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The trace element concentrations in tissues from the loggerhead sea turtle (*Caretta caretta*) from different life stages in Türkiye

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Abstract. Sea turtles can bioaccumulate essential and nonessential trace elements, making them useful bioindicators of the marine ecosystem. We analyzed the 11 trace element concentrations in kidney, liver, and muscle samples (n=175) of stranded loggerhead sea turtles, *Caretta caretta* (n=70). Our results for Fe, Cu, Zn, and Mn were lower, but Pb, Ni, and Cd levels were higher than those reported in the literature. Given the high levels of loggerhead turtles in specific foraging areas, long-term, concurrent trace element monitoring studies on a larger geographic scale are necessary to understand trace element exposure levels in loggerhead turtle tissues. Conservation of long-lived animals, such as sea turtles, depends on the sustainable monitoring of the pollutants in their environment. Although metal levels were lower than those reported in the literature, we view the Mediterranean Sea as a vast lake into which the 20 countries surrounding it discharge contaminants, and we recommend continuing temporal and spatial monitoring of the impact of pollutants on sea turtles.

Keywords: ecotoxicology, pollution, temporal variation, trace elements, Mediterranean.

Introduction

Essential and trace inorganic elements have critical physiological functions, but they can become toxic when they reach levels above acceptable levels (Keller et al. 2006). Maximum allowable concentrations in seawater are set by regulations; for example, lead (Pb) is set at 14 μg L−1; however, concentrations exceeding these levels are toxic (European Commission 2013). Coastal areas are affected by pollution from industry, commerce, agriculture, tourism, and urbanization. One of the prime causes of pollution is heavy metals and other non-essential elements in the environment resulting from intensifying human activities, such as metal mining, smelting, industrial activities, and foundries (Briffa et al. 2020). The bioaccumulation of trace elements, especially nonessential ones, has become a concern due to the potential for their transmission to the food chain, with the likelihood that this will negatively impact sea turtles' health (Aguirre et al. 2006, Ley-Quiñónez et al. 2011, Cortés-Gómez et al. 2017). Sea turtles have a long lifespan and immense migration ranges. They use different habitats during different life stages and are exposed to many anthropogenic threats. Thus, it is possible that sea turtles accumulate diverse contaminants over time and can therefore be used as bioindicators (Godley et al. 1999, Sakai et al. 2000).

Increased anthropogenic activities lead to higher levels of inorganic pollution in the sea. The sources of heavy metals in the marine environment include transportation and deposition of pollutants from the atmosphere; river discharges from land-based sources; discharges from mining; leaching from dumps and former industrial sites; marine vessels; and submarine activities, in different aquatic habitats (Singh & Steinnes, 2020). Monitoring trace element concentrations in sea turtles is considered a research priority, as they are long-lived organisms that feed at a high trophic level and may be subject to bioaccumulation and biomagnification of persistent toxic compounds (Hamann et al. 2010, Rees et al. 2016). Trace element concentrations in the various tissues of different sea turtle species have been the subject of several studies (Cortés-Gómez et al. 2017, Ross et al. 2017, Casini et al. 2018). The levels of pollutants vary among different geographic locations, seasons, trophic levels, tissues, body condition, sex, and age class (Keller 2013).

The loggerhead turtle (*Caretta caretta*, Linnaeus, 1758) is classified as vulnerable in the International Union for the Conservation of Nature (IUCN) Red List (Casale & Tucker 2015) and is the most common sea turtle species in the Mediterranean, in which it utilizes a variety of marine habitats (Casale et al. 2018). They are a generalist carnivorous species that feeds on a variety of prey (Bjorndal 1996). Thus, they are more prone to exposure to pollutants originating in different countries. The Mediterranean Sea, surrounded by 23 countries, is the world's largest semi-enclosed basin, with petrochemical and steel industries, large commercial and tourist harbors, domestic and industrial sewage, agricultural activities, and a high shipping rate (Casini et al. 2018), and is polluted by different contaminants (Aguilar et al. 2002). These are all sources contributing to pollution in the Mediterranean basin, which may overlap with loggerhead sea turtle habitats.

