate to a much greater extent. Green and loggerhead turtles exhibit high levels of fidelity to migratory routes, foraging areas, and wintering sites, both between and within years and after successive breeding migrations (Broderick et al. 2007). Additionally, Broderick et al. (2007) stated that their study females tracked for two consecutive migrations used highly similar routes to return to the same foraging locations. Many of the females in their study passed through suitable foraging habitats on their migration, and indeed some passed through areas used by other conspecific study animals. Broderick et al. (2007) explained the reasons for the turtles returning to a specific site - food resource limitations, territorial defense, or perhaps the proximity of good over-wintering sites can all define site selection. Alessandro & Antonello (2010) showed loggerhead migrations in the Mediterranean Sea, including the main routes, nesting beaches, and pelagic and demersal areas based on previous studies (Bentivegna 2002, Camiñas 2004, Maffucci et al. 2006, Broderick et al. 2007).

Similarly, Uçar et al. (2018) reported that on May 14, 2017, a dead-stranded sub-adult loggerhead turtle was found on Davultepe 100. Yıl (Gümüşkum) nesting beach in Mersin Bay on the eastern coast of Turkey. The turtle's journey covered the area between the Island of Ventotene (southwestern Italy) in the Tyrrhenian Sea and its final location in Turkey in the Eastern Mediterranean Sea. Uçar et al. (2018) stated that this loggerhead might actually have been foraging in the Tyrrhenian Sea and returning to the nesting area where it hatched in Turkey.

Considering the results of Alessandro & Antonello (2010) and the route of loggerhead in the study of Uçar et al. (2018), two main conclusions for the life cycles of the turtles in our

study could be made. (1) In the pelagic phase (summer), these loggerhead turtles may migrate from the Levantine Basin (Turkey) in the Eastern Mediterranean to the Ionian Sea in Italian waters in the Central Mediterranean during the spring migration; (2) in the demersal phase (winter), these loggerhead turtles may migrate from the Ionian Sea in Italian waters in the Central Mediterranean to the Levantine Basin (Turkey) in the Eastern Mediterranean during the autumn-winter migration.

Franzellitti et al. (2004) suggested that specific heavy metals could be used as "environmentally acquired markers" to investigate the feeding areas frequented by sea turtles and also as indicators of the animal-environment interaction. Mean values for As in the muscle and liver tissues in our study were compared to the As concentrations in turtles from the Mediterranean coasts of Turkey (Kaska et al. 2004) and eastern Italy (South Adriatic Sea) (Storelli et al. 1998). In the muscle tissue in particular, the mean concentration of As ($72.85 \pm 39.32 \mu\text{g g}^{-1}$ dry wt) in our study was higher than the level of As ($20.80 \pm 9.97 \mu\text{g g}^{-1}$ dry wt) recorded by Kaska et al. (2004) on the Mediterranean coast of Turkey. Additionally, the second highest level of As ($68.94 \pm 45.80 \mu\text{g g}^{-1}$ dry wt), which was not too different from our result, was recorded by Storelli et al. (1998) in eastern Italy (along the Apulian coasts, South Adriatic Sea) in Mediterranean areas (see Table 1). In liver tissue, the mean concentration of As ($37.60 \pm 37.61 \mu\text{g g}^{-1}$ dry wt) in our study was higher than the level of As ($14.23 \pm 7.60 \mu\text{g g}^{-1}$ dry wt) recorded by Kaska et al. (2004) on the Mediterranean coasts of Turkey. The second highest level of As ($21.67 \pm 17.22 \mu\text{g g}^{-1}$ dry wt) in liver tissue was recorded by Storelli et al. (1998) in eastern Italy (along the Apulian coasts, South Adriatic Sea) in Mediterranean areas (see Table 2). Godley et al. (1999) believed that further monitoring of metal burdens in marine turtles in the Mediterranean Sea region would be prudent, especially from those that range into more intensively industrialized regions such as Spain, Italy, and Greece. Therefore, in our study, the female loggerhead turtles appear to have passed through suitable foraging habitats between the Central and Eastern Mediterranean. Additionally, these female loggerhead turtles might actually have foraged in Italian waters and the Central Mediterranean, and then returned to the nesting area in Turkey where they hatched. The concentrations of heavy metals in the loggerhead turtles' tissues in our study may depend mainly on their feeding habits during their different life cycles and may be indicative of chronic exposure in the areas mentioned above.

