ENVIRONMENTAL ISSUES AFFECTING LOGGERHEAD TURTLES *CARETTA CARETTA*: A STUDY ON THE PRESENCE OF INORGANIC POLLUTANTS IN ORGANS, TISSUES, AND EGGS

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Abstract: Climate change significantly impacts nesting areas for sea turtles, leading to an expansion in the Western Mediterranean regions, particularly along the southern Italian beaches, in recent years. Furthermore, along these coasts, there is intense activity in recovering stranded turtles.

Within the One Health approach, which integrates health and environment, there is increasing interest in monitoring marine pollution and its effects on loggerhead turtles.

Caretta caretta (L., 1758), the most abundant sea turtle in the Mediterranean Sea, is globally categorized as "vulnerable" among endangered species. Due to their wide-ranging migrations, for extended periods, on a large geographical scale, sea turtles can accumulate marine pollutants including metals, PCBs, pesticides, PAHs and PFAS, in their liver, kidneys, and adipose tissue. Upon attaining sexual maturity, these toxicants may be transferred to offspring through the eggs, thus exposing developing embryos to potentially high doses of pollutants. This study aimed to quantify inorganic chemical contaminants in organs, tissues, and eggs of *C. caretta*.

Keywords: threatened species, marine environment, sea turtle, *Caretta caretta*

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Introduction

In the context of the One Health approach, which integrates human, animal, and environmental health, there is growing interest in monitoring marine pollution and its impacts on marine organisms.

Loggerhead turtle *Caretta caretta* (Linnaeus, 1758), the most abundant marine turtle species in the Mediterranean Sea, is globally classified as "vulnerable" on the International Union for Conservation of Nature's Red List of Threatened Species [2]. This species faces significant threats from high levels of anthropogenic activities in coastal areas, maritime traffic, macroscopic (plastic and other waste) and chemical pollution, fishing practices, as well as the ingestion of hooks and lines [16].

Since loggerhead turtles are omnivorous, consuming a diet rich in cephalopods, jellyfish, algae, and mollusks, they can accumulate high levels of contaminants, including metals [3,6]. This capability makes them excellent sentinel animals and bioindicators for monitoring marine water quality.

The 2008/56/EC Marine Strategy Framework Directive [7] established that the marine environment, a precious heritage, must be protected, safeguarded, and, where possible, restored. The aim is to maintain biodiversity and preserve the biodiversity and vitality of seas and oceans, ensuring they remain clean, healthy, and productive.

To achieve the objectives set by this Directive, it is necessary to consider the descriptors outlined in the Decision, including the need to maintain the contaminant concentrations at levels that do not result in pollution effects.

Toxic elements, such as mercury, lead, and cadmium, derived from both natural sources and widespread use in agriculture and industry, are pervasive in aquatic ecosystems [1].

These non-essential elements, also known as heavy metals, are particularly concerning due to their well-documented toxic effects on marine animals, including sea turtle [3].

Other inorganic elements such as arsenic, chromium, copper, manganese, nickel, selenium and zinc are found globally in aquatic systems and their potential transfer to marine turtles could pose a threat to their health [8]. Many of these elements are essential, but they can become toxic if their concentrations exceed certain values. Studies have shown carcinogenic and teratogenic effects, as well as toxicity to the neural, renal, reproductive and endocrine systems in sea turtles [19]. Additionally, positive correlations have been observed between some trace element concentrations and hatching success *Caretta caretta* nests [17].

Due to their peculiar ethological characteristics, longevity, feeding ecology, habitat use and migratory nature, sea turtles are considered good sentinel species for environmental assessment [15] and has been proposed as an indicator for Good Environmental Status (GES) to improve the effectiveness of conservation strategies [11].

Several studies have determined the levels of heavy metals, notably cadmium and lead, in various tissues of sea turtles. However, data on mercury are less common, despite its high toxic significant presence in the Mediterranean. Since the industrial Revolution, the Mediterranean Sea has received substantial amounts of mercury, which accumulates in sediments, water and biota. As a semi-enclosed basin, it has limited water exchange with the Atlantic Ocean, insufficient to dilute the high quantities of mercury discharged into it [5]. Finally, the low number of studies on mercury levels in turtles can be attributed to the specific analytical requirements for its determination [4].

Studies on the presence of potentially toxic elements in the tissues of sea turtles in the Western Mediterranean Sea are limited [1,8]. This lack of data is even more acute for turtle eggs, although climate change is leading to increased nesting activity along the western coasts of the Mediterranean [12].