The European Union's Marine Strategy Framework Directive (MSFD) has determined 11 descriptors to achieve a Good Environmental Status (GES) in European waters by

2020 (EC, 2017). Descriptor 8, defined as "Concentrations of contaminants are at levels not giving rise to pollution effects," the second criterion of the descriptor concerns the health of species and the condition of habitats. Although the main nesting sites of the loggerhead turtle are in the eastern Mediterranean, they migrate to different coastal neritic areas for foraging, including the western Mediterranean (Casale et al. 2018). They show high fidelity to their foraging areas (Broderick et al. 2007, Schofield et al. 2010, Rees et al. 2013, Haywood et al. 2020). Due to these characteristics, the loggerhead turtle is a good biological indicator for monitoring heavy metal pollution within the MSFD. The loggerhead turtle (Matiddi et al. 2019) and the green turtle (Sinaei et al. 2021) have been used as indicator species to monitor the pollution within the MSFD regions. Türkiye has one of the largest breeding loggerhead turtle colonies in the Mediterranean (Casale et al. 2018), which uses several foraging areas widely distributed across the Central Mediterranean (Cerritelli et al. 2022). As a result of their migration patterns, the loggerhead turtle population in Türkiye is an excellent representative sample for providing information about trace element pollution in the Mediterranean. Although Türkiye has a large number of nesting beaches along the Mediterranean coast and many studies have been conducted on stranded sea turtles (Türkozan et al. 2013, 2018, Tonay & Oruç 2016, Başkale et al. 2018, Sönmez 2018), which provide an excellent opportunity to study their biology and threats, especially on trace elements, studies on trace element accumulation in sea turtles in Türkiye are limited (Kaska et al. 2004, Yipel et al. 2017, Aymak et al. 2021).

Most literature reports trace element concentrations in sea turtle tissues from a region over a given period, while reviews compare or analyze metadata across different areas or species. When an animal is used as a bioindicator for long-term pollution monitoring, assessing temporal trends is essential. In this study, we aim to determine the differences of accumulation for 11 trace elements in the loggerhead sea turtles' kidney, liver, and muscle tissues, the same tissues sampled in the Kaska et al. 2004 study, from samples collected during the years of 2008 and 2016, to provide data for the long-term monitoring of pollutants on subadults and adults, males and nesting females stranded in the Dalyan region. Our aim is also to determine the maternal transfer levels of metals into the eggs and hatchlings.

Materials and methods

We collected stranded turtles along Dalyan beach, Mugla, Türkiye, in 2008 and 2016. The body decomposition of the sea turtles was assessed, and kidney, liver, and muscle samples from the turtles in good decomposition status, as determined by the methods used in the Darmon et al. (2022) study, were collected during necropsy. Sterilized laboratory equipment was used to prevent contamination. We classified the collected samples as adults if the curved carapace length (CCL) was greater than 65 cm, as in a recent study from the same region (Sözbilen et al. 2021). An individual with a carapace below 65 cm was categorized as subadult. The sex of adult individuals was determined according to the study by Wibbels (1999). A total of 21 subadults and 49 adults (30♀ + 19♂) samples were collected based on the total stranded turtles available in those years. We also collected 21 randomly selected hatchling samples (one from each nest) based on availability in 2016, along with yolk, liver, muscle, and eggshell samples, to assess possible metal levels from Dalyan beach, Türkiye. The randomization was done to ensure samples were collected from nests in different parts of the beach.

All the examined tissue samples were removed from the carcasses during necropsies and transferred to sterile vials; however, collection of all three tissues was not possible for all turtles, so not all tissue types were analyzed for some turtles. We analyzed 175 tissue samples from dead loggerhead turtles collected in 2008 and 2016. The samples were then dried in an oven at 50℃ for 4-7 days, to a constant weight. The dried tissue aliquots (0.050 - 0.500 mg) were placed in 50 ml round-bottom flasks and digested by adding 10 ml 65% HNO_3 (Merck, Suprapur grade) and treating them overnight on a hot plate in a fume cupboard until they looked clear. After cooling, the clear samples were diluted to 10 ml using distilled water.