The amount of data available on the chemical contamination of sea turtles and the effects on their health is still relatively limited (D'Illo et al. 2011). Kaska et al. (2004) reported that the levels of essential elements such as Fe and Cu were found to be the highest in most of the stranded loggerhead and green turtle specimens from the southwestern coast of Turkey, with significant levels of other heavy metals (Cd, Pb, Cr, Ni, Se, Sb, and As) also present, which would likely affect the health of these endangered turtle species in the Mediterranean. We were able to compare the study of Kaska et al. (2004) to our study for Cu, Cr, Pb, As, and Cd, both in muscle and liver tissues; the mean concentrations of As and Cu in our study were higher, and the mean concentrations of Cd, Pb, and Cr were lower than the levels in Kaska et al.

(2004). According to the compared results and the view of Kaska et al. (2004), we agree that the significant levels of heavy metals found to be present in turtle tissues are likely to affect the health of loggerhead turtles in the Mediterranean. Additionally, on Kazanlı beach, located near the industrialized city of Mersin, the concentrations of heavy metals (Cd, Pb, Fe, Cr, Ni, Se, Sb, As, and Cu) were investigated in the nesting environment of green turtles and in the tissues of plants growing on the beach and in the adjacent environment (beach sand, sea ground sediment, and sea grass as well as eggshells from the nests), and the results showed no significantly high levels of heavy metals (Çelik et al. 2006). In addition, Çelik et al. (2006) also stated that herbivorous green turtles might be affected by heavy metal concentrations in the future since they feed mainly on sea grass, and the accumulation of heavy metals via rivers into the sea might cause some pollution problems. In another study on Kazanlı, Sangün & Özdilek (2007) stated that the extensive Cr and Ni levels found in soil that had been used for building material is likely to impair public and environmental health in the area, including that of sea turtles. A toxicological risk assessment based on water quality criteria indicated that aquatic toxicity from both acute and chronic Ni and Cr does not exceed estimated ecotoxicology levels (Sangün & Özdilek 2007). Nevertheless, the toxic effects of heavy metals and pollutants on both loggerhead and green turtles must be investigated.

Heavy metal correlations between tissues

García-Fernández et al. (2009) measured metal levels among the different tissues (liver, kidney, pectoral muscle, brain, and bone) of loggerhead turtles from the southwestern Mediterranean coastline (Andalusia, Spain), and found positive correlations between hepatic and renal Cd ($p=0.03$, $\rho=0.718$) and hepatic and renal Zn ($p=0.06$, $\rho=0.673$). Jerez et al. (2010) examined dead loggerhead sea turtles from the Western Mediterranean shores of Murcia (Spain), and established the concentrations of six elements (Hg, Cd, Pb, As, Zn, and Se, all potentially toxic at high concentrations) in different tissues and organs (liver, kidney, muscle, bone, blood, central nervous system, and skin). They found a statistically significant positive correlation between Zn in liver and kidney tissue ($p<0.05$) and a statistically significant negative correlation between Se in muscle and liver tissue ($p<0.05$).

Along the Mediterranean coast of Turkey, differences in Cr ($p=0.000$) and Cu ($p=0.001$) concentrations among the tissues (liver, bladder, kidney, lung, muscle) of loggerhead turtles have been reported, and no statistical differences in Pb ($p>0.05$) concentrations among the tissues of loggerhead turtles could be found. Ni, Fe, Se, Cd, Sb, and As were statistically different among the tissues of loggerhead turtles (Kaska et al. 2004). However, in our study, a significant difference between muscle and liver tissues ($p<0.05$) was detected only for the concentration of Pb, and not for other element concentrations (Cu, Zn, Cr, Mn, As, and Cd). Additionally, from the southern Tyrrhenian coast of Italy (Western Mediterranean Sea), Maffucci et al. (2005) reported that sex had no significant influence on trace element (Cd, Cu, Hg, and Zn) concentrations in the liver, kidney, and pectoral muscle tissues of loggerhead turtles. In our study, the influence of sex on the correlations of heavy metal concentrations in tis-

sues was not tested because all of our specimens were female.

