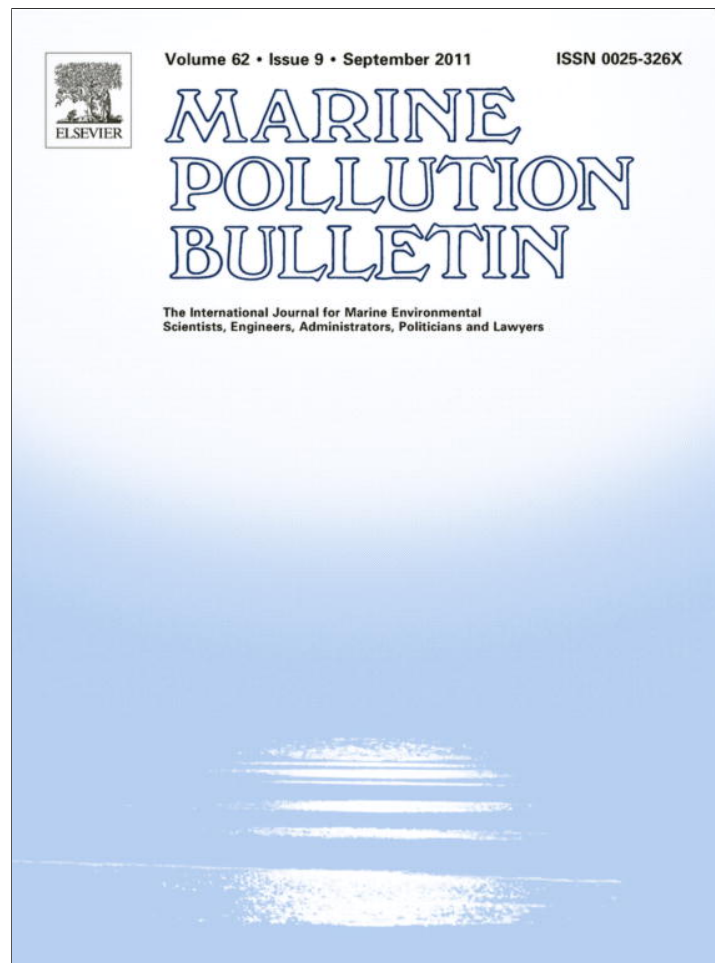


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Baseline heavy metals and metalloid values in blood of loggerhead turtles (*Caretta caretta*) from Baja California Sur, Mexico

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ABSTRACT

Environmental pollution due to heavy metals is having an increased impact on marine wildlife accentuated by anthropogenic changes in the planet including overfishing, agricultural runoff and marine emerging infectious diseases. Sea turtles are considered sentinels of ecological health in marine ecosystems. The objective of this study was to determine baseline concentrations of zinc, cadmium, copper, nickel, selenium, manganese, mercury and lead in blood of 22 clinically healthy, loggerhead turtles (*Caretta caretta*), captured for several reasons in Puerto López Mateos, Baja California Sur, Mexico. Zinc was the most prevalent metal in blood ($41.89 \mu\text{g g}^{-1}$), followed by Selenium ($10.92 \mu\text{g g}^{-1}$). The mean concentration of toxic metal Cadmium was $6.12 \mu\text{g g}^{-1}$ and $1.01 \mu\text{g g}^{-1}$ respectively. Mean concentrations of metals followed this pattern: $\text{Zn} > \text{Se} > \text{Ni} > \text{Cu} > \text{Mn} > \text{Cd} > \text{Pb}$ and Hg. We can conclude that blood is an excellent tissue to measure in relatively non-invasive way baseline values of heavy metals in *Caretta caretta*.

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The pollution of the oceans represents a problem of great impact to the health of wildlife, humans and ecosystems (Wilcox and Aguirre, 2004). Among the environmental contaminants of great relevance due to their toxicity are those of chemical origin such as pesticides, halogenated hydrocarbons, PCBs, organochlorines, carbamates, dioxin, solid waste and heavy metals (Aguirre et al., 2006; Clark, 2001; Friend and Franson, 1999). In particular, heavy metals have a great relevance in ecotoxicology because they are highly persistent and their potential toxicity to all organisms (Storelli et al., 2005). In addition, they are not biodegradable through bacterial metabolic pathways in a short period of time.