The concentrations of chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), iron (Fe), selenium (Se), cadmium (Cd), antimony (Sb), zinc (Zn), manganese (Mn) and Arsenic (As) in the tissues were measured using the same methods from the previous, Kaska et. al 2004 paper, by flame atomic absorption spectrophotometry (Perkin Elmer Analyst 200 Model). A flame atomic absorption spectrometer (PerkinElmer AAnalyst 200) equipped with a deuterium lamp background corrector and an air-acetylene flame was used for burning. The acetylene flow rate was 2.5 mL min^{-1} and the nebulizer flow rate was 10.0 mL min^{-1}. The operating parameters for working trace elements were set as recommended by the manufacturer.

Certified reference materials, blanks, and replicate samples were used for quality control. Quantitative analysis was performed using a four-point linear calibration of a solution containing trace metals. Calibration standard solutions of the above elements were prepared by stepwise dilution of each certified standard trace metal solution at 1000 mg L−1 (TraceCERT® CRM, certified reference metals for AAS, Sigma-Aldrich). The coefficients from the calibration lines were>0.9971. Recovery and reproducibility were at high levels (>91%). Procedural blanks were analyzed for each batch of four samples. The analysis results were reliable when the repeat-sample (3-sample) analysis error was below 5%, and the analytical precision for replicate samples was within ±9%. The concentrations of trace elements in diluted samples were determined directly from calibration graphs, after correction for the absorbance of an appropriate reagent blank. All of the samples were analyzed in triplicate for trace element concentrations. The metal concentrations were calculated by multiplying the spectrometer value by a 10-fold dilution factor and dividing by the tissue dry weight.

Due to the number of samples available, the data were normally distributed (Kolmogorov–Smirnov D test; all $P > 0.05$), allowing comparisons using parametric tests. Accumulation levels in the tissues of male and female, and adult and immature turtles, were compared with the Student's t-test. Homogeneity of data for all trace elements and tissues was tested with the Levene test, and all samples showed homogeneous variance ($P > 0.05$). We used a two-way ANOVA to determine whether element levels differed among tissues and sampling years (Kaska et al. 2004). If any differences were found, Tukey's HSD post hoc test was used to identify the groups that differed. The hatchling and adult tissues were compared with a t-test. All statistical analyses were performed with Minitab version 18.1.

In this study, all analyses were performed on a dry-weight (dw) basis, and results are reported on a dw basis. However, trace element concentrations were reported in wet weight (ww) in most of the literature (Cortés-Gómez et al. 2017). Therefore, we converted our results into ww to compare with previous studies. We used the moisture content of 64% for the kidney, 75% for the liver, and 80% for the muscle, in accordance with the study by García-Fernández et al.

(2009). These values were cross-checked with a previous study that found similar average moisture contents in loggerhead sea turtle tissues in Cyprus: 78% for the liver, 72% for the kidney, and 79% for the muscle (Godley et al. 1999).

Results

The number of sample distributions and their results of descriptive statistics are presented in Table 1. There was no difference in trace element concentrations between males and females in adult individuals (P>0.05). In addition, we found no statistical differences between adult and immature individuals across age classes (P>0.05). Therefore, we only present descriptive data for different sampling years. The mean CCL of the first sampling period was 67.43 cm ±9.90 (min= 47 cm, max= 80 cm) [31], and the mean CCL of the following sampling periods was 65.74 cm ±11.20 (min= 26.80 cm, max= 85.30 cm).