Eggs also represent a valuable and sensitive biomarker that can indicate the body burden of sea turtles resulting from their exposure to various chemicals, while simultaneously minimizing the impact of tissue sampling on live specimens [14]. In this paper, we report the levels of trace element in the liver, muscle tissue, and eggs of *Caretta caretta* turtles from the Western Mediterranean Sea.

Material and Methods

In 2022, 26 loggerhead turtles were found stranded on the beaches of Campania region in Southern Italy. Twenty-four carcasses, which showed no evidence of decomposition, were subjected to necropsy and biometric parameters were recorded. Length parameters, including straight line carapace length (SCL), curved carapace length (CCL), straight line width (SCW), curved carapace width (CCW), head width (HW) and curved plastron length (CPL), were measured to the nearest 1 cm using a flexible tape measure. Sex determination was carried out by visual examination of the gonads and in case of doubt, confirmed by histological examination. Liver and muscle tissue were collected and kept frozen at -20°C until analysis. The yolk and albumen samples were obtained from unhatched eggs collected from 20 nests located along the beaches of the Campania region in Southern Italy (Figure 1). A single unhatched egg was taken from each of the 20 nests located in Campania, and analyses were carried out on the albumen and yolk separately.

For the determination of trace elements, 0.5 g of homogenized samples of liver, muscle tissue and egg content (albumen and yolk) were weighed and subjected to acid mineralization with 5.0 mL of 70 % nitric acid for trace element analysis, 2.5 mL of 30% hydrogen peroxide, and 2.5 mL of ultrapure water using a microwave digestion system ultraWAVE (Milestone, Bergamo, Italy) following a modified EPA Method 200.8 (US EPA, 1994) and EPA Method 3051A (US EPA, 2007). After digestion, the sample was brought to room temperature and then diluted with ultrapure water. Trace elements were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) mod. NexION 350X (Perkin Elmer, Waltham, MA, USA). Standard solution containing arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), strontium (Sr) and zinc (Zn) at 1000 mg L^{-1} (Perkin Elmer, Waltham, MA, USA) was used to obtain calibration curves. Standard solution of rhodium as internal standard at a concentration of 200 ng mL^{-1} was added to standard and sample solutions by means of online mixing.

Total mercury (Hg tot) was determined using the gold amalgamation combustion atomic absorption spectrometry technique with a Direct Mercury Analyzer® DMA 80 evo (Milestone, Sorisole, Italy).

Figure 1 – Nests and stranding sites of the loggerhead turtles *Caretta caretta* in 2022.

For the analysis, 100 mg of homogenized sample was weighed directly into nickel boats and transferred to DMA. Total mercury concentrations were calculated by interpolation of absorbance ($\lambda = 253.7$ nm) in external analytical curves obtained by analysis of solutions of mercury at different concentrations (0.005, 0.010, 0.025, 0.050, 0.100, 0.200, 1.0 mg L^{-1}) prepared by dilution with ultrapure water of a 1000 ± 2 mg L^{-1} standard solution of inorganic mercury (Merck KGaA, Darmstadt, Germany).

Mussel tissue ERM®- CE278k (Joint Research Centre, Belgium) was used as a certified reference material (CRM) for validation of method (assessment of trueness and repeatability) and quality assurance.

Results

A total of 24 specimens of *Caretta caretta* (12 females, 10 males, 2 non determined) found stranded in 2022 along the coast of the Campania region in Southern Italy were analyzed in this study. The measured biometric parameters are reported in Table 1.

Table 1 – biometric parameters (cm) measured in 24 *Caretta caretta* specimens from Campania region.

	Mean	***SD***	***Median***	***Min***	***Max***
SCL	58.4	16.3	66.1	27.5	82.6
CCL	62.4	16.2	69.0	31.0	88.0
SCW	45.5	11.8	49.4	23.7	62.8
CCW	57.3	15.5	62.5	28.0	80.0
HW	11.0	2.9	11.5	6.2	15.7
CPL	44.6	11.1	50.8	24.0	56.5

The body weight of the turtles ranged from 3.8 kg to 75.0 kg with a mean value of 31.4 kg. Regarding age, 10 specimens were classified as juveniles and 14 as adults.

During 2022, a total of 20 unhatched eggs from nests located along the beaches of Campania region were collected, and the content (yolk and albumen) analysed for the levels of trace elements.

The characteristics of the nests sampled are reported in Table 2, including municipality, geographic coordinates (latitude and longitude), clutch size (number of eggs laid), Hatching Success (HS%), which is the percentage of eggs that successfully hatch into hatchlings out of the total number of eggs laid, and Emerging Success ES%, which indicates the proportion of hatchlings that emerge from the nest chamber and reach the surface of the beach. As usually happens, ES% (mean value 70 %) is slightly lower than HS% (mean value 75 %) because not all hatchlings reach the beach surface.

