Chapter 9
Ecotoxicological Studies of Metal Pollution in Sea Turtles of Latin America

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9.1 Introduction

Metals are natural components of rocks and soil. Natural processes like weathering and erosion are responsible for their entering into water bodies. However, rapid urban development and increases in fertilizer and pesticide use, as well as industrial activities such as mining and smelting, fossil fuel use, and many forms of waste disposal, have drastically raised the amount of such elements in the environment (Athar and Vohora 1995). Estuaries and coastal regions generally act as the final receiving body of these substances, and their increased concentrations tend to accumulate, concentrate, and biomagnify through the food chain since the organisms are not able to completely eliminate the metals absorbed (Storelli et al. 2005; Camacho et al. 2013).

Some metals, such as Al, As, Co, Cr, Cu, Se, and Zn, play a crucial role in animal metabolism and growth pathways, but deviations above the normal range result in metal toxicity, while concentrations below the range can also be detrimental to the functioning of the organism (Keller et al. 2006). Other metals (Pb, Hg, Cd, As) have no function in the organism, and their accumulation can pose a threat to the wildlife that interacts with them. This interaction occurs through ingestion, inhalation, and/or absorption. However, the most common route for these elements is from dietary intake (Anan et al. 2001). In sea turtles, the bioaccumulation of heavy metals is

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critical, due to their long life spans, high daily consumption rates, and extended periods of time spent in coastal foraging grounds near sources of pollution.

Most sea turtle populations around the globe are listed as threatened or endangered (IUCN 2019), facing both natural and anthropogenic stressors linked to dramatic ontogenetic life cycle changes. Although physically robust and able to accommodate severe physical damage, sea turtles appear surprisingly susceptible to biological and chemical insults. The adverse effects include compromised physiology, chronic stress, impaired immune function, and an increase in disease susceptibility, like fibropapillomatosis (Aguirre et al. 1994; Aguirre and Lutz 2004).

On the other hand, sea turtles present several particular advantages as indicators of heavy metal pollution (Lam et al. 2004). Sea turtles have a wide distribution range during their life cycle, which begins in the terrestrial environment, and then moving to a pelagic phase and returning to coastal areas to feed, they may serve as a good proxy for overall ecosystem health (Kampalath et al. 2006). Furthermore, the development within a wide range of environments increases the possibility of interacting with anthropogenic impacts including pollution, fishing, or changing environments (Marcovaldi et al. 2006; Gilman et al. 2007).

In addition to their high mobility throughout different ecosystems, the long life history of sea turtles enables an extended exposure period to contaminants, both spatially and temporally. As they occupy different trophic levels, from herbivorous to carnivorous, they can provide a fairly comprehensive profile of contamination throughout the food chain (Anan et al. 2001). Besides that, levels of metals in sea turtles may reveal a better picture of hazards to humans than measurements taken in the physical environment, plants, or invertebrates (Anan et al. 2002).

Sea turtles are also considered sentinel species (Aguirre and Lutz 2004) since they suffer from disease processes related to environmental conditions as the worldwide distributed fibropapillomatosis, a skin cancer associated with a herpesvirus 5 (ChHV5) which affects especially green turtles.

Thus, sea turtles can be useful as sentinel species and bioindicators for diverse pollutants, and they can help us to understand the risk not only to the species themselves but also to the ecosystem at large.

9.2 Sea Turtles of Latin America

Latin America contains 13 dependencies and 20 countries, which cover an area that stretches from the northern border of Mexico to the southern tip of South America, including the Caribbean. Of the seven species of sea turtles, six are found in the coast of Latin America; these include *Chelonia mydas* (and *Chelonia mydas agassizii*), *Eretmochelys imbricata*, *Caretta caretta*, *Lepidochelys olivacea*, *Lepidochelys kempii*, and *Dermochelys coriacea*.