The correlations of heavy metal concentrations in tissues and turtle size

In several studies, the influence of turtle size on element concentrations in tissues has been evaluated using standard carapace length (Saeki et al. 2000, Gardner et al. 2006, García-Fernández et al. 2009, Jerez et al. 2010), MSCL (Franzellitti et al. 2004) and CCL (Maffucci et al. 2005). Saeki et al. (2000) reported that for green turtles, the As concentrations in muscle tissue decreased significantly with SCL ($P<0.005$), but the relationship between As and SCL could not be determined in the loggerhead and hawksbill turtles due to the small sample size. García-Fernández et al. (2009) tested for correlation between metal concentrations (Zn, Cd, Pb, and Cu) in each tissue (liver, kidney, pectoral muscle, brain, and bone) and loggerhead turtle SCL, and the only positive results was for Zn in muscle tissue ($p=0.017$, $\rho=0.59$), where one would expect Pb and Cd to accumulate with age. Gardner et al. (2006) analyzed the correlation between metal concentrations (Pb, Fe, Cd, Ni, Cu, Zn, and Mn) in each tissue (liver, kidney, adipose, and pectoral muscle tissues) and the SCL of loggerhead turtles from northwestern Mexico. Positive correlations with SCL were detected for Cd and Cu measured in kidney tissue, and the Ni concentration was positively correlated with the SCL in both kidney and liver tissue. Jerez et al. (2010) did not register correlation between the concentrations of the elements (As, Cd, Hg, Pb, Se, and Zn) analyzed in different fluids and tissues (liver, kidney, muscle, bone, blood, central nervous system, and skin) of loggerhead sea turtles and the phase of development. This was probably due to the unequal distribution of individuals in the classification of body size (SCL) and the limited number of specimens available. However, the higher average levels observed in adult specimens (Pb in bone; Cd in kidney; As in kidney; Cd, Hg, Pb, and Zn in muscle; As, Hg, Pb, and Se in central nervous system tissue) may be indicative of chronic exposure (Jerez et al. 2010). In our study, the influence of size on the element concentrations was not tested using SCL. Instead, a highly negative linear relationship ($p<0.05$) was found between the concentration of As in muscle and turtle size (CCL, CCW, MSCL, and SCW), but the other element concentrations showed no apparent correlation with turtle size ($p>0.05$).

In their study along the Adriatic Sea coast, Franzellitti et al. 2004 reported a negative correlation ($p<0.05$) between MSCL and Zn in liver tissue while Cd, Cu, Fe Mn, and Ni showed widely variable values with no apparent correlation with MSCL. No statistically significant correlation was noted in muscle between metal concentrations and the size of the individuals (Franzellitti et al. 2004). In our study, in accordance with Franzellitti et al. (2004), the six female loggerhead turtles were classified into only one size class, with benthonic sub-adults and adults, depending on the MSCL (55–65 cm). Therefore, the relationship between heavy metal concentrations in muscle and liver tissues and the size classification of the loggerheads was not evaluated depending on the MSCL range. Furthermore, the turtles' ages were not determined. Therefore, neither size nor age were used to evaluate a potential relationship between heavy metal concentrations

and growth.

From the southern Tyrrhenian coast of Italy (Western Mediterranean Sea), Maffucci et al. (2005) reported that Cd, Cu, Hg, and Zn concentrations in the liver, kidney, and pectoral muscle tissues of loggerhead turtles were not influenced by the size (CCL) of the specimen except for Se in the liver, which was negatively correlated with the CCL ($p < 0.001$). Additionally, they stated that the size effect in their 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, respectively). In our study, the concentrations of Cu, Zn, Cd, Cr, Mn, and Pb showed no apparent correlation with the CCL and the other carapace measurements (CCW, MSCL, and SCW) ($p > 0.05$). A highly negative linear relationship ($p < 0.05$) was found only between the concentration of As in muscle tissue and CCL, CCW, MSCL, and SCW.

A relationship between heavy metal concentrations and growth was not observed in our samples due to the limited number of specimens classified into only one size class and the absence of young and very old turtles. However, in parallel with Jerez et al. (2010), we believe that the higher average level of As in the muscle tissue in our specimens (ben-thonic sub-adults and adults) may be indicative of chronic exposure.

Causes of stranded loggerhead turtle mortalities

Clustering of loggerhead turtles strandings based on the season. The highest stranding rates during the summer months, with numbers varying between 10–95 strandings per year in the area used as a foraging habitat by loggerhead turtles, were observed by Affronte & Scaravelli (2001) in the north-western Adriatic Sea. In recent studies on the coasts of Turkey, Yalçın Özdilek et al. (2018) reported that stranded loggerhead turtles were found most frequently in the summer (32.4%), followed by autumn (29.7%), spring (18.9%), and winter (18.9%). Başkale et al. (2018) stated that the stranded loggerhead turtles were found every month, but recordings peaked between June and August. On Kazanlı beach, stranded female loggerhead turtles were mostly found during the nesting seasons by our research team between 2009 and 2016 (in unpublished reports). This shows that in the breeding season, female individuals approached the nesting beaches but died for different reasons. Furthermore, sometimes outside of the nesting seasons, stranded female loggerhead turtles were found on the coasts of Mersin, e.g., the six dead-stranded female loggerhead turtles in our study were all found at the same time, on February 14, 2009.

