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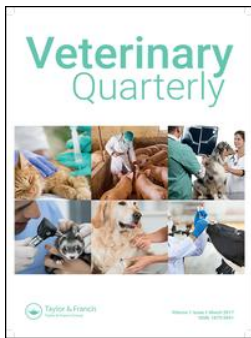
Toxic metals in Loggerhead sea turtles (*Caretta caretta*) stranded freshly dead along Sicilian coasts / Cammilleri, Gaetano; Galluzzo, Francesco Giuseppe; Pulvirenti, Andrea; Pantano, Licia; Calabrese, Vittorio; Gentile, Antonino; Cumbo, Valentina; Macaluso, Andrea; Macaluso, Vito; Vella, Antonio; Ferrantelli, Vincenzo. - In: THE VETERINARY QUARTERLY. - ISSN 0165-2176. - 43:1(2023), pp. 1-10. [10.1080/01652176.2023.2169781]

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To cite this article: Gaetano Cammilleri, Francesco Giuseppe Galluzzo, Andrea Pulvirenti, Licia Pantano, Vittorio Calabrese, Antonino Gentile, Valentina Cumbo, Andrea Macaluso, Vito Macaluso, Antonio Vella & Vincenzo Ferrantelli (2023): Toxic metals in Loggerhead sea turtles (*Caretta caretta*) stranded freshly dead along Sicilian coasts., Veterinary Quarterly, DOI: [10.1080/01652176.2023.2169781](https://doi.org/10.1080/01652176.2023.2169781)

To link to this article: <https://doi.org/10.1080/01652176.2023.2169781>



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## Toxic metals in Loggerhead sea turtles (*Caretta caretta*) stranded freshly dead along Sicilian coasts.

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### Abstract

**Background:** The Loggerhead sea turtle (*Caretta caretta*) is a marine reptile belonging to a monophyletic group of chelonians. As these animals are long-lived, they have the ability to accumulate pollutants.

**Aim:** To collect epidemiological data on toxic metals in marine Loggerhead sea turtles.

**Materials and Methods:** Forty Loggerhead sea turtles comprising 25 males and 15 females stranded freshly dead between 2013 and 2018 along the coasts of Sicily, Southern Italy, were examined for arsenic, cadmium, and lead accumulation in muscle and adipose tissues by means of a validated ICP-MS method. A modified K index as a growth condition factor, namely Fulton's K index, was used. Samples were tested in duplicate. A Wilcoxon rank sum test was carried out to evaluate metal contents differences between muscle and adipose tissues and between genders.

**Results:** The Fulton's K index suggested a good body condition of the *C. caretta* recovered with mean values of  $5.34 \pm 3.40$  ( $n=40$ ;  $\pm SD$ ). Detectable concentrations of lead were found in 70% of the samples analysed with mean values of  $0.65 \pm 1.67$  mg/kg wet weight and  $0.51 \pm 1.29$  mg/kg wet weight in muscle and adipose tissues, respectively. No significant differences in arsenic, cadmium, and lead were detected between genders. In addition, no significant correlation was found between modified K index and concentrations of arsenic, cadmium, and lead.

**Clinical relevance:** Findings on muscle and adipose tissues suggest chronic exposure of *Caretta caretta* to high concentrations of especially lead which might negatively affect health and welfare of these marine turtles although body condition was good.

**Keywords:** *Caretta caretta*; Loggerhead sea turtle; Sicily; heavy metals; lead; cadmium; arsenic

## 1. Introduction

The pollution by toxic metals in the aquatic environment has given rise to much discussion because of the risk of toxicity and bioaccumulation in the marine food chain (Sakai et al. 2000; Gochfeld 2003; García-Fernández et al. 2009; Jerez et al. 2010; Novillo et al. 2017). Natural and anthropogenic sources, including industrial and domestic sewage, and atmospheric deposits account for sources of toxic metals in the aquatic ecosystem (Slooff 1982; Sankar et al. 2006; Fazio et al. 2014; Cammilleri et al. 2018). Most of the waste accumulated in the sea comes from land and inevitably interacts with marine organisms (Galgani et al. 2014). The toxic metals accumulated in living organisms reflect the degree of environmental pollution (Canbek et al. 2007). The Loggerhead sea turtle (*C. caretta*) is a marine reptile belonging to a monophyletic group of chelonians containing seven extant species adapted to live in the marine environment, with a wide distribution in the Mediterranean Sea (Naro Maciel et al. 2008). *C. caretta* is a long living vertebrate species that behave as carnivorous generalist feeders foraging principally on fish, crustaceans, molluscs, and other invertebrates (Godley et al. 1997). When turtles become adults, they return to natal beaches to reproduce and feed on coastal benthic habitats (McClellan and Read 2007). The most important areas of reproduction are Greece, Turkey, Libya, and Cyprus (Nagelkerken et al. 2003), whereas the most important feeding areas are the Tunisian continental platform and the Adriatic and Ionian seas (Mingozzi et al. 2008). In Italy, the most important nesting areas are the Ionic part of southern Calabria; smaller sites are located in the Pelage Islands and Southern Sicily (Mingozzi et al. 2008). *C. caretta*, according to its biological factors (i.e., age, gender, migration habits), could bioaccumulate toxic inorganic elements and compounds from contaminated food, marine sediment, and seawater (Cortés-Gómez et al. 2017). High concentration of trace elements in Loggerhead sea turtles could result in permanent physiological dysfunctions, however there are no data on the toxicity thresholds (Finlayson et al. 2016; Cortés-Gómez et al. 2018; Cortés-Gómez et al. 2020; Canzanella et al. 2021).

Toxicological effect of toxic metals include cell death via apoptosis and necrosis, modification in the fertilization and survival of the eggs, immunosuppression and, skeletal malformations (García-Fernández et al. 2009; Cortés-Gómez et al. 2017). Studies have verified the distribution and evasion flux of toxic elements at the atmosphere/sea interface in Sicilian coasts (Southern Italy), illustrating that the intense industrial activity of the past has led to high pollution in these areas (Copat et al. 2012; Ministero ambiente 2008).

Arsenic (As) occurs in seawater mainly as inorganic arsenic in the trivalent and pentavalent states. Many algae can accumulate and biotransform inorganic arsenic to arsenosugars (Llorente-Mirandes et al. 2010). Regarding lead (Pb), the slowing down of aerosol Pb concentration decrease in the troposphere, is related to the implementation of antipollution policies and the limitation of Pb use as an additive in gasoline in the Mediterranean water column (Nicolas et al. 1994; Migon et al. 2008). The concentration of cadmium (Cd) in the marine environment is low, but it tends to accumulate in living organisms perturbing embryonic development and triggering stress protein synthesis, synthesis of globulins, reduce red blood cell production, and induce detoxification genes (Rie et al. 2001; García-Fernández et al. 2009; Komoroske et al. 2011; Camacho et al. 2013; Perrault et al. 2017; Cortés-Gómez et al. 2018). Polluted Cd areas were revealed in the Mediterranean sea around Sicily island, with concentrations comprising between  $0.56 \times 10^{-3}$  and  $80.4 \times 10^{-3}$   $\mu\text{g/L}$  (Censi et al. 2002). At present, few studies are focused on the presence of these elements in Mediterranean sea turtles and most of them did not investigate the correlation between growth parameters and bioaccumulation of these

elements considering that these animals are long-lived and have the ability to accumulate pollutants (Maffucci et al. 2005; et al. 2005; Storelli et al. 2008). Recent studies have verified a possible relation between growth condition factor, such as Fulton's K condition indices, and the bioaccumulation of xenobiotics in aquatic organisms but the latter have focused only on organisms of commercial interest, not considering organisms useful in environmental monitoring (Mohamed Harris et al. 2019).