Many coastal areas are important foraging and nesting sites for these species. However, some of them are exposed to environmental pollution via anthropogenic (industries, mining activities, oil and gas) or natural sources (naturally enriched soil and sediment, runoff, and atmospheric deposition) which could affect the development, health, reproduction, and survival of these animals.

Sea turtles are also an important food source in many coastal communities, because they can provide an easy-access source of protein and income, especially for low-income families or communities. Moreover, hunting sea turtles is a traditional practice for certain indigenous populations in Guyana, Mexico, and Nicaragua, for whom turtles are culturally important. The collection and sale of eggs and/or adult turtles has, with limitations, been allowed in certain countries, including Costa Rica, Guatemala, and Honduras (Ross et al. 2017). However, little is known about the potential exposure to heavy metal intake through the consumption of sea turtles and their eggs.

Studies regarding the toxicology of heavy metals in sea turtles from Latin America have assessed five species, but *Chelonia mydas* and *Lepidochelys olivacea* are more highlighted (Fig. 9.1). It differs from the studies made all over the world, where the most studied species are *Chelonia mydas* and *Caretta caretta* (Cortés-Gomes et al. 2017). Due to their toxicity risk, cadmium, lead, and mercury are among the most studied metals.

The researchers have identified a range of contaminants in various sea turtle tissues (liver, kidney, muscle, adipose tissue, heart, brain, bone, salt gland), but noninvasive and nonlethal methods of monitoring are crucial to allow a large number of samples, including live and healthy animals. That is why some authors have aimed assessing the use of blood, scutes, and/or eggs as a predictive matrix for the pollutants metabolized by the organism.

9.3 Biomarkers of Ecotoxicological Assessment

The term biomarker refers to body fluids, cells, or tissues and behavioral, physiological, or energetic responses of organisms that may indicate the presence of or exposure to contaminants (Livingstone 1993). In the following section, the biomarkers used in sea turtle toxicological studies are described and the publications about them are pointed out.

9.3.1 Blood

The use of blood in assessing the exposure to contaminants is a good option because it is quick and easy to collect and the animals can return to the environment immediately following venipuncture. Blood has been recognized as an indicator of recent exposure to pollutants in sea turtles, since it is the first means of transport of these elements throughout the body and before targeting organs (Day et al. 2005).

Many evidences indicate that the route of entry of pollutants in sea turtles occurs mainly through food intake. Thus, blood samples could provide an approach on