In recent years several studies have demonstrated the effects of heavy metals in coastal areas adding their accumulation effects on the immune system of several species (García-Fernández et al., 2008; Keller et al., 2004; Richardson et al., 2010; Ruelas-Inzunza et al., 2008; Soto-Jimenez et al., 2003; Storelli et al., 1998; Szefer et al., 2006). Therefore, it is important to understand the effects of

heavy metals (Cd, Hg, Pb, Zn, Cu, Mn and Ni) and some metalloids (As and Se) on the health and immunology of species placed in higher trophic levels of the food web, as biomagnification processes could have devastating pathologic effects (Tabor and Aguirre, 2004).

The effects of heavy metals and other contaminants in sea turtles have been previously documented (Day et al., 2007; Godley et al., 1999; Maffucci et al., 2005). In addition, they have been listed as one of the potential synergic etiologies of marine turtle fibropapillomatosis (Aguirre et al., 1994). With current anthropogenic changes in marine ecosystems, heavy metals are a threat to marine turtle populations worldwide (Maffucci et al., 2005).

Loggerhead turtles (*Caretta caretta*) are categorized as endangered of extinction by Mexican law due to several factors including the illegal harvest of turtles and their eggs, nesting habitat destruction and pollution (Gardner and Nichols, 2001; Lutcavage et al., 1997). Unfortunately, there is no published information on pollution levels and their effects in sea turtle populations in Mexico, particularly the Pacific loggerhead turtle (Gardner et al., 2006). Due to its carnivorous diet, the species may be susceptible of acquiring high levels of heavy metals by bioaccumulation as previously reported (Sakai et al., 2000a; Storelli and Marcotrigiano, 2003). It is an established fact that carnivores tend to have higher levels of heavy metals than herbivores (Storelli et al., 1998).

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Blood has been successfully used as a tissue to measure heavy metals and metalloids due to the vital functions performed by blood cells and their susceptibility to intoxication (Caurant et al., 1999; Goye and Clarkson, 2001). The processes of absorption, accumulation and circulation of heavy metals that can be correlated to their bio-availability and potential toxicity can be observed in blood (Du Laing et al., 2007). This study reports baseline levels of selected heavy metals in blood of Pacific loggerhead turtles in Baja California Sur (BCS), Mexico.

Clinically healthy loggerhead turtles were captured by hand using snorkel that we denominate “turtle rodeo” along the coast-line of Puerto López Mateos, BCS (25°00'0"N; 12°30'0"W). All turtles were measured with a one-meter caliper, weighed (standard Carapace length: 49.0–83.5 cm; mean 68.9 ± 10.0) following sampling procedures described earlier (Bolten, 1999). All turtles were released unharmed into the ocean. Briefly, 8–10 mL blood samples were collected from the dorsal postoccipital sinus (Owens, 1999) using 10 mL heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ), and 21" needles for different purposes including hematology, plasma biochemistry, hormones and heavy metal analysis. All specimens were kept cool at 4 °C either in wet ice, blue ice or a refrigerator then kept in an ultrafreezer at –70 °C until processing.

All the laboratory materials used were thoroughly acid-washed to prevent contamination of samples (Paez-Osuna et al., 2010). Whole blood was subjected to an acid digestion using a mixture of 5 ml HNO₃, HCl and H₂O₂ in a proportion of 2:2:1 to 0.5 g of each blood specimen (wet weight). Digestions were performed in a microwave system (ANTON PAAR). Likewise, the reference equipment TORT-2 (National Research Council of Canada, Ottawa) was used per triplicate to determine the percentage of recuperation and evaporation, assuring that the analyzed values were within certified values (Gardner et al., 2006). The percentage of the recovered metals was approximately 95%. To ensure the efficiency of the equipment while reading the concentrations and to observe if the analysis matrix could generate interference, 0.06 µL of SIMA 6000 (Perkin-Elmer) multi-element standard was added to 0.5 g of two blood specimens and a third one in deionized water; obtaining about 93% accordance in results. Digestions were aforated in a volumetric pipette with 25 mL of deionized water.