In this study, we examined Loggerhead sea turtles stranded on the Sicilian coasts (south Mediterranean Sea) for the detection of arsenic (As), cadmium (Cd), and lead (Pb) by an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method. We analysed their distribution and accumulation in adipose and muscle tissues of this species and their correlation with morphometric parameters. Epidemiological data on the presence of toxic metals in South Mediterranean ecosystems were obtained.

## 2. Materials and methods

### 2.1. Sampling

Forty freshly dead *C. caretta* turtles stranded along 18 localities of the Sicilian coasts were recovered by the National Center of Turtles and Cetaceans Recovery from 2013 to 2018. The recovering sites (Palermo, Trapani, Mazara del Vallo, Gela, Modica, Avola, Catania, Messina, Lipari, Ustica) are shown in Figure 1. All the specimens were transferred to the Istituto Zooprofilattico Sperimentale della Sicilia (Via Gino Marinuzzi 3, 90129, Palermo, Italy) and subjected to necropsy, biometry (straight carapace length, curved carapace length, etc.) and gender assessment by visual examination (Wyneken 2001; Casale et al. 2005). A modified Fulton's K index was calculated with each loggerhead turtle recovered by the formula:

$$\left(\frac{\text{body mass}}{SCL^3}\right) * 10^4$$

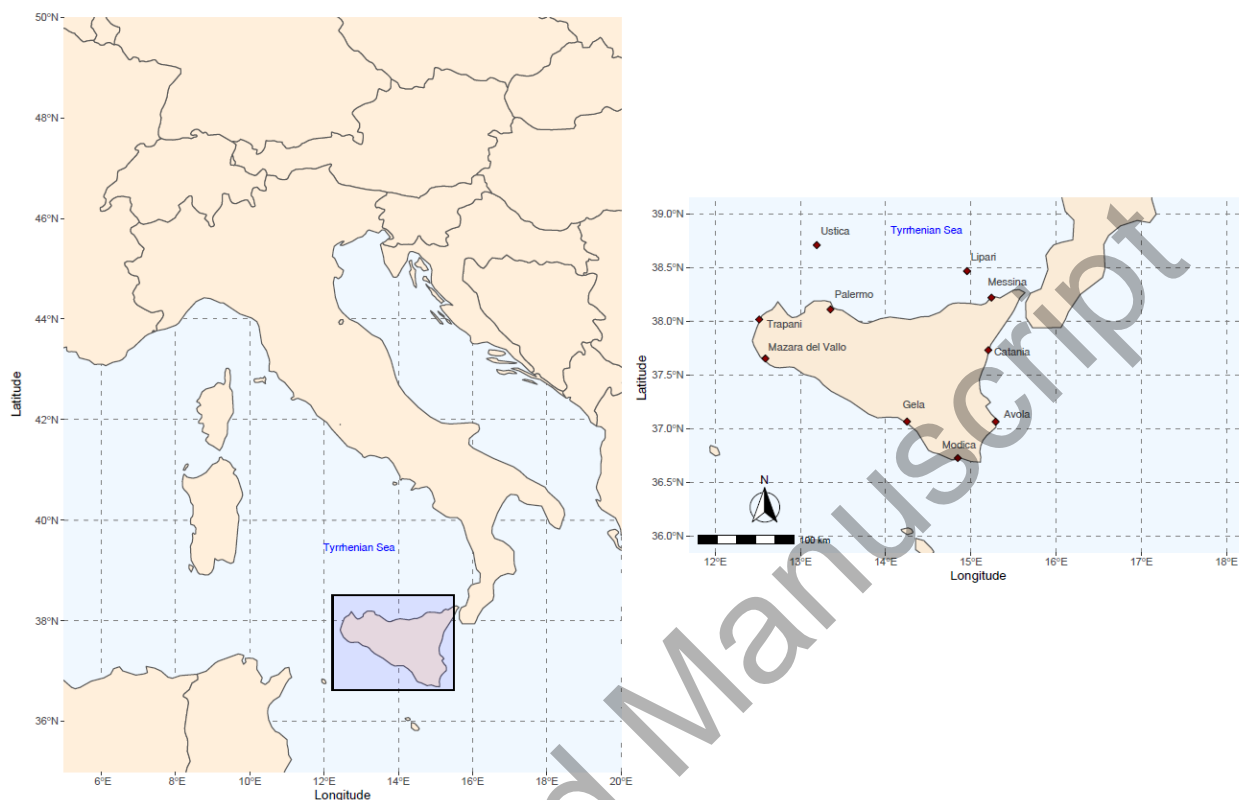
Where SCL is the straight carapace length (cm) and body mass is expressed in Kg (Bjorndal et al. 2000). Muscle from limbs and adipose tissue from carapace depots were collected during necropsies and stored at  $-20^{\circ}\text{C}$  until chemical analysis. All the turtles were recovered freshly dead, with no visual evidence of tissue decomposition (Wyneken 2001; Heithaus et al. 2007). The morphometrics of the *C. caretta* specimens examined are summarised in Table 1. Initial inspections allowed us to identify the gender of the specimens examined, revealing 25 males and 15 females.

**Table 1.** Biometric parameters of the 40 specimens of *Caretta caretta* sampled. Body weight (kg), SCL (straight carapace length, cm) and CCW (curved carapace width, cm) are shown.

	N	Mean $\pm$ SD	Min	Max
Weight	40	22.75 $\pm$ 11.26	10.5	52
SCL	40	51.21 $\pm$ 11.56	28	74.5
CCW	40	41.66 $\pm$ 9.83	24	59

Modified K	40	$6.03 \pm 3.58$	2.55	18.72
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**Figure 1.** Map of the recovering sites of the loggerhead turtles (*Caretta caretta*) stranded between 2013 and 2018 in coastal areas of Sicily, Southern Italy.



## 2.2. Reagents and standards

All solutions were prepared with analytical ultra-pure grade reagents. Water for chromatography LiChrosolv<sup>®</sup> was purchased from Merck KgaA (Darmstadt, Germany). Ultrapure nitric acid 60% was purchased from Merck KgaA (Darmstadt, Germany). Standard solutions: the multielement calibration solutions (As, Cd, Pb) were prepared at different concentration levels (0.001 - 50 µg/L) from 1000 mg/L single element ICP-MS grade standard from VWR International LTD (Randon, Pennsylvania, USA). A tuning solution for ICP-MS, capable of covering a wide range of masses (Ce, Co, Li, Mg, Tl, and Y 1 µg/l) were purchased from Agilent Technologies (Santa Monica, CA, USA) to optimize the performance of ICP-MS instrument before use. Internal standard solution: 100 mg/L standard stock solution of scandium (Sc), yttrium (Y), indium (In), terbium (Tb), rhodium (Rh), lutetium (Lu), lithium 6 (Li), indium (In), germanium (Ge) and bismuth (Bi) was purchased from Agilent Technologies (Santa Monica, CA, USA). Ultrapure grade carrier gas of argon, helium and hydrogen (99.9995% pure) were purchased from SOL S.p.A (Monza, Mi, Italy).