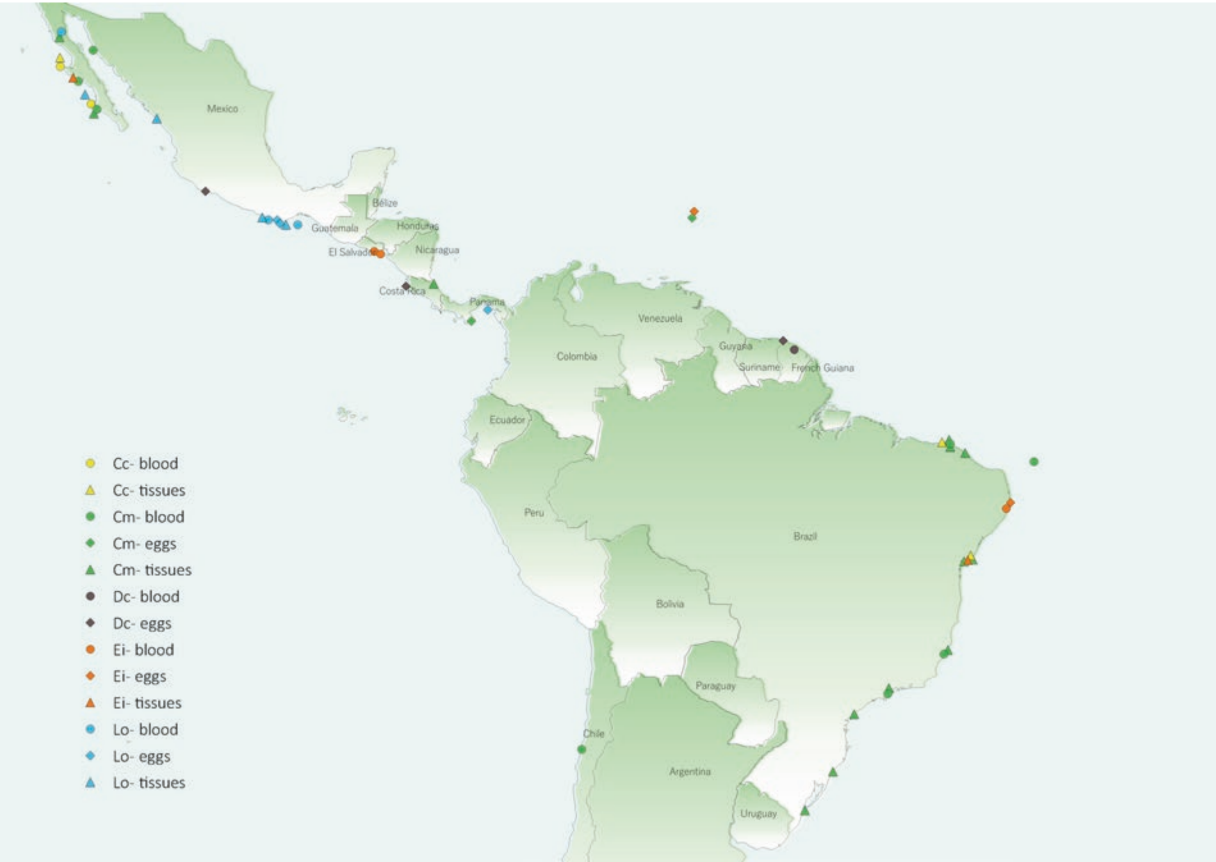

Fig. 9.1 Location of the studies presented in this review. Different symbols were used to represent studies that sampled blood, tissues, and eggs. Different colors were used to represent each species of sea turtles (*Caretta caretta, Cc; Chelonia mydas, Cm; Dermochelys coriacea, Dc; Eretmochelys imbricata, Ei; Lepidochelys olivacea, Lo*)

contamination from the site where turtles feed. During the nesting season, sea turtles have rarely been observed foraging; nevertheless, females seem to ingest at least a significant volume of water to decrease their body temperature in warm waters of nesting tropical beaches (Southwood et al. 2005) and to ensure egg production (albumen is mainly composed of water) (Wallace et al. 2006), which could be related to their contamination during this period. Besides allowing the determination of different chemicals, blood can also be used to assess clinical parameters that could be adversely modulated by the pollutants (Camacho et al. 2013).

In Latin America 20 studies used blood as a biomarker (Table 9.1). The most investigated species is *Lepidochelys olivacea*, with nine publications from Mexico. There are four publications on *Chelonia mydas* (three from Brazil and one from Chile), one publication on *Chelonia mydas agassizii* from Mexico, three publications on *Eretmochelys imbricata* (two from El Salvador and one from Brazil), and two publications on *Caretta caretta* from Mexico and one publication on *Dermochelys olivacea* from French Guiana.

Only ten publications correlated the levels of contaminants with a toxicological endpoint. The majority of these publications investigated health parameters (Ley-Quiñónez et al. 2017; Álvarez-Varas et al. 2017; Tauer et al. 2017; Cortés-Gómez et al. 2017, 2018a), three publications correlated with oxidative stress (Labrada-Martagón et al. 2011; Silva et al. 2016; Cortés-Gómez et al. 2018b), two publications with fibropapillomatosis (Silva et al. 2016; Prioste 2016), and one with carapace asymmetry (Cortés-Gómez et al. 2018c).