The results (mean value, standard deviation, min and max) of the determination of trace element concentrations in albumen, yolk, muscle tissue and liver are reported in Table 3.

Table 2 – Characteristics of nests sampled for the study: municipality, latitude and longitude, clutch size, HS% (Hatching Success) and ES% (Emerging Success).

Nest	*Municipality*	*Lat.*	*Long.*	*Clutch size*	*HS %*	*ES %*
N02	Camerota	40.00085	15.38069	95	58.9	58.9
N03	Eboli	40.5462	14.907	129	69	65.9
N04	Castel Volturno	40.89958	14.03233	82	81.7	81.7
N05	Camerota	40.02963	15.31913	123	90.2	84.6
N06	Camerota	40.00075	15.38092	92	21.7	20.7
N07	Castel Volturno	40.93824	14.00989	63	93.7	71.4
N08	Eboli	40.51271	14.92557	96	86.5	75
N09	Camerota	40.02896	15.32028	77	98.7	98.7
N10	Camerota	40.02594	15.32345	112	50.9	50
N11	Castel Volturno	40.99533	13.9577	74	50	41.9
N12	Acciaroli	40.19066	15.02087	105	22.9	22.9
N13	Centola	40.04788	15.28393	100	89	87
N14	Castel Volturno	40.93573	14.01151	53	79.2	77.4
N15	Eboli	40.54536	14.90767	101	92.1	65.3
N16	Camerota	40.02658	15.32308	72	97.2	97.2
N18	Camerota	40.02012	15.32881	97	71.1	57.7
N19	Pollica	40.18484	15.0242	60	93.3	93.3
N20	Camerota	40.00013	15.38468	81	91.4	85.2
N21	Centola	40.04801	15.28397	88	92	92
N23	Centola	40.04899	15.28349	77	81.8	57.1

Table 3 – trace element concentrations (mean ± sd in mg kg^{-1}, range min-max) in muscle tissue, liver, yolk, and albumen of eggs of loggerhead turtles.

	Albumen	***Yolk***	***Muscle***	***Liver***
	mean ± sd (min-max)	**mean ± sd** (min-max)	**mean ± sd** (min-max)	**mean ± sd** (min-max)
As	**0.220 ± 0.125** (0.035-0.401)	**0.904 ± 0.606** (0.183-2.11)	**21.05 ± 14.3** (3.29-68.0)	**8.55 ± 4.81** (1.27-20.1)
Cd	< LOQ	**0.005 ± 0.003** (0.001-0.008)	**0.026 ± 0.028** (0.001-0.129)	**1.35 ± 0.63** (0.53-3.18)
Co	**0.007 ± 0.002** (0.004-0.014)	**0.013 ± 0.002** (0.011-0.016)	**0.020 ± 0.032** (0.003-0.127)	**0.041 ± 0.042** (0.015-0.201)
Cr	**0.297 ± 0.254** (0.049-0.880)	**0.311 ± 0.202** (0.107-0.801)	**0.369 ± 0.369** (0.029-1.30)	**0.110 ± 0.109** (0.019-0.51)
Cu	**0.974 ± 0.961** (0.053-3.04)	**1.46 ± 0.546** (0.688-3.0)	**0.743 ± 0.373** (0.385-1.92)	**8.51 ± 5.73** (1.61-23.0)
Fe	**3.56 ± 1.78** (0.859-6.75)	**20.2 ± 5.5** (12.0-31.8)	**45.2 ± 56.9** (8.6-229)	**461.7 ± 418.2** (59-2003)
Hg	< LOQ	**0.022 ± 0.014** (0.011-0.060)	**0.067 ± 0.028** (0.023-0.160)	**0.243 ± 0.162** (0.070-0.801)
Mn	**0.077 ± 0.044** (0.026-0.159)	**0.530 ± 0.283** (0.226-1.34)	**1.38 ± 2.45** (0.06-9.30)	**3.32 ± 8.80** (0.51-44.5)
Ni	**0.175 ± 0.145** (0.034-0.512)	**0.172 ± 0.113** (0.054-0.447)	**0.156 ± 0.184** (0.014-0.655)	**0.060 ± 0.044** (0.021-0.196)
Pb	**0.016 ± 0.008** (0.007-0.032)	**0.040 ± 0.012** (0.024-0.061)	**0.054 ± 0.047** (0.014-0.223)	**0.080 ± 0.061** (0.022-0.123)
Se	**0.282 ± 0.183** (0.038-0.585)	**1.15 ± 0.40** (0.30-1.72)	**1.38 ± 0.52** (0.36-2.31)	**3.45 ± 1.88** (1.20-9.28)
Sr	**5.06 ± 2.41** (1.00-8.86)	**38.6 ± 10.9** (17.2-59.1)	**2.35 ± 1.21** (0.61-5.25)	**16.1 ± 28.3** (1.17-99.0)
Zn	**3.93 ± 3.93** (13.6-0.15)	**31.1 ± 26.2** (8.7-137.3)	**18.0 ± 8.7** (8.8-38.5)	**18.4 ± 6.6** (11.2-37.7)