### 2.3. Analytical methods

The digestion process of the samples was carried out according to protocols reported before (Fazio et al. 2014; Cammilleri et al. 2020). Briefly, ~1 g of the samples were transferred into previously decontaminated PTFE (poly-tetrafluoroethylene-tetrafluoroethylene) tubes with 3 ml of Ultrapure nitric acid 60% (V/V) and 5 mL of water. The sample digestion was carried out using a microwave digester Multiwave 3000 (Anton Paar, Graz, Austria). The digestion conditions for muscle and fat are reported in Table 2.

**Table 2.** Microwave digestion conditions for the muscle and fat samples analysed.

Phase	Power (W)	Ramp (min)	Hold (min)	FAN
1	600	10:00	40	1
2	0	0	15	3

#### 2.3.1. ICP-MS analysis

The elements were determined using a 7700x series ICP-MS (Agilent Technologies, Santa Monica, CA, USA). The sample extracted were pumped by a peristaltic pump from tubes arranged on an autosampler ASX-500 Series (Agilent Technologies, Santa Monica, CA, USA), combined with a quartz cyclonic spray chamber (water cooled at 2° C). The instrumental settings and operative conditions can be found in Supplementary Material (S1).

A calibration curve based on 8 standard additions (BlankCal - 0.01 – 0.05 – 0.1 – 0.5 – 1 – 5 – 10 – 50 µg/L) was made to evaluate the linearity. A pool of digested samples was used for this test. The linearity of the calibration curve was considered acceptable for  $r^2 > 0.999$ . The method limits of detection and quantification (LOD and LOQ) were calculated by the  $3\sigma$  and  $10\sigma$  approaches (ISO 17025:2005; Barwick et al. 2014). LOD is defined as the lowest concentration of an analyte in a sample that can be consistently detected with a stated probability (typically at 95% certainty), while LOQ is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision (Armbruster and Pry 2008). Digested samples were used to calculate method LOD and LOQ according to protocols reported previously. The trueness of the method was assessed using the recovery of 3 concentration levels for each element (Fazio et al. 2014; Cammilleri et al. 2020). An acceptance limit between 90 and 110% was chosen (Barwick et al. 2014). A range of recovery between 97% and 102% was obtained for all the concentration levels of the analytes examined. Each sample was tested in duplicate.

#### 2.4. Data collection and statistical analysis

All the results under the LOQ of the method were considered for the statistical analysis as half of the LOQ values (Hornung and Reed 1990; Lubin et al. 2004; Helsel 2005; Zota et al. 2014; Goniewicz et al. 2018). All the As, Cd, and Pb concentrations were expressed as wet weight (w.w.) and converted to dry weight (d.w.), using the mean

water content (79%) for muscle according to Maffucci et al. (2005), for the comparison with other studies. The conditions of normal distribution and homogeneity of variances were not met. Therefore, a Wilcoxon rank sum test was carried out to evaluate As, Cd and Pb contents differences between muscle and adipose tissues and between genders with *W* as the Wilcoxon test statistic or the sum of the ranks in one of both groups and *S* as the sum of all squared rank differences. The relationship between the modified *K* index of the loggerhead turtles and toxic metals contents was examined via Spearman's correlation method using the R<sup>®</sup> 4.1.2 software (freeware available on <https://cran.r-project.org/>). The null hypothesis was rejected with a *P*-value less or equal than 0.05.

### 3. Results

The Modified Fulton's *K* calculation suggested a good body condition of the *C. caretta* recovered, with mean values of  $5.34 \pm 3.40$ . The LOD-LOQ values for the analytes were: 0.001-0.002 mg/kg for As, 0.0008-0.001 mg/kg for Cd, and 0.002-0.006 mg/kg for Pb. The linear regression model was acceptable for all the analytes in the defined range, with a satisfactory correlation coefficient ( $r^2 \geq 0.999$ ). The As, Cd, and Pb concentration (wet weight) of the loggerhead turtles examined are shown in Table 3.

**Table 3.** As, Cd, and Pb concentrations (mg/kg w.w.) detected in the tissues of the loggerhead turtles analysed. The fourth column shows the values of the samples above the limit of detection (>LOD).

Element	Sample type	N	>LOD	Mean $\pm$ SD (mg/kg)	Median	min-max (mg/kg)
As	Adipose tissue	40	8 (20)	$0.69 \pm 1.92$	1.1	<LOD-7.5
	Muscle tissue	40	13 (32,5)	$0.76 \pm 1.79$	1.78	<LOD-9.1
Cd	Adipose tissue	40	10 (25)	$0.02 \pm 0.05$	0.07	<LOD-0.08
	Muscle tissue	40	15 (37,5)	$0.07 \pm 0.24$	0.02	<LOD-1.3
Pb	Adipose tissue	40	18 (45)	$0.51 \pm 1.29$	0.29	<LOD-6.12
	Muscle tissue	40	19 (47,5)	$0.65 \pm 1.67$	0.35	<LOD-6.9

Toxic metal concentrations were detected in 13 (32.5%), 15 (37.5%) and 19 (47.5%) muscle samples for As, Cd and Pb, respectively. The muscle tissue of the Loggerhead sea turtles showed the highest occurrence and mean concentrations examined. However, no significant differences were found between adipose and muscle tissues for all the analytes examined (*W* = 11.5, *P*-value = 0.641 for Pb; *W*=5, *P*-value=0.381 for As; *W*=4.5, *P*-value=0.2555 for Cd). Initial inspections allowed us to identify the gender of the specimens examined, revealing 25 males and 15 females. Pb, Cd, and As were mostly found in male specimens, showing mean concentrations of  $0.68 \pm 1.83$  mg/kg,  $0.11 \pm 0.24$  mg/kg, and  $1.05 \pm 2.46$  mg/kg, respectively in the muscle samples. However, no significant differences were found between males and females for all the toxic metals analysed (*W*=8, *p*-value=0.8831 for Pb; *W*=6, *p*-value=0.872 for Cd; *W*=3, *p*-value=0.3333 for As). The highest Cd concentration (1.3 mg/kg) was found

in a muscle sample of a male loggerhead turtle of 19.2 kg stranded along the coasts of Catania. Instead, the highest concentration of Pb (6.90 mg/kg) and As (9.1 mg/kg) were detected in the muscle tissue of a male turtle of 31.5 kg stranded along the coasts of Milazzo. No significant correlation was found between modified K index and the analytes (S=22433, p-value=0.163 for Pb, S=26493, p-value=0.7483 for As, S=28271, p-value=0.8855 for Cd; Figure 2).