In Mexico, Ley-Quiñónez et al. (2017) investigated associations among metals and biochemical parameters from 22 *Caretta caretta* captured in Bahía Magdalena, finding significant positive correlations between Cd, As, and Mn and ALP. The authors argument that this increase in ALP activity may be caused by Cd and As accumulation suggesting a possible liver damage. Other possibility may occur due to an incorrect excretion process of these elements, resulting in the development of pathologies.

However, they highlight that the explanation of such correlations remains speculative, due to probable involvement of immune responses and the lack of baseline information regarding the toxicological effects of metal accumulation in sea turtle and their immune response to it. Cortés-Gómez et al. (2018a) also found significant relationships between metals and biochemical substances in *Lepidochelys olivacea* from Oaxaca. In this study, Sr and As had positive correlations with AST and urea, while As and Cd had negative correlations with glucose. Esterase activity (EA) was the parameter with most negative correlations (with Pb, Ti, As, Cr, and Se), which reinforce the results of other researchers regarding the possible inhibition of EA by metals. The authors concluded that the several correlations detected between the elements analyzed and biochemical parameters indicate that these contaminants may have a negative effect on the health of these turtles.

Cortés-Gómez et al. (2017) also examined the concentrations of EA and cortisol in *Lepidochelys olivacea* from a Mexican population and evaluated the possible correlations with metals. The authors found significant correlations between EA and important metals, such as Cd, Pb, Ti, and Al, and between cortisol and Sr, Se, and

Table 9.1 Metal levels (mean ± SD, μg.g^{-1} wet weight) in blood of sea turtles at different areas in Latin America

						Essential metals						Nonessential metals					
Study site[a]	Sp	n	CCL	Capture	Method	Cr	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	Sr	Source
Mexico – BS	*Cc*	22	69.0	DV	ICP-AES	–	2.8 ± 0.3	0.6 ± 0.7	1.5 ± 1.9	6.14 ± 1.4	44.8 ± 2.7	4.0 ± 1.2	1.8 ± 0.4	–	–	–	Ley-Quiñónez et al. (2011)
Brazil – CE	*Cm*	24	48.4	IC	ICP-MS	–	–	–	–	29.99 ± 126	7.58 ± 4.17	0.93 ± 0.90	0.016 ± 0.016	0.009 ± 0.008	0.029 ± 0.027	–	Prioste (2016)[b]
Brazil – ES	*Cm*	68	42.8	CN	ICP-MS	–	–	–	–	1.19 ± 1.85	21.53 ± 46.08	2.16 ± 5.15	0.009 ± 0.011	0.010 ± 0.017	1.12 ± 2.04	–	(Prioste (2016)[b]
Brazil – PE	*Cm*	31	72.0	I	ICP-MS	–	0.757	0.061	–	0.424	14.05	0.366	0.014	–	0.027	–	Prioste et al. (2015)[b]
Brazil – PE	*Cm*	71	68.7	DV	ICP-MS	–	–	–	–	0.331 ± 0.37	8.31 ± 4.26	5.04 ± 39.7	0.010 ± 0.009	0.0002 ± 0.0002	0.027 ± 0.020	–	Prioste (2016)[b]
Brazil – SP	*Cm*	70	39.3	IC	ICP-MS	–	–	–	–	1.23 ± 1.96	6.94 ± 5.79	2.17 ± 3.97	0.012 ± 0.013	0.022 ± 0.037	0.034 ± 0.029	–	Prioste (2016)[b]
Brazil – SP	*Cm*	13	37.0	DV/IC	AAS	–	0.92	–	8.94	–	0.68	–	0.078	–	0.954	–	Silva et al. (2016)[c]
Chile – AT	*Cm*	7	66.5	EN	AAS	–	2.26 ± 0.10	–	–	–	–	–	–	–	1.11 ± 0.06	–	Álvarez-Varas et al. (2017)
Mexico – BS	*Cm*	42	67.2[d]	FN	FAAS	–	–	–	76.47	1.59	13.92	–	0.06	–	–	0.28	Labrada-Martagón et al. (2011)
Mexico – BS	*Cm*	14	61.2[d]	FN	FAAS	–	–	–	73.30	1.81	13.58	–	0.03	–	–	0.18	Labrada-Martagón et al. (2011)
Mexico – SO	*Cma*	12	67.2[d]	DV/FN	ICP-OES	–	1.71 ± 0.73	1.22 ± 0.99	1.03 ± 1.01	7.66 ± 3.19	63.58 ± 17.0	–	0.99 ± 0.35	–	–	–	Ley-Quiñónez et al. (2013)
French Guiana – SLM	*Dc*	78	160	DN	ICP-MS/DMA	–	1.34 ± 0.28	–	–	9.98 ± 0.05	11.10 ± 0.28	–	0.08 ± 0.03	0.011 ± 0.003	0.18 ± 0.05	–	Guirlet et al. (2008)
Brazil – PE	*Ei*	18	–	DN	XRF	0.085 ± 0.02	0.951 ± 2.005	–	1.711 ± 0.35	–	–	–	–	0.027 ± 0.012	0.729 ± 0.488	–	Simões (2016)[b]
ElSalvador–US	*Ei*	28	84.0	DN	GFAAS/AAS	–	–	–	–	–	–	0.24 ± 0.38	–	0.019 ± 0.010	0.06 ± 0.009	–	Bardales and Benavides (2016)
ElSalvador–US	*Ei*	66	84.9	DN	GFAAS	–	–	–	–	–	–	0.245	–	0.008	0.045	–	Tauer et al. (2017)