Analyses were conducted of the temporal distribution of trace element concentrations in kidney, liver, and muscle tissues. The mean concentrations of the elements in the kidney samples for the years studied are in the following sequence, from highest to lowest: As > Cd > Fe > Ni > Zn > Se > Pb > Sb > Cr > Cu > Mn. In the liver samples, the sequence of elements differs from that in the kidney samples: Fe > As > Se > Ni > Cd > Zn > Pb > Cu > Cr > Mn > Sb. The order of elements in the muscle samples was Fe > As > Ni > Se > Cd > Pb > Zn > Cu > Cr > Sb > Mn. Accumulation levels of each trace element significantly differed across all tissues, and only Ni levels differed among years. ($F_{2,\ 49}$=9.61; P<0.001). The kidney samples from 2008 had significantly higher Cu levels than those from 2016 ($F_{2,\ 49}$=30.50; P<0.001). We found statistically significant differences in Ni levels and Sb levels ($F_{2,\ 49}$=3.48; P<0.05) between the 2008 and 2016 kidney samples. Only Sb levels were significantly different in liver samples among the years ($F_{2,\ 67}$=20.89; P<0.001). The liver samples from 2008 had significantly lower Sb levels than those from 2016. The muscle samples from 2016 had significantly higher Sb levels ($F_{2,\ 50}$=15.81; P<0.001). We did not find any statistically significant differences in the remaining trace element concentrations across the different tissue samples over the years. The data relating to 21 eggs and hatchlings are presented in Table 2. We analyzed element levels in adults and hatchlings. Between the adult liver samples and hatchling liver samples, there are statistically significant differences in Fe (t=4.093; df=89; P<0.001), Cr (t=10.352; df=76; P<0.001), Ni (t=23.875; df=74; P<0.001), Se (t=17.239; df=89; P<0.001) and As (t=8.561; df=49; P<0.001). We also found statistically significant differences between the adult muscle samples and hatchling muscle samples for Fe (t=2.348; df=69; P<0.05), Cu (t=8.238; df=69; P<0.001), Cr (t=9.683; df=69; P<0.001), Ni (t=16.072; df=69; P<0.001), Cd (t=11.724; df=62; P<0.001), Pb (t=6.758; df=65; P<0.001), Se (t=16.891; df=69; P<0.001) and As (t=6.308; df=29; P<0.001).

Discussion

Generally, trace elements and heavy metals were used interchangeably, but they were classified by atomic weight. The term 'heavy metal' has been used to describe metallic chemical elements and metalloids that are toxic to the environment and to humans (Briffa et al. 2020). Some metalloids and lighter metals, such as selenium, arsenic, and aluminum, are also toxic. We also measured Chromium, Manganese, Iron, Nickel, Copper, Zinc, Arsenic, Selenium, Cadmium, Lead, and Antimony. Some of these metals, such as cadmium, nickel, and arsenic, can alter DNA repair mechanisms, whereas zinc, iron, and selenium can play vital roles in enzymatic activities (Walker et al. 2012). Antimony has no known biological role and can be toxic (Burford 2012). These essential elements can be divided into three groups: major elements needed for the body, macro minerals, and trace elements. Heavy metals can interact with nuclear proteins and DNA, potentially altering their structure. The toxicity of heavy metals depends on the metal and its nature, the biological role, exposure duration, and life-stage of the organisms, and it is also connected to other organisms through food webs. Environmental conditions can change at any time, and all biotas can be affected. These could be related to the different pollutants, changes in anthropogenic activities, climate change, or the introduction of new invasive species into the environment. The other life stages of sea turtles have been the subject of trace element monitoring (Savoca et al. 2022), as no long-term study data are available in the same population of sea turtles. Stranded animals give us an opportunity to understand the health status and threats to the endangered species. Determining the cause of death or injury is vital to identifying threats. However, this is usually problematic (Matiddi et al. 2019) because, in the marine environment, unhealthy animals are subject to multiple threats of varying degrees. This could be related to weakening of their immune system due to trace-element accumulation (Reinero et al. 2022) or to different levels of pollution, such as marine debris (Darmon et al. 2022). Based on previous studies (e.g., Sakai et al. 2000, Jerez et al. 2010), the fluctuation in results suggests differences in the baseline levels of metal contamination across the different areas studied or in the feeding habits of the individual specimens. García-Fernández et al. (2009) reported metal levels in the tissues (liver, kidney, pectoral muscle, brain, and bone) of loggerhead turtles from Spain. They found positive correlations between hepatic and renal Cd levels ($p = 0.03$, $\rho = 0.718$) and between hepatic and renal Zn levels ($p = 0.06$, $\rho = 0.673$). Jerez et al. (2010) also reported that tissues from dead loggerhead sea turtles in Spain showed 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). The differences may occur in different years within the same country.

The essential and non-essential elements we have examined can play important roles in metabolic processes, but they can also cause health problems by altering metabolic pathways. For instance, different forms of Cd, Cr, and Cu play complex roles in various metabolic pathways and can ultimately cause serious health problems, such as cancer, and generate free radicals (Valko et al. 2005). Cr also plays a role

Table 1. Descriptive statistics and statistical comparison of trace element concentrations and their comparison with literature. Bolded values indicate statistically significant differences between the years for the trace element in that tissue.