Figure 2. Boxplot, scatterplot, and correlation plot of the morphometric parameters reordered and As, Cd, Pb concentrations sorted by matrices (muscle in red and fat in blue obtained from 40 specimens). SCL=straight carapace length; CCL=curved carapace length. In the scatterplots: triangles=muscle, circle=adipose tissues. In the main diagonal the boxplot divided by group (muscle in red, fat in blue). The six-scatter plot below the principal diagonal showed a linear correlation SCL and weight, CCL and weight, SCL and CCL. The Spearman correlation coefficients between each pair of variables is above the principal diagonal. The correlation between weight and SCL is 0.805 (first row, second column) and supports the conclusion that as SCL increases, weight increase. No correlation was found between trace elements and the other parameters.

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#### 4. Discussion

The results of this work showed elevated concentrations of Pb in 47.5% of muscles and 45% of adipose tissue of the Loggerhead sea turtles examined, revealing a chronic exposure to higher concentrations of this element compared to specimens from the Adriatic Sea (Eastern Mediterranean; below detection limit-0.09 mg/kg w.w.) (Storelli et al. 2005; Andreani et al. 2008), from East Spain coasts ( $0.04 \pm 0.05$  mg/kg w.w.) (Jerez et al. 2010), and Southern coasts of the Tyrrhenian sea (below detection limit-0.11 mg/kg w.w.) (Esposito et al. 2020). Our findings are surprising if one considers the regulation that limited the leaded gasoline consumption applied in European countries since the 1970s (Nicolas et al. 1994; Tian and Ruiz Pino 1995) and seem to disagree with what was assumed by Jerez et al. (2010) regarding a reduction of Pb concentrations in the Mediterranean. On this basis, a statistical simulation of the surface and bottom layers of the Mediterranean carried out by Tian and Ruiz Pino (1995) predicted an increase in Pb concentrations in the Eastern Basin, confirmed by the high concentrations of this element found in small pelagic fish and other marine organisms (Tigano et al. 2009; Copat et al. 2012). Furthermore, a clear distinction between the anthropogenic inputs of lead on the Sicilian and Murcia coasts should be considered (Jerez et al. 2010; Copat et al. 2012; Ministero ambiente 2008).

The main sources of Pb pollution in Sicilian coasts are attributable to refining activities and/or oil leaks in the Augusta roadstead; presence of numerous chemical and petrochemical industrial plants, refineries, as well as a purification plant for the disposal of industrial and civil wastewater located along the Sicilian coasts (Romano et al. 2013; Cosentino et al. 2013; Romano et al. 2016). Augusta roadstead (East Sicily, Italy) represents one of the largest European petrochemical poles that is considered highly contaminated by several industrial discharges that were reversed directly into the sea when environmental legislation was not active (Rothwell and Croudace 2015; Benedetti et al. 2022).

This has inevitably led to a continuous and prolonged supply of pollutants to the sea over time, both through the aquifer and the numerous discharges (Ministero ambiente 2008). Most metals, including Pb, are not subject to bacterial degradation. Therefore, they are an essentially permanent addition to marine ecosystems. Consequently, they accumulate in the sediments and are bio magnified up the food chain, with the top predators receiving the most significant dose of conservative substances (Kirpichtchikova et al. 2006). On this regards, our Pb results seem comparable with those found by Russo et al. (2003) in the muscle tissues of 10 juveniles and subadults *C. caretta* stranded along the Sicilian coasts, confirming the high Pb burdens in this area and the absence of correlation between the size of Loggerhead sea turtles and the accumulation of toxic elements. Although not directly comparable, our data follow previous studies on the bioaccumulation of toxic metals in other marine organisms sampled along the Sicilian coastline (Tigano et al. 2009; Copat et al. 2012). Furthermore, our results are similar to those detected in Loggerhead sea turtles from the northern Cyprus (Godley et al. 1999), confirming a greater exposure to this element in the South eastern Mediterranean.

The Pb concentrations observed in this work are higher than those found in other studies on Loggerhead sea turtles stranded on Atlantic French and Japanese coasts (Sakai et al. 1995; Caurant et al. 1999; Sakai et al. 2000), which revealed average values between 0.03 and  $0.09 \pm 0.03$  mg/kg w.w. in muscle and adipose tissues. The high Pb concentrations found in this study could explain the lack of a significant distinction between muscle and fat tissue accumulation due to this species' physiology, in particular, to the activation of metal detoxification mechanisms such as the expression

of Metallothioneins (MTs). MTs play a special part in regulating the intracellular availability of metals. The binding by MT of xenobiotic metals in an organism protects it from their toxic effects (Bioaccumulation of Metals in Tissues of Marine Animals, Part I). MTs are activated by metal regulatory transcription factor 1 (MTF1), present in both adipocytes and muscle cells (Morão et al. 2022). A lack of significant differences between muscle and fat samples for Cd and Pb was also found by Novillo et al. 2017 in *C. caretta* specimens from Spanish coasts. The epidemiological data obtained in this work could be helpful for further toxicological studies describing the threshold concentrations of Pb above which harmful effects would be likely, in accordance to what was stated by Novillo et al. 2017.

As opposed to Pb, the Cd concentrations detected in the muscle and adipose tissues of the Loggerhead sea turtles analysed in this work were slightly lower than those found in specimens analysed in the Adriatic Sea (Franzellitti et al. 2004; Storelli et al. 2005), Atlantic coasts of France (Caurant et al. 1999) and Japan (Sakai et al. 1995; Sakai et al. 2000). The Cd mean concentrations found in this study were comparable to those found in muscle tissues of Loggerhead sea turtles stranded in Cyprus (Godley et al. 1999) but up to twenty times lower than loggerhead turtles stranded on Atlantic coasts (Torrent et al. 2004; Nicolau et al. 2017). No significant correlation was found between cadmium concentration and the morphometric parameters of the specimens examined, in contrast to what was reported by Storelli et al. (1998) but in accordance to the findings of Esposito et al. (2020) in *C. caretta* specimens from the Tyrrhenian sea. Our results suggest that the body burden of cadmium in Loggerhead sea turtles is not mainly dependent on accumulation phenomena as described by Catsiki et al. (1994), also given that Cd is not bio-magnified (Gray 2002). The low concentrations of Cd could be mainly attributed to individual variations in diet. Squids are important cadmium accumulators and a source of this element for predators at the top of the marine food web, such as Loggerhead sea turtles (Bustamante et al. 1998; Storelli et al. 2005; Jerez et al. 2010). Contrary to Loggerhead sea turtles of other areas, which include squids in their diet, Loggerhead sea turtles in the eastern Mediterranean Sea feed upon organisms with low Cd concentrations, such as benthic molluscs and crustaceans (Godley et al. 1997).