Heavy metal concentrations (based on wet weight) were determined using an optical emission inductively coupled plasma atomic spectrophotometer (ICP-AES) model OPTIMA 4300™ DV (Perkin-Elmer). Analytical wavelengths (nm) were Cd = 228.8; As = 188.9; Ni = 231.6; Hg = 253.6; Pb = 220.3; Zn = 206.2; Cu = 327.3; Mn = 257.6; and Se = 196.0. In addition, a concentration of 0.48 µL in 25 mL of the standard reference in order to determine that concentrations were below the detection level of the calibration curve being used. Blanks were also analyzed in eight samples to check for contamination. Detection limits for each element were 0.01 µg g⁻¹ for Cd, As, Ni, Hg and Pb, and 0.04 µg g⁻¹ for Zn, Cu, Mn and Se.

All statistical analyses were performed using StatSoft STATISTICA 8.0.550. Reported statistics are arithmetic means and standard deviation (SD) in micrograms per gram on wet weight. The correlations among the different heavy metals in blood were determined using a simple regression model R² 50% used as a statistical indicator. The CCL and weight were used as a relative indicator of age determination in sea turtles (Gardner et al., 2006). Standard, one-way analysis of variance (ANOVA) was used to determine significant differences in the size of the turtles captured.

No correlations were found between of heavy metals and metalloids analyzed in blood and the size of the turtles in this study. Table 1 summarizes the concentrations of heavy metals and metalloids analyzed in loggerhead turtles. Essential elements like zinc and selenium were the most common metals identified followed

Table 1

Concentration (µg g⁻¹ wet weight) of heavy metals and metalloids in blood of loggerhead turtles (*Caretta caretta*) collected in Puerto López Mateos, BCS, Mexico.

Element	Mean ± SD	Range (n ^a)
Zn	4481 ± 1753	28.02–75.00
Cu	283 ± 062	<LOD – 1.094 (13)
Mn	061 ± 055	<LOD – 2.65 (16)
Ni	159 ± 242	<LOD – 13.25 (15)
Cd	18 ± 063	1.33–3.78
As	409 ± 256	0.35–23.61
Se	614 ± 358	1.22–14.40

LOD – Limit of detection; n^a – Number of samples above the LOD in parenthesis if ≤ 16.

by cadmium and arsenic. Nickel and manganese were present in very low concentrations. Pb and Hg were below the detection levels of the calibration curve being used.

Metals like Zn, Cu and Mn are related based on physico-chemical similarities that interact in different physiological pathways (Frías-Espéricueta et al., 2006). During this study, the concentration of these metals were higher than levels reported in muscle of sea turtles; however, the concentration of Cu and Mn in blood was lower than the ones reported in other regions for liver and kidney, and the Zinc was higher than previous studies (Caurant et al., 1999; García-Fernández et al., 2008; Sakai et al., 2000b; Storelli et al., 2005; Torrent et al., 2004) (Table 2). The concentration of Zn (58.4 µg g⁻¹), was higher in blood of Kemp's ridley turtles (*Lepidochelys olivacea*) while levels of Cd and Cu were similar (Paez-Osuna et al., 2010). Goye and Clarkson (2001) described that Zn can remain in blood and bones for several days before being fixed or excreted. Concentrations of Zn in our study suggest a major exposure to this element in loggerhead turtles from BCS than other turtle species previously reported in the literature. These levels matched the results reported by Gardner et al. (2006). It may be possible to determine the low affinity of these elements in particular Cu, for muscle tissue (Storelli et al., 2005); while the highest concentrations are concentrated in liver and kidney (Goye and Clarkson, 2001) (Table 2). Storelli et al. (2008) reported that high levels of Zn in liver could act as a possible regulation mechanism; however, the relationship of high Cu levels and their affinity to liver is practically unknown. In the case of manganese, there are few studies reporting accumulation in sea turtles (Gardner et al., 2006; Sakai et al., 2000a).