		KIDNEY					LIVER					MUSCLE					Max values reported	Reference
	Years	N	Mean	S.E.	Min	Max	N	Mean	S.E.	Min	Max	N	Mean	S.E.	Min	Max		
As	2008	4	17.11	3.440	7.14	22.89	4	14.92	0.542	13.99	16.13	5	22.21	3.960	8.34	32.57	72.85	Aymak et al 2021
	2016	10	17.40	2.720	3.11	31.48	11	15.05	0.298	13.58	16.69	5	23.83	2.890	16.43	33.63	68.94	Storelli et al 2005
Cd	2008	11	17.52	1.180	14.13	25.78	11	10.56	1.560	5.73	24.25	11	3.15	0.247	2.00	4.08	51.2	Jerez et al 2010
	2016	26	17.15	0.712	14.14	32.39	31	9.47	1.070	2.36	20.10	14	3.27	0.258	2.01	5.04	38.3	Sakai et al 2000
Cr	2008	11	2.33	0.088	2.05	2.92	11	2.17	0.043	2.06	2.57	11	1.28	0.156	0.34	2.45	2.77	Kaska et al 2004
	2016	26	2.26	0.033	2.04	2.78	31	2.30	0.017	2.15	2.50	14	1.46	0.159	0.51	3.12	1.43	Storelli et al 2005
Cu	2008	11	**0.48**	0.064	0.13	0.93	11	2.39	0.398	0.95	4.69	11	1.54	0.160	0.18	1.98	55.3	Sakai et al 2000
	2016	26	**0.66**	0.145	0.05	3.35	31	2.28	0.216	0.82	5.46	14	1.73	0.191	0.40	3.51	27.72	Aymak et al 2021
Fe	2008	11	16.03	1.460	9.92	22.81	11	16.25	3.360	3.19	34.31	11	22.53	3.600	4.82	44.86	37.96	Torrent et al 2004
	2016	26	17.10	0.868	9.54	26.13	31	17.83	2.540	0.87	57.58	14	23.44	6.210	3.67	79.17	36.83	Yipel et al 2017
Mn	2008	11	0.58	0.084	0.12	1.20	11	1.19	0.305	0.23	3.69	11	0.29	0.191	0.02	2.18	7.48	Andreani et al 2008
	2016	26	0.57	0.069	0.08	1.36	31	1.31	0.141	0.33	3.59	14	0.11	0.024	0.01	0.34	5.08	Aymak et al 2021
Ni	2008	11	**10.33**	0.070	10.05	10.78	11	11.52	0.960	8.50	17.61	11	9.91	0.807	4.05	14.54	5.81	Torrent et al 2004
	2016	26	**11.29**	0.174	8.24	14.28	31	12.62	0.117	11.53	14.20	14	10.40	0.863	3.14	15.56	5.23	Yipel et al 2017
Pb	2008	11	4.35	0.613	0.73	9.01	11	3.11	0.234	1.38	4.45	11	2.40	0.414	0.03	4.09	3.55	Kaska et al 2004
	2016	26	4.65	0.445	0.96	12.56	31	3.32	0.084	1.59	4.23	14	2.53	0.325	0.07	4.03	2.75	Jerez et al 2010
Sb	2008	11	**2.59**	0.114	2.21	3.39	11	**0.58**	0.083	0.17	1.11	11	**0.38**	0.074	0.05	0.76	24.70	Mondragon et al 2023
	2016	26	**2.75**	0.081	2.16	3.61	31	**0.79**	0.063	0.33	1.58	14	**0.97**	0.163	0.11	1.96	12.27	Mondragon et al 2023
Se	2008	11	7.89	0.555	6.03	11.74	11	12.38	0.733	8.86	18.85	11	8.22	0.292	6.40	9.53	7.52	Yipel et al 2017
	2016	26	8.20	0.355	1.48	12.74	31	12.74	0.418	8.90	21.55	14	8.49	0.435	6.13	12.20	5.98	Yipel et al 2017
Zn	2008	11	7.83	1.900	1.95	25.06	11	7.95	1.330	1.19	14.65	11	1.33	0.246	0.16	2.85	119.0	Sakai et al 2000
	2016	20	8.15	2.120	1.13	42.75	31	8.53	0.739	1.39	19.36	14	0.89	0.140	0.12	1.89	113.29	Jerez et al 2010

Table 2. Descriptive statistics of loggerhead turtle eggshell and hatchling tissues (N=21). Bolded values indicate statistically significant differences between the hatchlings and adults.