Very low As concentrations were detected in this study, in contrast to what was found in Loggerhead sea turtles stranded in the South Adriatic Sea ( $15.47 \pm 11.91$  mg/kg w.w.) (Storelli et al. 1998; Storelli and Marcotrigiano 2000), Portugal ( $14.7 \pm 1.47$  mg/kg w.w.) (Nicolau et al. 2017) and Canarias ( $7.35 \pm 1.37$  mg/kg w.w.) (Torrent et al. 2004), even though the diet of southeast Mediterranean Loggerhead sea turtles is primarily based on small crustaceans which usually show high concentrations of total As (Norin et al. 1985; Godley et al. 1997). Our findings on As concentrations seem to be similar to what was found by Abdallah and Abd Allah (2010) in Loggerhead sea turtles stranded on Egyptian coasts. Besides dietary differences, bioaccumulation of contaminants in marine biota can be viewed as a result of the interaction of numerous biological factors comprising age, trophic level, locality of sampling, and reproductive state (Storelli and Zizzo 2014; Cammilleri et al. 2017).

The toxic metal concentrations of the samples analysed did not differ between genders, suggesting that overall metal loads are similar for males and females. However, the lack of significant differences could be due to methodological limitations linked to the unbalanced number of males and females examined in this work. Maternal metal transfer has been documented in sea turtles (Ikonomopoulou et al. 2011), so the higher toxic metal concentrations found in males may result from females' ability to offload a significant portion of the metal burden into their offspring. Offloading maternal transfer, is known for both oviparous and live-bearing reproductive strategies. Thus, as

the female ages, it can offload its metals burden to her eggs, thereby decreasing her tissue concentrations. On the contrary, males do not have a similar offloading mechanism, so they continually accumulate metals; although not significantly different, this could explain the higher toxic metals values found in the male specimens examined. Differences in metal concentrations between genders may be related to age. For this purpose, further studies based on age determination by skeleton chronological analysis, coupled with morphometric analysis, are needed to verify the accumulation of these substances over time.

## **Conclusions**

A reliable ICP-MS method was carried out to detect As, Cd, and Pb in muscle and adipose tissues of Loggerhead sea turtles stranded along the Sicilian coasts. To the best of our knowledge, the present work reported for the first time the presence of arsenic in loggerhead turtles stranded on the shores of Sicily (Southern Italy). About 48% of the samples analysed showed Pb concentrations over the LOD, with very high concentrations (up to 16 times the data reported in the literature). The results obtained confirmed that toxic metals principally accumulate in muscle tissue and showed lower Cd and As contents than reported in other studies. No significant differences were found between the genders. Our data also confirmed the possibility to monitor *C. caretta* as a bioaccumulator of toxic metals exposure in the aquatic environment thereby assessing the health of wildlife species. The findings presented here provide interesting epidemiological data to increase the knowledge of pollution impacts on marine turtles inhabiting contaminated areas.

## **Acknowledgements**

We would like to thank the Coast Guard of Sicily for reporting the strandings of the loggerhead turtles examined.

## **Conflicts of Interest**

The authors declare no conflict of interest.

## References

- Abdallah MAM, Abd-Allah MAM. Bioaccumulation of toxic metals in loggerhead turtles from Mediterranean Sea coast, Egypt. In: 10th International Conference on the Mediterranean Coastal [Internet]. Rhodes, Greece: E. Özhan (Ed.); [accessed 2019 Mar 20]. [https://www.researchgate.net/publication/286059247\\_Bioaccumulation\\_of\\_toxic\\_metals\\_in\\_loggerhead\\_turtles\\_from\\_Mediterranean\\_Sea\\_coast\\_Egypt](https://www.researchgate.net/publication/286059247_Bioaccumulation_of_toxic_metals_in_loggerhead_turtles_from_Mediterranean_Sea_coast_Egypt)
- Andreani G, Santoro M, Cottignoli S, Fabbri M, Carpenè E, Isani G. 2008. Metal distribution and metallothionein in loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. *Science of The Total Environment*. 390(1):287–294. <https://doi.org/10.1016/j.scitotenv.2007.09.014>
- Armbruster DA, Pry T. 2008. Limit of blank, limit of detection and limit of quantitation. *The clinical biochemist reviews*. 29(Suppl 1):S49.
- Barwick V, Bravo PPM, Ellison SL, Engman J, Gjengedal EL, Lund UO, Magnusson B, Müller H-T, Patriarca M, Pohl B. 2014. The fitness for purpose of analytical methods.
- Benedetti M, Romano E, Ausili A, Fattorini D, Gorbi S, Maggi C, Salmeri A, Salvagio Manta D, Sesta G, Sprovieri M, Regoli F. 2022. 10-year time course of Hg and organic compounds in Augusta Bay: Bioavailability and biological effects in marine organisms. *Front Public Health*. 10:968296. <https://doi.org/10.3389/fpubh.2022.968296>
- Bioaccumulation of Metals in Tissues of Marine Animals, Part I. *Pol J Environ Stud*. 20(5):1117–1125.
- Bjorndal KA, Bolten AB, Chaloupka MY. 2000. Green Turtle Somatic Growth Model: Evidence for Density Dependence. *Ecological Applications*. 10(1):269–282. [https://doi.org/10.1890/1051-0761\(2000\)010\[0269:GTSGME\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0269:GTSGME]2.0.CO;2)
- Bustamante P, Caurant F, Fowler SW, Miramand P. 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Science of The Total Environment*. 220(1):71–80. [https://doi.org/10.1016/S0048-9697\(98\)00250-2](https://doi.org/10.1016/S0048-9697(98)00250-2)
- Camacho M, Orós J, Boada LD, Zaccaroni A, Silvi M, Formigaro C, López P, Zumbado M, Luzardo OP. 2013. Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): Results from a nesting colony from Cape Verde, West Africa. *Marine Environmental Research*. 92:15–22. <https://doi.org/10.1016/j.marenvres.2013.08.002>
- Cammilleri G, Calvaruso E, Pantano L, Cascio GL, Randisi B, Macaluso A, Vazzana M, Caracappa G, Giangrosso G, Vella A, Ferrantelli V. 2017. Survey on the presence of non-dioxine-like PCBs (NDL-PCBs) in loggerhead turtles (*Caretta caretta*) stranded in south Mediterranean coasts (Sicily, Southern Italy). *Environmental Toxicology and Chemistry*. 36(11):2997–3002. <https://doi.org/10.1002/etc.3866>
- Cammilleri G, Galluzzo P, Pulvirenti A, Giangrosso IE, Lo Dico GM, Montana G, Lampiasi N, Mobilia MA, Lastra A, Vazzana M, et al. 2020. Toxic mineral elements in *Mytilus galloprovincialis* from Sicilian coasts (Southern Italy). *Natural Product Research*. 34(1):177–182. <https://doi.org/10.1080/14786419.2019.1610963>
- Cammilleri G, Vazzana M, Arizza V, Giunta F, Vella A, Dico GL, Giaccone V, Giofrè SV, Giangrosso G, Cicero N, Ferrantelli V. 2018. Mercury in fish products: what's the best for consumers between bluefin tuna and yellowfin tuna? *Natural Product Research*. 32(4):457–462. <https://doi.org/10.1080/14786419.2017.1309538>
- Canbek M, Demir T, Uyanoglu M, Bayramoglu G, Emiroglu Ö, Arslan N, Koyuncu O. 2007. Preliminary assessment of heavy metals in water and some Cyprinidae species from the Porsuk River, Turkey. *Journal of Applied Biological Sciences*. 1(3):91–95.
- Canzanella S, Danese A, Mandato M, Lucifora G, Rivero C, Federico G, Gallo P, Esposito M. 2021. Concentrations of trace elements in tissues of loggerhead turtles (*Caretta caretta*) from the Tyrrhenian and the Ionian coastlines (Calabria, Italy). *Environ Sci Pollut Res*. 28(21):26545–26557. <https://doi.org/10.1007/s11356-021-12499-4>