This metal is considered a nutritionally essential trace metal for some plants, bacteria and invertebrates (Goye and Clarkson, 2001). Ni values in blood where higher in our study than those reported (0.01–0.35 µg g⁻¹) in several tissues by Gardner et al. (2006) (Table 1). Our values correlated with other studies with similar values (Aguirre et al., 1994; Sakai et al., 2000a; Sakai et al., 2000b). Perhaps due to the low concentrations of Ni in the environment these tend not to concentrate in sea turtles (Goye and Clarkson, 2001).

The primary pathway of Cd poisoning is through the food chain (Maffucci et al., 2005; Storelli et al., 2008,2005). Our study reported higher levels of Cd than Gardner et al. (2006) in muscle, adipose tissue and liver. However, not for renal values as they reported the highest levels in any aquatic organism for Mexico (Table 2). For other regions, higher levels are reported in liver and kidney and lower for muscle (Caurant et al., 1999; García-Fernández et al., 2008; Sakai et al., 2000a; Storelli et al., 1998,2005; Torrent et al., 2004). This correlates to the physiology and accumulation process of the metal in liver where we identify the highest accumulations in any marine organism. Storelli et al. (2005) studied a population of *C. caretta* in the Adriatic Sea, concluding that the low concentrations of Cd may be correlated to the foraging areas where the metal is present in extremely low concentrations (Frías-Espéricueta et al., 2006; Sakai et al., 2000a; Storelli and

Table 2
Concentrations (Mean ± SD) of heavy metals and As reported in several countries from selected tissues of loggerhead turtles (*Caretta caretta*).

Site	Zn	Cu	Cd	As	Author
<i>Liver</i>					
France	25.00 ± 9.50	8.25 ± 6.59	2.58 ± 4.12	NA	Caurant et al. (1999)
Italy	27.90 ± 6.50	7.40 ± 3.90	2.84 ± 0.72	NA	Franzellitti et al. (2004)
Italy	29.3 ± 7.71	7.69 ± 4.63	3.36 ± 1.94	NA	Storelli et al. (2005)
Spain	13.48 ± 1.70	15.02 ± 2.07	2.53 ± 0.45	NA	Torrent et al. (2004)
Spain	26.82 ± 20.63	5.40 ± 2.01	5.85 ± 13.42	NA	García-Fernández et al. (2008)
Mexico	69.14	33.94	1.75	NA	Gardner et al. (2006)
Japan	28.10 ± 4.70	17.70 ± 8.90	28.10 ± 4.70	NA	Sakai et al. (2000b)
Japan	NA	NA	NA	6.32	Saeki et al. (2000)
Italy	NA	NA	NA	6.7	Storelli and Marcotrigiano (2000)
<i>Kidney</i>					
Italy	30.90 ± 8.00	1.50 ± 0.40	0.36 ± 0.11	NA	Franzellitti et al. (2004)
Italy	29.3	7.69	3.36	NA	Storelli et al. (2005)
Spain	27.88 ± 26.77	3.77 ± 3.50	31.47 ± 70.75	NA	García-Fernández et al. (2008)
Mexico	32.47	4.35	73.11	NA	Gardner et al. (2006)
Italy	23.1 ± 4.53	1.21 ± 0.54	8.35 ± 4.83	NA	Storelli et al. (2005)
Japan	25.4 ± 4.39	1.30 ± 0.21	38.3 ± 17.5	NA	Sakai et al. (2000b)
Japan	NA	NA	NA	6.32	Saeki et al. (2000)
<i>Muscle</i>					
Italy	30.90 ± 8.00	1.50 ± 0.40	0.36 ± 0.11	NA	Franzellitti et al. (2004)
Italy	29.3	7.69	3.36	NA	Storelli et al. (2005)
Spain	65.39 ± 28.3	5.04 ± 1.93	0.20 ± 0.14	NA	García-Fernández et al. (2008)
Mexico	31.11	0.41	0.1	NA	Gardner et al. (2006)
Japan	25.0 ± 3.50	0.81 ± 0.28	0.06 ± 0.03	NA	Sakai et al. (2000a)
Italy	NA	NA	NA	15.47	Storelli and Marcotrigiano (2000)

Concentration in µg g⁻¹; NA = Not analyzed; ND = Not detected.