	Fe		Cu		Cr		Ni		Cd		Pb		Sb		Se	
	Mean	S. E.	Mean	S. E.	Mean	S.E.	Mean	S. E.	Mean	S. E.	Mean	S. E.	Mean	S. E.	Mean	S. E.
Hatchling Liver	**6.81**	1.060	2.16	1.176	**0.19**	0.027	**1.80**	0.315	4.62	1.060	0.15	0.023	0.52	0.069	**1.88**	0.216
Hatchling Muscle	**13.10**	2.390	**0.26**	0.202	**0.15**	0.029	**0.31**	0.060	**0.03**	0.006	**0.03**	0.005	0.40	0.117	**0.91**	0.218
Yolk	6.60	0.227	0.79	0.056	0.08	0.007	0.60	0.045	1.37	0.066	0.10	0.024	0.33	0.047	1.26	0.416
Egg shell	5.74	0.789	0.67	0.082	0.06	0.006	0.34	0.038	0.18	0.018	0.22	0.024	0.47	0.093	1.26	0.220

in calcium (Ca) metabolism and can alter Ca levels. The spleen, bone marrow, lungs, lymph nodes, liver, and kidneys are the organs that receive the most Cr. In our study, Cr levels were almost equal in the kidney and liver, but lower in the muscle. Pb is also a well-known toxic element that causes severe health problems, such as disrupting Ca metabolism, bone illness, cardiovascular issues, hypertension, and reproductive system problems (Atsdr 2007a). It is also one of the well-studied trace elements in all organisms and is known to cause various cancer types in humans due to its effects on metabolic pathways. Different animal models have shown that mechanisms of action similar to those in humans could be involved (Atsdr 2007b). Mn is an element that can cause oxidative stress by blocking ATP synthesis in the nervous system (Engwa et al. 2019). Ni is known to affect calcium channels in cells and can eventually cause deficiencies in cell growth and even apoptosis. Ni is generally found at higher levels in the brain, lungs, adrenals, kidneys, and liver. Ni is also known to have adverse effects on the reproductive system. Zn is one of the most important essential elements, serving as a coenzyme in approximately 300 enzymes, but exceeding exposure levels can also cause health problems such as anemia, digestive problems, and impaired immune function. Likewise, Se is another important essential element that plays a role in antioxidant metabolism in the body. At high levels, Se can cause problems in protein and cell metabolism. These metals are non-biodegradable and can remain in the environment for a very long time. They can also react with other elements, becoming more toxic (Walker et al. 2012). There is no such record for sea turtles, but the monitoring of such elements is usually carried out on stranded turtles, when we do not know the exact cause of mortality. The metals and their toxicity capacities are explained in detail by the study by Briffa et al. (2020).