Size and sex of loggerhead turtles. Kaska et al. (1998) reported that the number of male adults found was low, and this might be explained with the end of the mating season or males' preference for deeper waters as feeding areas and the fact that females generally dominate in hatchlings populations. Along the Mediterranean coasts of Turkey, in the summer of 2001 (Kaska et al. 2004), the majority of dead-stranded turtles were recorded on the easternmost beaches, such as Kazanlı (32 turtle carcasses), Erdemli (Mersin), and Samandağ (Hatay). Most of these turtles were loggerhead turtles, and only 16 of them were green turtles stranded on Erdemli and Samandağ beaches. Only two adult male green turtles were recorded at Erdemli (Mersin), and one adult

male was found loggerhead at Kazanlı (Mersin). All of the others were categorized as females or juveniles. Additionally, most of the stranded green turtles were either adults or juveniles close to being sub-adults, and all of the loggerhead turtles found stranded were adults or sub-adults (Kaska et al. 2004).

Yalçın Özdilek et al. (2018) observed that different sized and adult sea turtles were found stranded in the Marmara Sea, sub-adult sea turtles were stranded in the northern Aegean Sea; 15 (55.6%) of the loggerhead turtles were identified as adults and 12 (44.4%) as sub-adults. One of the adults was identified as male (6.6%). Başkale et al. (2018) stated that the majority of dead loggerheads in the Fethiye-Göcek Specially Protected Area (Western Mediterranean coast of Turkey) were adult females (49.02%), followed by immature individuals (22.55%), adult males (19.61%) and undetermined adults (8.82%). Sönmez (2018) reported that of the stranded loggerhead turtles found on Samandağ beach on the Eastern Mediterranean coast of Turkey, 29 (25.2%) of the individuals were adults, 81 (70.5%) were sub-adults, and five (4.3%) were in the oceanic stage. Two of the adult loggerhead turtles were males. In the previous studies on Kazanlı beach, strandings represent mainly adults and sub-adults (Aureggi 2001, Türkozan et al. 2013). Similarly, in our study, all of the dead-stranded loggerhead turtles were adults and sub-adults, and all of the stranded samples were female.

Fishing activities that might cause the death of turtles. On the Mediterranean coast of Turkey, the leading causes of death and injury in loggerhead turtles and green turtles were determined as fishing activities, incidental catching in fishing equipment, getting caught by trawling nets, intentional harm cases or deliberately killing (mainly due to head trauma) by fishermen on the grounds that they damage nets, spoil catch or remove bait, marine pollution, marine vehicle collisions and undetermined reasons (Oruç et al. (1997), Godley et al. (1998), Kaska et al. (2004), Sönmez (2018), Başkale et al. (2018), Yalçın Özdilek et al. (2018)).

One interesting issue is that Kazanlı is one of the most important sites for green turtles, and a small number of loggerhead turtle nests are found regularly in the Mersin province in Turkey (Türkozan & Kaska, 2010). However, most dead-stranded loggerhead turtles were found on this beach (Aureggi 2001, Kaska et al. 2004, Türkozan et al. 2013). This, of course, raises some questions: (1) Although Kazanlı beach is the nesting beach of green turtles, why are most dead-stranded loggerhead turtles found on this beach? (2) Is there a possibility that loggerhead turtle individuals died off the coast of Mersin and drifted towards Kazanlı beach due to onshore currents? For Kazanlı beach, Aureggi (2001) reported that the cause of death and the region they came from were unknown because most of strandings had already died several days earlier, and the turtles were washed on the beach already decomposed. Likewise, in our study, the dead-strandings were loggerhead turtles, and an advanced state of decomposition and decay was observed in the samples. No holes were observed on the heads or carapaces, and during the necropsy, no hooks, plastic bags, etc. were found in the digestive tracts. However, the study of Kaska et al. (2004), which also includes Kazanlı beach, reported that the main causes of turtle mortalities were fishing-industry related injuries, with trawl nets drowning turtles, and around 80

adult turtle deaths could be attributed to incidental captures in one fishing season along the Turkish coasts. Furthermore, Kaska et al. (2004) speculated that the carnivorous loggerhead turtles' and fishing activities overlap and cause many incidental captures since the majority of the stranded turtles were loggerheads. Green turtles are vegetarians and prefer sea-grass beds for feeding.

As a result, in light of the data reported by the above-mentioned authors who performed research on the coasts of Turkey, the leading cause of loggerhead turtles strandings was fishing activities. In parallel with the data from these studies, we suspect that the six dead-stranded female loggerhead turtles in our study, which were found at the same time on February 14 (i.e., outside of the trawling ban, between April 15 and August 31), encountered trawl nets and died by drowning off the coast of Mersin, and then drifted to Kazanlı beach due to onshore currents.

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