Marcotrigiano, 2003). However, for *C. caretta*, diet may not have any relevance as this may be related to the physiology of each species and perhaps age may play a more important role in the accumulation process (Caurant et al., 1999; Saeki et al., 2000; Storelli et al., 2005).

The correlations of Cd with other metals in tissues of sea turtles have been emphasized in the study performed by Gardner et al. (2006). Positive correlations of Cd with Ni, Mn and As were observed in blood of analyzed specimens (Fig. 1). Other studies have observed correlations between Cd and Zn and Cu in liver. Apparently both metals play a Cd detoxifying role (Gardner et al., 2006; García-Fernández et al., 2008; Storelli et al., 2008). These correlations were not observed in this study. Correlations between Cd and Mn were observed in liver of *L. olivacea* y *C. Caretta*, (Gardner et al., 2006). The correlation of Cd and other metals, specifically the one with As was not possibly established due to lack of knowledge in this ecotoxicological research. In the literature, most of the studies are directed to measure correlations among Cd and Zn or Ca and Cu particularly in blood. However, there are no studies of correlating Cd and Mn. Therefore, more studies are warranted to determine possible functions or effects of these metals in blood.

Together with Cd, Pb and Hg are the metals with great ecotoxicologic importance. In our study, the concentrations of Pb and Hg were below the detection levels of the calibration curve being used; however, in other study in blood of *C. caretta*, low concentrations of Hg and the negative correlation found *ex vivo* between mercury and lymphocyte numbers and mercury and B-cell proliferative responses suggest subtle negative impacts of mercury on sea turtle immune function (Day et al., 2007).

There are extremely few studies in the literature about As in sea turtles (Storelli and Marcotrigiano, 2000). The average levels reported here in were lower than those reported somewhere else (Table 2) for muscle and liver of *C. caretta* (Agusa et al., 2008; Saeki et al., 2000; Storelli et al., 1998; Storelli and Marcotrigiano, 2000). In all these studies, the highest concentration of As was recorded in muscle, while the inorganic form tends to accumulate in liver (Storelli and Marcotrigiano, 2003). Concentrations of As tend to be high in crustaceans and this may represent a risk to sea turtle

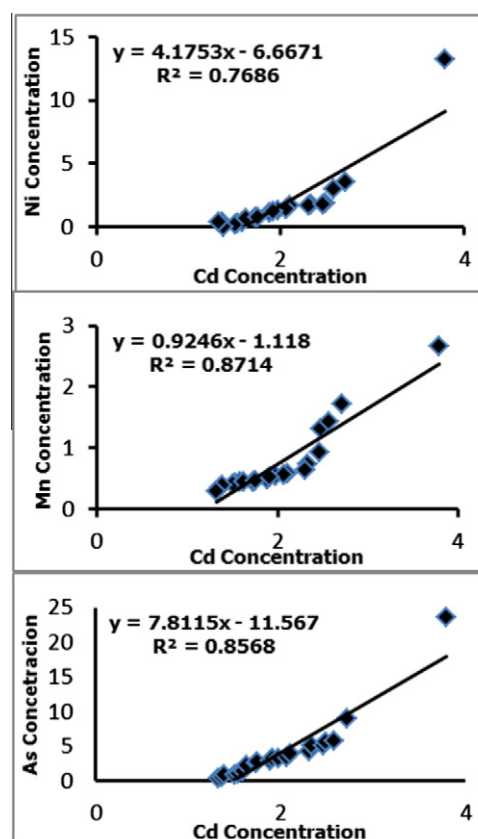


Fig. 1. Correlations of blood concentrations (µg g⁻¹) between Cd and other metals in *C. caretta* captured in Baja California Sur, Mexico.

species feeding in crabs like loggerheads. The most toxic is the inorganic form that accumulates in liver (Storelli and Marcotrigiano, 2003). Further studies are necessary to explain the levels in blood for sea turtles.