The trace element accumulation levels in the kidney, liver, and muscle tissues of the loggerhead turtle population in Türkiye were lower than or in a similar range of levels previously reported from populations in different parts of the world (Franzellitti et al. 2004, Maffucci et al. 2005, Storelli et al. 2005, Andreani et al. 2008, Jerez et al. 2010, Cortés-Gómez et al. 2017, Novillo et al. 2017, García-Fernández et al. 2009). Although we found some variation among the three sampling years, there was no clear trend in trace element accumulation in most cases over the 8 years. In addition, trace element concentrations were significantly different across the three types of tissue samples examined, as previously reported (Cortés-Gómez et al. 2017) and referenced therein. Given the high fidelity of loggerhead turtles to specific foraging areas, we may assume that trace element contamination levels in the habitats used by the population from Türkiye did not change. This may be due to their foraging and migration patterns in the region, as well as to the fact that a significant portion of individuals use the western coasts of Türkiye in the Mediterranean (Sözbilen et al. 2021, Cerritelli et al. 2022). The loggerhead turtles nesting in Greece also use the feeding grounds on the western coast of Türkiye (Schofield et al. 2010, Patel et al. 2015, Rees et al. 2017). All sea turtle species prepare for reproduction while foraging (Miller et al. 2003). In this period, they store energy and required nutrients for vitellogenesis and egg production. After storing energy for migration for several years, they migrate to the breeding areas (Schroeder et al. 2003). When we take this into account, the bioaccumulation of heavy metals depends mainly on the sea turtles' foraging habitat, and it may take several years. The western coast of Türkiye is primarily composed of tourism regions with few extensive industrial facilities and only a small number of large rivers that could convey trace elements into the marine environment. Conversely, the eastern Mediterranean coast of Türkiye has industrial areas and large rivers that run off land-based pollutants. Studies conducted in this region reported that As, Cd, Cr, and Pb levels were considerably lower than our results, while Cu, Fe, Mn, Ni, Se, and Zn levels were higher in kidney and liver samples (Yipel et al. 2017). This difference may have occurred because sea turtle populations use different foraging areas in distant regions, and because the regions differ geographically. There are suitable feeding zones for loggerhead turtles in almost all neritic coastal areas of the Mediterranean (Almpanidou et al. 2022). Sea turtles may also store these toxic metals in their scutes, and females may be able to transfer them to their eggs, but as this was not part of our study, we focused only on the stranded ones. A study of loggerhead turtles stranded on the eastern Mediterranean coast of Türkiye showed that a significant portion of the individuals examined belonged to the loggerhead turtle population nesting in Cyprus (Türkozan et al. 2018). This may indicate that loggerhead turtles on the western coast of Türkiye use different foraging and nesting areas than those on the eastern coast. In addition, a recent study from Northern Cyprus investigated essential and nonessential elements in juvenile loggerhead and green turtles and found that nonessential elements were lower than our results for juvenile loggerhead turtles, especially for As, Cd, Cr, and Pb (Çelik et al. 2023). However, the individuals in our study are considerably larger than those in the North Cyprus study, and therefore, the results, including those for juveniles, being lower than those in our study may be considered normal for bioaccumulation.

We also found no differences in trace element concentrations across tissues, sexes, and age classes, except for Mn. Previous studies have shown that sex does not affect trace element accumulation (Register 2011). The majority of the foraging grounds of adult loggerhead turtles are located in the coastal neritic zones of the Mediterranean (Almpanidou et al. 2022). In addition, immature individuals sampled in this study were included in the age class settled in neritic areas, suggesting that adults and immature individuals feed on similar food sources in the same foraging areas.

The trace element concentrations in the loggerhead turtle tissues, especially nonessential ones in the northwest Mediterranean, were higher than our results; e.g., Cd levels in kidney, liver, and muscle (Franzellitti et al. 2004, Maffucci et al. 2005, Storelli et al. 2005, Andreani et al. 2008, Jerez et al. 2010, Cortés-Gómez et al. 2017, Novillo et al. 2017, García-Fernández et al. 2009). The essential elements, such as Se, Zn, Mn, and Fe, were reported at levels similar to or lower than those reported in the literature cited above. This could be related to foraging-ground preferences and even to individual differences in the use of essential elements and their metabolic activities. Bioaccumulation and bioavailability are considered jointly, and bioaccumulation was defined as the direct link between contaminants in the environment and exposed organisms (Wang 2016). As generalist carnivores, loggerhead turtles feed on diverse prey from different trophic levels, including jellyfish, crustaceans, fish, mollusks, tube worms, and even vegetation (Bjorndal 1996). Hence, they can be exposed to heavy metals from different available sources in the marine environment. Some elements may be related to their bioavailability in the foraging grounds rather than to individual size or their accumulation in tissues (Cortés-Gómez et al. 2017). Our results support the conclusion that heavy metal levels may be more closely related to environmental bioavailability. Growth rate is another critical factor that affects bioaccumulation and is influenced by environmental conditions, such as temperature and food availability (Wang 2016). The Mediterranean is expected to be among the regions most affected by climate change (Lionello & Scarascia 2018). Therefore, changes in environmental conditions, such as temperature, salinity, and food availability, can alter the rate of bioaccumulation of trace elements in the region. Thus, our results can serve as a baseline reference for future biomonitoring studies and for assessing changes in the marine environment. Sea turtles are ecotransformers, since they feed in the sea and transfer their eggs (yolk and eggshells) to the beaches. In our investigation of the effects of pollution, identified as one of the global research priorities (Hamann et al. 2010), our results clearly show significant differences between hatchlings and adults; however, further studies are needed to understand maternal transfer of pollutants better. Further studies can be conducted to determine whether contaminants in eggs and hatchlings were transferred directly from the mother or were due to localized pollution at the nesting site.