- Casale P, Freggi D, Basso R, Argano R. 2005. Size at Male Maturity, Sexing Methods and Adult Sex Ratio in Loggerhead Turtles (*Caretta caretta*) from Italian Waters Investigated Through Tail Measurements. *The Herpetological Journal*. 15(3):145–148.
- Catsiki VA, Bel F, Nicolaidou A. 1994. Size dependent metal concentrations in two marine gastropod species. *Netherlands Journal of Aquatic Ecology*. 28(2):157–165. <https://doi.org/10.1007/BF02333986>
- Caurant F, Bustamante P, Bordes M, Miramand P. 1999. Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts. *Marine Pollution Bulletin*. 38(12):1085–1091. [https://doi.org/10.1016/S0025-326X\(99\)00109-5](https://doi.org/10.1016/S0025-326X(99)00109-5)
- Censi P, Mazzola S, Alonzo G, Saiano F, Patti B, Sprovieri M, Bonanno A, Spoto SE. 2002. Trace element distributions in the Strait of Sicily (Central Mediterranean Sea). I. Evidence of rock-water interactions and pollution. *Periodico di Mineralogia*. 71:255–272.
- Copat C, Bella F, Castaing M, Fallico R, Sciacca S, Ferrante M. 2012. Heavy Metals Concentrations in Fish from Sicily (Mediterranean Sea) and Evaluation of Possible Health Risks to Consumers. *Bull Environ Contam Toxicol*. 88(1):78–83. <https://doi.org/10.1007/s00128-011-0433-6>
- Cortés-Gómez AA, Romero D, Girondot M. 2017. The current situation of inorganic elements in marine turtles: A general review and meta-analysis. *Environmental Pollution*. 229:567–585. <https://doi.org/10.1016/j.envpol.2017.06.077>
- Cortés-Gómez A.A., Romero D, Girondot M. 2018. Carapace asymmetry: A possible biomarker for metal accumulation in adult olive Ridley's marine turtles? *Mar Pollut Bull*. 129:92–101.
- Cortés-Gómez AA, Romero D, Santos J, Rivera-Hernández JR, Girondot M. 2020. Inorganic elements in live vs dead nesting olive ridley marine turtles in the Mexican Pacific: Introducing a new statistical methodology in ecotoxicology. *Sci Total Environ*. 761:143249.
- Cortés-Gómez Adriana A., Tvarijonavičiute A, Girondot M, Tecles F, Romero D. 2018. Relationship between plasma biochemistry values and metal concentrations in nesting olive ridley sea turtles. *Environ Sci Pollut Res*. 25(36):36671–36679. <https://doi.org/10.1007/s11356-018-3467-z>
- Cosentino C, Pepe F, Scopelliti G, Calabrò M, Caruso A. 2013. Benthic foraminiferal response to trace element pollution-the case study of the Gulf of Milazzo, NE Sicily (Central Mediterranean Sea). *Environ Monit Assess*. 185(10):8777–8802. <https://doi.org/10.1007/s10661-013-3292-2>
- Esposito M, De Roma A, Sansone D, Capozzo D, Iaccarino D, di Nocera F, Gallo P. 2020. Non-essential toxic element (Cd, As, Hg and Pb) levels in muscle, liver and kidney of loggerhead sea turtles (*Caretta caretta*) stranded along the southwestern coasts of Tyrrhenian Sea. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 231:108725. <https://doi.org/10.1016/j.cbpc.2020.108725>
- Fazio F, Piccione G, Tribulato K, Ferrantelli V, Giangrosso G, Arfuso F, Faggio C. 2014. Bioaccumulation of Heavy Metals in Blood and Tissue of Striped Mullet in Two Italian Lakes. *Journal of Aquatic Animal Health*. 26(4):278–284. <https://doi.org/10.1080/08997659.2014.938872>
- Finlayson KA, Leusch FDL, van de Merwe JP. 2016. The current state and future directions of marine turtle toxicology research. *Environment International*. 94:113–123. <https://doi.org/10.1016/j.envint.2016.05.013>
- Franzellitti S, Locatelli C, Gerosa G, Vallini C, Fabbri E. 2004. Heavy metals in tissues of loggerhead turtles (*Caretta caretta*) from the northwestern Adriatic Sea. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 138(2):187–194. <https://doi.org/10.1016/j.cca.2004.07.008>
- Galgani F, Claro F, Depledge M, Fossi C. 2014. Monitoring the impact of litter in large vertebrates in the Mediterranean Sea within the European Marine Strategy Framework Directive (MSFD): Constraints, specificities and recommendations. *Marine Environmental Research*. 100:3–9. <https://doi.org/10.1016/j.marenvres.2014.02.003>