Although being an essential metal for living organisms, Se can be toxic in high levels of exposure (Aguirre et al., 1994). Previous publications on Se related to accumulation in different tissues of *C. Caretta* report lower levels than the ones found in our study ($1.19\text{--}3.54\ \mu\text{g g}^{-1}$) (Caurant et al., 1999; Storelli et al., 1998, 2005). A previous publication (Aguirre et al. 1994) reporting values of Se in kidney ($0.16\ \mu\text{g g}^{-1}$) and liver ($0.57\ \mu\text{g g}^{-1}$) in green turtles, *Chelonia mydas* from Hawaii, described that higher Se levels were identified in the pelagic phase of the species, when turtles are carnivorous compared to adults that have mostly a herbivorous diet. During the same study, the author reported a hepatic concentration of $3.39\ \mu\text{g g}^{-1}$ in a pelagic specimen of *C. caretta*. Those concentrations are lower than those reported herein. However, Storelli et al. (2005) reported that the accumulation of this metal in some marine mammals, is always elevated, but in aquatic reptiles like sea turtles is difficult to establish whether concentrations of Se can be toxic.

This study reports selected metal and metalloid values in blood of a wild population of *C. Caretta*. It is important to emphasize the lack of ecotoxicological research in marine organisms and that the few studies published provide just a glimpse of the complexity of toxic metals and their interaction with the ocean environment. For example, Zn was found in high concentrations compared to levels reported in other parts of the world. For now, we can conclude that levels correlate to exposure of this metal in populations of sea turtles in the Eastern Pacific and correlate with the previous study by Gardner et al. (2006). In addition, we could observe higher concentrations of Cu and Cd in blood as they relate to concentrations in muscle; however, previous published studies report the low affinity of these metals to muscle. We were able to observe correlations of Cd among As, Ni and Mn. There is a big gap of information in the correlation of different metal values in marine organisms. In addition, the function of Mn in the detoxification process is mostly unknown. We can conclude that blood is an excellent tissue to measure in a relatively non-invasive way baseline values of heavy metals in *C. Caretta*. Further research is necessary to determine the role of these heavy metals in the physiology and immunology of this species and their role in the health parameters from the individual to the population levels and their impact to ecosystems. Also, is necessary to study the effects of toxic metals like Hg and Pb in sea turtle immunity and reproduction even at low concentrations.

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Corrigendum

Corrigendum to ‘Baseline heavy metals and metalloid values in blood of loggerhead turtles (*Caretta caretta*) from Baja California Sur, Mexico’ [Mar. Pollut. Bull. 62 (2011) 1979–1983]

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The authors regret that the decimal points were not displayed correctly in table 1 of their article. The correct version of the table appears below.

The authors would like to apologise for any inconvenience caused.

Table 1

Concentration ($\mu\text{g g}^{-1}$ wet weight) of heavy metals and metalloids in blood of loggerhead turtles (*Caretta caretta*) collected in Puerto López Mateos, BCS, Mexico.

Element	Mean \pm SD	Range (n ^a)
Zn	44.81 \pm 17.53	28.02-75.00
Cu	2.83 \pm 0.62	<LOD-1.094 (13)
Mn	0.61 \pm 0.55	<LOD -2.65 (16)
Ni	1.59 \pm 2.42	<LOD -13.25 (15)
Cd	1.8 \pm 0.63	1.33-3.78
As	4.09 \pm 2.56	0.35-23.61
Se	6.14 \pm 3.58	1.22-14.40

LOD- Limit of detection; n^a- Number of samples above the LOD in parenthesis if ≤ 16 .

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