Sea turtle movement in the marine environment and their protection are significant. A recent study conducted a metadata analysis of large-scale tracking data from around the world and investigated connectivity among sea turtles' different habitats. The Mediterranean Sea is the smallest basin, where only 5% of sea turtles live, and sea turtle movements in the Mediterranean are comparatively shorter than those in other parts of the world (Kot et al. 2022). The Mediterranean Sea is bordered by 20 countries, and long-lived animals such as loggerhead turtles are found in each MSFD region, except the Black Sea. The Mediterranean Sea has changed over the last two decades, but our results on trace elements in loggerhead turtle tissues were stable in most cases. Although pollution levels have been increasing, due to human traffic and industrial development, transfer to large marine organisms may be slower than to smaller animals such as mollusks or small fish. Therefore, monitoring trace elements plays a crucial role in assessing pollution in the marine environment. As reviewed by Furness (2018), trace elements change in the aquatic environment. We can see that element levels in different species depend on their diets and on changes in diet over time. Sometimes changes in diet can lead to a reduction in some trace elements rather than an increase.

Most essential and non-essential elements examined in our study have adverse effects on the reproductive system. Although most studies on the impact of basic and non-essential elements on health have been carried out in humans and mammals, most animals use similar metabolic pathways. Higher-than-normal levels of trace elements in sea turtles could reduce or impair reproductive activities, such as eggshell formation, sperm quality, and other mineral processes, including Ca, an essential secondary messenger in all cells. This is particularly relevant in the presence of Ni or Mn. Therefore, monitoring such elements not only on stranded animals but also on living specimens in their natural environment, especially reproductively active ones, or at rescue centers, is very important for the conservation of sea turtles. Moreover, female sea turtles may also transfer some of the stored trace elements into their eggs and embryos. High levels of trace element contamination can negatively affect embryonic development and ultimately reduce hatching success. Even though our results alert us to any immediate emergency, further studies with larger sample sizes are needed. This, together with the potential effects of trace elements on reproductive systems, altered hatching success, and weakened immune systems, is a threat that could negatively impact the future survival rate of a "conservation-dependent species" such as the Mediterranean loggerhead sea turtle population.

Conclusions

Heavy metal pollution is gaining worldwide attention because these metals are present in the Earth’s crust but may emerge due to anthropogenic activities. These metals are discarded into the environment and end up in the oceans. Toxic metals, particularly metals that are non-essential, are harmful to all living things, even at low concentrations. Some of these metals affect biological activity, while others accumulate in different organs and may cause health problems for those organisms. Metals can serve as essential elements for enzymatic activities, be detoxified by proteins for long-term storage, or be excreted by organisms, as in mother turtles potentially transferring the metals to their eggs. Still, there is no such option for male turtles.

A total of 8 of 11 trace elements did not show temporal changes over 8 years. This indicates that there have been minimal changes in most metals, with some exceptions, in trace element accumulation in the foraging grounds of the loggerhead turtle population of the western Mediterranean coasts of Türkiye. The changes in some of the exceptions could be further explored in other studies to determine whether these differences across years are due to a trend or pollution events in the region. This study will serve as a basis for future studies monitoring environmental pollutants, especially in the central and eastern MFSD areas, since the turtles found on the western coasts of Türkiye exhibit lower and more stable trace element accumulation than those in other parts of the Mediterranean. As the loggerhead turtle is a migratory species, individuals' foraging grounds may differ from one breeding season to the next. In addition, individuals in a breeding population within a single breeding season are usually a mixture of populations from different foraging grounds.

The monitoring of heavy metal accumulation in different tissues with the same methodology for at least three successive breeding seasons, using additional research tools such as mixed-stock and stable isotope analyses, to determine the loci of the foraging areas of the different individuals, would therefore be valuable and crucial research for identifying the most contaminated regions of the Mediterranean. We thus recommend that further research be undertaken to compare trace-element accumulation across specific foraging areas, using additional tools such as stable-isotope analysis to validate the origin of stranded turtles.

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