- García-Fernández AJ, Gómez-Ramírez P, Martínez-López E, Hernández-García A, María-Mojica P, Romero D, Jiménez P, Castillo JJ, Bellido JJ. 2009. Heavy metals in tissues from loggerhead turtles (*Caretta caretta*) from the southwestern Mediterranean (Spain). *Ecotoxicology and Environmental Safety*. 72(2):557–563. <https://doi.org/10.1016/j.ecoenv.2008.05.003>
- Gochfeld M. 2003. Cases of mercury exposure, bioavailability, and absorption. *Ecotoxicology and Environmental Safety*. 56(1):174–179. [https://doi.org/10.1016/S0147-6513\(03\)00060-5](https://doi.org/10.1016/S0147-6513(03)00060-5)
- Godley BJ, Smith SM, Clark PF, Taylor JD. 1997. Molluscan and crustacean items in the diet of the loggerhead turtle, *Caretta caretta* (Linnaeus, 1758) [Testudines: Cheloniidae] in the eastern Mediterranean. *J Molluscan Stud*. 63(3):474–476. <https://doi.org/10.1093/mollus/63.3.474>
- Godley BJ, Thompson DR, Furness RW. 1999. Do Heavy Metal Concentrations Pose a Threat to Marine Turtles from the Mediterranean Sea? *Marine Pollution Bulletin*. 38(6):497–502. [https://doi.org/10.1016/S0025-326X\(98\)00184-2](https://doi.org/10.1016/S0025-326X(98)00184-2)
- Goniewicz ML, Smith DM, Edwards KC, Blount BC, Caldwell KL, Feng J, Wang L, Christensen C, Ambrose B, Borek N, et al. 2018. Comparison of Nicotine and Toxicant Exposure in Users of Electronic Cigarettes and Combustible Cigarettes. *JAMA Netw Open*. 1(8):e185937. <https://doi.org/10.1001/jamanetworkopen.2018.5937>
- Gray JS. 2002. Biomagnification in marine systems: the perspective of an ecologist. *Marine Pollution Bulletin*. 45(1):46–52. [https://doi.org/10.1016/S0025-326X\(01\)00323-X](https://doi.org/10.1016/S0025-326X(01)00323-X)
- Heithaus MR, Frid A, Wirsing AJ, Dill LM, Fourqurean JW, Burkholder D, Thomson J, Bejder L. 2007. State-dependent risk-taking by green sea turtles mediates top-down effects of tiger shark intimidation in a marine ecosystem. *Journal of Animal Ecology*. 76(5):837–844. <https://doi.org/10.1111/j.1365-2656.2007.01260.x>
- Helsel DR. 2005. Nondetects and data analysis. Statistics for censored environmental data. Nondetects and data analysis Statistics for censored environmental data [Internet]. [accessed 2018 Aug 27]. <https://www.cabdirect.org/cabdirect/abstract/20053102639>
- Hornung RW, Reed LD. 1990. Estimation of Average Concentration in the Presence of Nondetectable Values. *Applied Occupational and Environmental Hygiene*. 5(1):46–51. <https://doi.org/10.1080/1047322X.1990.10389587>
- Ikonomopoulou MP, Olszowy H, Limpus C, Francis R, Whittier J. 2011. Trace element concentrations in nesting flatback turtles (*Natator depressus*) from Curtis Island, Queensland, Australia. *Marine Environmental Research*. 71(1):10–16. <https://doi.org/10.1016/j.marenvres.2010.09.003>
- ISO E. 2005. IEC 17025: General requirements for the competence of testing and calibration laboratories. International Organization for Standardization and International Electrotechnical Commission.
- Jerez S, Motas M, Cánovas RÁ, Talavera J, Almela RM, del Río AB. 2010. Accumulation and tissue distribution of heavy metals and essential elements in loggerhead turtles (*Caretta caretta*) from Spanish Mediterranean coastline of Murcia. *Chemosphere*. 78(3):256–264. <https://doi.org/10.1016/j.chemosphere.2009.10.062>
- Kirpichtchikova TA, Manceau A, Spadini L, Panfili F, Marcus MA, Jacquet T. 2006. Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence, EXAFS spectroscopy, chemical extraction, and thermodynamic modeling. *Geochimica et Cosmochimica Acta*. 70(9):2163–2190. <https://doi.org/10.1016/j.gca.2006.02.006>
- Komoroske LM, Lewison RL, Seminoff JA, Deheyn DD, Dutton PH. 2011. Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. *Chemosphere*. 84(5):544–552. <https://doi.org/10.1016/j.chemosphere.2011.04.023>
- Llorente-Mirandes T, Ruiz-Chancho MJ, Barbero M, Rubio R, López-Sánchez JF. 2010. Measurement of arsenic compounds in littoral zone algae from the Western Mediterranean Sea. Occurrence of arsenobetaine. *Chemosphere*. 81(7):867–875. <https://doi.org/10.1016/j.chemosphere.2010.08.007>

- Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L, Hartge P. 2004. Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. *Environmental Health Perspectives*. 112(17):1691–1696. <https://doi.org/10.1289/ehp.7199>
- Maffucci F, Caurant F, Bustamante P, Bentivegna F. 2005. Trace element (Cd, Cu, Hg, Se, Zn) accumulation and tissue distribution in loggerhead turtles (*Caretta caretta*) from the Western Mediterranean Sea (southern Italy). *Chemosphere*. 58(5):535–542. <https://doi.org/10.1016/j.chemosphere.2004.09.032>
- McClellan CM, Read AJ. 2007. Complexity and variation in loggerhead sea turtle life history. *Biol Lett*. 3(6):592–594. <https://doi.org/10.1098/rsbl.2007.0355>
- Migon C, Robin T, Dufour A, Gentili B. 2008. Decrease of lead concentrations in the Western Mediterranean atmosphere during the last 20 years. *Atmospheric Environment*. 42(4):815–821. <https://doi.org/10.1016/j.atmosenv.2007.10.078>
- Mingozzi T, Masciari G, Paolillo G, Pisani B, Russo M, Massolo A. 2008. Discovery of a regular nesting area of loggerhead turtle *Caretta caretta* in southern Italy: a new perspective for national conservation. In: Hawksworth DL, Bull AT, editors. *Biodiversity and Conservation in Europe* [Internet]. Dordrecht: Springer Netherlands; p. 277–299. [https://doi.org/10.1007/978-1-4020-6865-2\\_20](https://doi.org/10.1007/978-1-4020-6865-2_20)
- Ministero ambiente. [accessed 2019 Mar 20]. [https://www.minambiente.it/sites/default/files/archivio/allegati/bonifiche/accordo\\_priolo\\_07\\_11\\_08.pdf](https://www.minambiente.it/sites/default/files/archivio/allegati/bonifiche/accordo_priolo_07_11_08.pdf)
- Mohamed Harris J, Vinobaba P, Kularatne RKA, Kankanamge CE. 2019. An assessment of heavy metal levels in brackish water shrimps: Impact on sexes and the relationship between metal pollution index and Fulton's K condition indices. *Human and Ecological Risk Assessment: An International Journal*. 25(8):1968–1979. <https://doi.org/10.1080/10807039.2018.1478717>
- Morão IFC, Lemos MFL, Félix R, Vieira S, Barata C, Novais SC. 2022. Stress response markers in the blood of São Tomé green sea turtles (*Chelonia mydas*) and their relation with accumulated metal levels. *Environ Pollut*. 293:118490. <https://doi.org/10.1016/j.envpol.2021.118490>
- Nagelkerken I, Pors LPJJ, Hoetjes P. 2003. Swimming behaviour and dispersal patterns of headstarted loggerhead turtles *Caretta caretta*. *Aquatic Ecology*. 37(2):183–190. <https://doi.org/10.1023/A:1023924631480>
- Naro-Maciel E, Le M, FitzSimmons NN, Amato G. 2008. Evolutionary relationships of marine turtles: A molecular phylogeny based on nuclear and mitochondrial genes. *Mol Phylogenet Evol*. 49(2):659–662. <https://doi.org/10.1016/j.ympcv.2008.08.004>
- Nicolas E, Ruiz-Pino D, Buat-Ménard P, Bethoux JP. 1994. Abrupt decrease of lead concentration in the Mediterranean Sea: A response to antipollution policy. *Geophysical Research Letters*. 21(19):2119–2122. <https://doi.org/10.1029/94GL01277>
- Nicolau L, Monteiro SS, Pereira AT, Marçalo A, Ferreira M, Torres J, Vingada J, Eira C. 2017. Trace elements in loggerhead turtles (*Caretta caretta*) stranded in mainland Portugal: Bioaccumulation and tissue distribution. *Chemosphere*. 179:120–126. <https://doi.org/10.1016/j.chemosphere.2017.03.108>
- Norin H, Vahter M, Christakopoulos A, Sandström M. 1985. Concentration of inorganic and total arsenic in fish from industrially polluted water. *Chemosphere*. 14(3):325–334. [https://doi.org/10.1016/0045-6535\(85\)90060-8](https://doi.org/10.1016/0045-6535(85)90060-8)
- Novillo O, Pertusa JF, Tomás J. 2017. Exploring the presence of pollutants at sea: Monitoring heavy metals and pesticides in loggerhead turtles (*Caretta caretta*) from the western Mediterranean. *Science of The Total Environment*. 598:1130–1139. <https://doi.org/10.1016/j.scitotenv.2017.04.090>
- Perrault JR, Stacy NI, Lehner AF, Poor SK, Buchweitz JP, Walsh CJ. 2017. Toxic elements and associations with hematology, plasma biochemistry, and protein electrophoresis in nesting loggerhead sea turtles (*Caretta caretta*) from Casey Key, Florida. *Environmental Pollution*. 231:1398–1411. <https://doi.org/10.1016/j.envpol.2017.09.001>