LOQ: limit of quantification.

Discussion

The loggerhead turtles stranded on the beaches of Southern Italy showed levels of toxic elements similar to those found in previous studies, although a slight decrease in levels should be noted. The most abundant metals in eggs are strontium, zinc and iron, as already reported by Esposito et al. [9]. Compared to the albumen, the yolk contains over 80 % of these elements. This distribution has also been observed for other elements, such as arsenic and manganese. On the other hand, for toxic elements such as nickel and chromium, there is an almost equal distribution in the two matrices of the egg. The interesting result is the low mean level of two toxic metals, lead and mercury, in the yolk (0.040 and 0.022 mg kg^{-1}, respectively) but especially in the albumen (0.016 and <0.001 mg kg^{-1}, respectively).

The average concentration of arsenic in the yolk is similar to that determined in our previous study on whole eggs collected on the coasts of southern Italy [9] while on muscle and liver, values lower than those reported for loggerhead turtle from Mediterranean Sea were detected [1,4,13].

Copper and zinc are two essential elements involved in enzymatic activities, transport processes, and energy production, as well as for egg formation. A study has highlighted how the hatching success of *Caretta caretta* is influenced by their concentrations [17]. In our study, copper levels in the yolk are slightly higher than those in the egg white, while for zinc the concentrations in the yolk are approximately ten times higher than in the albumen (31.1 vs 3.93 mg kg^{-1}). Cobalt plays a fundamental role in many biological processes; at high levels it has been recognized as a possible carcinogen (group 2B) for humans. Low levels of cobalt were recorded in all matrices analyzed.

In the liver, similar to what was observed in eggs, the most abundant metals are iron, zinc, and strontium, followed by arsenic and copper. The presence of cadmium is also relevant (1.35 mg kg^{-1}) in liver which is confirmed to be the one of target organ for the accumulation of this element. The values found are in agreement with those reported by other studies although a slight decrease in levels should be noted [10,18,19]. However, the measured concentrations indicate long term exposure to cadmium but at the same time they highlight a decrease in hepatic cadmium concentration which is lower than all previous studies.

As mentioned above, data on the presence of mercury in *Caretta caretta* turtles from Mediterranean Sea is very scarce, however, compared to the value of 0.26 mg kg^{-1} recorded in turtles stranded between 2014 and 2020 in southern Italy [1] the value of 0.24 mg kg^{-1} determined in this study in the same area on stranded specimens in 2022, appears slightly lower. This decrease is even more evident when compared with the study of Storelli which found in 2003, a value of 0.43 mg kg^{-1} of mercury in the liver [18].

According to the results, nickel had the lowest concentration in the liver compared to other studies on loggerhead turtle [3,10,19], while comparisons for selenium and chromium are more difficult due to lack of data. Manganese plays a fundamental role in biochemical processes; in our study, the levels in the muscle are similar to the values reported in *C. caretta* from Cyprus (1.38 vs 1.402 mg kg^{-1}) but in the liver, the concentration of manganese is much lower than that recorded in the Eastern Mediterranean and the Adriatic Sea (1.53 vs 7.999 and 6.23 mg kg^{-1}, respectively) [3,18].

Conclusion

In this study, different tissues and organs of *C. Caretta* were analyzed to determine the levels of toxic and potentially toxic elements.

The results still showed high values of cadmium and mercury, particularly in the target organ liver, where these metals accumulate and can pose a health risk to these animals. The presence of trace elements in the eggs confirms that metals accumulated in the females are transferred to the eggs.

The results of this study enrich the limited database of inorganic contaminants available for the *Caretta caretta* species in the Mediterranean Sea. On the other hand, they allowed us to compare the concentrations obtained from previous studies and to verify that, in some cases, there was a significant decrease in metals considered toxic, which could reflect a decrease in contamination of the Mediterranean Sea.

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