- Rie M, Lendas K, Callard I. 2001. Cadmium: Tissue distribution and binding protein induction in the painted turtle, *Chrysemys picta*. *Comp Biochem Physiol Part C Toxicol Pharmacol*. 130:41–51.
- Romano E, Bergamin L, Ausili A, Celia Magno M, Gabellini M. 2016. Evolution of the anthropogenic impact in the Augusta Harbor (Eastern Sicily, Italy) in the last decades: benthic foraminifera as indicators of environmental status. *Environ Sci Pollut Res Int*. 23(11):10514–10528. <https://doi.org/10.1007/s11356-015-5783-x>
- Romano E, Bergamin L, Magno MC, Ausili A. 2013. Sediment characterization of the highly impacted Augusta harbour (Sicily, Italy): modern benthic foraminifera in relation to grain-size and sediment geochemistry. *Environ Sci Process Impacts*. 15(5):930–946. <https://doi.org/10.1039/c3em30824c>
- Rothwell RG, Croudace I w. 2015. Micro-XRF Studies of Sediment Cores: A Perspective on Capability and Application in the Environmental Sciences. In: Croudace IW, Rothwell RG, editors. *Micro-XRF Studies of Sediment Cores: Applications of a non-destructive tool for the environmental sciences* [Internet]. Dordrecht: Springer Netherlands; [accessed 2022 Dec 5]; p. 1–21. [https://doi.org/10.1007/978-94-017-9849-5\\_1](https://doi.org/10.1007/978-94-017-9849-5_1)
- Russo G, Bella C, Loria G, Insacco G, P. P, Violani C, Zava B. 2003. Notes on the influence of human activities on sea chelonians in Sicilian waters. *Journal of Mountain Science*. 7 (Suppl.):37–41.
- Sakai H, Ichihashi H, Suganuma H, Tatsukawa R. 1995. Heavy metal monitoring in sea turtles using eggs. *Marine Pollution Bulletin*. 30(5):347–353. [https://doi.org/10.1016/0025-326X\(94\)00185-C](https://doi.org/10.1016/0025-326X(94)00185-C)
- Sakai H, Saeki K, Ichihashi H, Suganuma H, Tanabe S, Tatsukawa R. 2000. Species-Specific Distribution of Heavy Metals in Tissues and Organs of Loggerhead Turtle (*Caretta caretta*) and Green Turtle (*Chelonia mydas*) from Japanese Coastal Waters. *Marine Pollution Bulletin*. 40(8):701–709. [https://doi.org/10.1016/S0025-326X\(00\)00008-4](https://doi.org/10.1016/S0025-326X(00)00008-4)
- Sankar TV, Zynudheen AA, Anandan R, Viswanathan Nair PG. 2006. Distribution of organochlorine pesticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India. *Chemosphere*. 65(4):583–590. <https://doi.org/10.1016/j.chemosphere.2006.02.038>
- Slooff W. 1982. Skeletal anomalies in fish from polluted surface waters. *Aquatic Toxicology*. 2(3):157–173. [https://doi.org/10.1016/0166-445X\(82\)90013-3](https://doi.org/10.1016/0166-445X(82)90013-3)
- Storelli MM, Barone G, Storelli A, Marcotrigiano GO. 2008. Total and subcellular distribution of trace elements (Cd, Cu and Zn) in the liver and kidney of green turtles (*Chelonia mydas*) from the Mediterranean Sea. *Chemosphere*. 70(5):908–913. <https://doi.org/10.1016/j.chemosphere.2007.06.069>
- Storelli MM, Ceci E, Marcotrigiano GO. 1998. Distribution of Heavy Metal Residues in Some Tissues of *Caretta caretta* (Linnaeus) Specimen Beached Along the Adriatic Sea (Italy). *Bull Environ Contam Toxicol*. 60(4):546–552. <https://doi.org/10.1007/s001289900660>
- Storelli MM, Marcotrigiano GO. 2000. Total Organic and Inorganic Arsenic from Marine Turtles (*Caretta caretta*) Beached Along the Italian Coast (South Adriatic Sea). *Bull Environ Contam Toxicol*. 65(6):732–739. <https://doi.org/10.1007/s0012800184>
- Storelli MM, Storelli A, D’Addabbo R, Marano C, Bruno R, Marcotrigiano GO. 2005. Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation. *Environmental Pollution*. 135(1):163–170. <https://doi.org/10.1016/j.envpol.2004.09.005>
- Storelli MM, Zizzo N. 2014. Occurrence of organochlorine contaminants (PCBs, PCDDs and PCDFs) and pathologic findings in loggerhead sea turtles, *Caretta caretta*, from the Adriatic Sea (Mediterranean Sea). *Science of The Total Environment*. 472:855–861. <https://doi.org/10.1016/j.scitotenv.2013.11.137>
- Tian RC, Ruiz-Pino D. 1995. Simulation and prediction of anthropogenic lead perturbation in the Mediterranean Sea. *Science of The Total Environment*. 164(2):135–150. [https://doi.org/10.1016/0048-9697\(95\)04455-A](https://doi.org/10.1016/0048-9697(95)04455-A)

Tigano C, Tomasello B, Pulvirenti V, Ferrito V, Copat C, Carpinteri G, Mollica E, Sciacca S, Renis M. 2009. Assessment of environmental stress in *Parablennius sanguinolentus* (Pallas, 1814) of the Sicilian Ionian coast. *Ecotoxicology and Environmental Safety*. 72(4):1278–1286. <https://doi.org/10.1016/j.ecoenv.2008.09.028>

Torrent A, González-Díaz OM, Monagas P, Orós J. 2004. Tissue distribution of metals in loggerhead turtles (*Caretta caretta*) stranded in the Canary Islands, Spain. *Marine Pollution Bulletin*. 49(9):854–860. <https://doi.org/10.1016/j.marpolbul.2004.08.022>

Wyneken J. 2001. *The Anatomy of Sea Turtles*. US Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470.172.

Zota AR, Calafat AM, Woodruff TJ. 2014. Temporal Trends in Phthalate Exposures: Findings from the National Health and Nutrition Examination Survey, 2001–2010. *Environmental Health Perspectives*. 122(3):235–241. <https://doi.org/10.1289/ehp.1306681>

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