Mexico – OA	*Lo*	17	27.98	DB	ICP-OES	0.5 ± 0.91	1.0 ± 0.9	–	0.06 ± 0.06	7.1 ± 4.5	–	0.9 ± 0.6	7.0 ± 2.0	–	0.08 ± 0.1	1.3 ± 1.8	Cortés-Gómez et al. (2018c)
Mexico – OA	*Lo*	25	66.4	DA	GFAAS/ CV-AAS	–	0.47 ± 0.08	–	0.56 ± 0.26	–	11.68 ± 0.94	–	0.09 ± 0.04	0.001 ± 0.000	0.019 ± 0.03	–	Páez-Osuna et al. (2010a, b, 2011)[c]
Mexico – OA	*Lo*	41	65.50	DA	ICP-OES	–	0.61 ± 0.11	0.59 ± 0.10	0.04 ± 0.02	5.75 ± 2.48	10.55 ± 3.68	1.16 ± 0.70	0.17 ± 0.08	–	0.02 ± 0.01	–	Cortés-Gómez et al. (2014)
Mexico – OA	*Lo*	44	65.89	DN	ICP-OES	–	–	–	–	6.54 ± 3.10	10.88 ± 2.33	1.05 ± 0.53	0.15 ± 0.06	–	0.07 ± 0.04	1.03 ± 0.29	Cortés-Gómez et al. (2017)
Mexico – OA	*Lo*	20	–	DA	ICP-OES	0.73 ± 0.10	0.56 ± 0.35	–	0.06 ± 0.03	7.26 ± 5.52	8.06 ± 4.70	1.41 ± 1.62	0.13 ± 0.08	–	0.01 ± 0.01	1.02 ± 0.62	Cortés-Gómez et al. (2018b)
Mexico – OA	*Lo*	100	–	DA	ICP-OES	0.17 ± 0.07	0.52 ± 0.2	0.41 ± 0.12	0.07 ± 0.07	6.72 ± 3.0	7.7 ± 2.4	1.27 ± 0.9	0.12 ± 0.05	–	0.02 ± 0.01	1.02 ± 0.45	Cortés-Gómez et al. (2018a)
Mexico – SI	*Lo*	19	63.15	–	ICP-AES	–	1.02	2.77	1.35	11.15	37.12	2.44	1.33	–	BDL	–	Zavala-Norzagaray et al. (2014)

As arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Se* selenium, *Sr* strontium, *Zn* zinc, *Cc Caretta caretta*, *Cm Chelonia mydas*, *Cma Chelonia mydas agassizii*, *Dc Dermochelys coriacea*, *Ei Eretmochelys imbricata*, *Lo Lepidochelys olivacea*, *CN* casting net, *DA* during “arribada,” *DB* dead in the beach, *DN* during nesting, *DV* diving, *I* intentionally, *IC* incidental capture, *EN* entanglement nets, *FN* fishing net, *AAS* atomic absorption spectrophotometry, *CV-AAS* cold vapor atomic absorption spectrometry, *CVAFS* cold vapor atomic fluorescence spectrometry, *DMA* direct mercury analysis, *FAAS* flame atomic absorption spectrophotometry, *GFAAS* graphite furnace atomic absorption spectroscopy, *ICP-AES* inductively coupled plasma-atomic emission spectrometry, *ICP-OES* inductively coupled plasma-optical emission spectrophotometry, *ICP-MS* inductively coupled plasma-mass spectrometry, *XRF* X-ray fluorescence spectrometer, *BDL* below detection limits

[a] Country-state/province/department abbreviation

[b] μg.mL^{-1}

[c] Results originally published in dry weight transformed into wet weight using the humidity percentage reported by Guirlet et al. (2008) (blood, 80%)

[d] Original publication in straight carapace long (SCL), transform into CCL using the following formula: $Cm = -0.028 + 1.051$ (SCL) (Bjorndal and Bolten 1989)

As, which has already been reported. According to them, it could be expected that during an acute stress episode, cortisol and EA levels increase and present positive correlation. However, a strong negative correlation between EA and cortisol was observed in this study. The authors correlated this information with previous studies in the same population (Cortés-Gómez et al. 2014) and assumed that these animals were chronically exposed to different inorganic elements, such as Pb and Cd. They also suggested that a prolonged period under a stressful condition generated by pollution drives to a higher consumption of esterase and to a prolonged cortisol elevation, which could explain the results they found. Therefore, they emphasize the need of further research to clarify this topic.

Indicators of oxidative stress (antioxidant enzyme activities and lipid peroxidation levels) and levels of metals and organochlorine pesticides (OC) were evaluated by Labrada-Martagón et al. (2011) along with body condition of *Chelonia mydas* caught alive by monofilament fishing nets. Turtles were captured in Punta Abreojos (PAO) and Bahía Magdalena-Almejas (BMA), Mexico.

The results showed higher concentrations of Si and Cd in turtles captured in PAO, while turtles from BMA had higher levels of OCs. Additionally, sea turtles captured in PAO had enzymatic antioxidants mostly correlated to the concentration of pesticides, while in individuals from BMA, the antioxidant enzyme activities were correlated with the trace element concentrations. The authors attributed these regional differences to the influence of habitat conditions. The location of PAO and its direct connection to the Pacific Ocean could explain the concentration of trace elements and higher frequency of OC residuals in sea turtles, in contrast to the inland channels of BMA. However, the highest concentration of OCs found in the sea turtles from BMA, compared to PAO, could be the result of the agriculture activity developed in the last 50 years in the region.

Cortés-Gómez et al. (2018b) also related the transcription rate and/or enzymatic activities of some antioxidant enzymes (SOD, CAT, and GR) and metallothionein (MT) to metals in blood samples and tissues (liver and kidney) of 40 *Lepidochelys olivacea* from Mexico. Gene expression of *sod*, *cat*, and *gr* was higher in blood than the liver and kidney, which could be influenced by the fact that tissues were collected from dying turtles. However, most of the significant correlations of gene expression and enzyme activities were found in the liver. This must be related to the role of this structure as the first filter organ, with all metals passing through it before going to their target organs and accumulate.

Additionally, the authors found very high Cd levels and several positive relationships of *sod*, *cat*, and *gr* gene expression in different tissues. This could mean that the turtles were responding to the metals inducing production of ROS and damage through high transcription levels of these antioxidant enzymes. They argued that multiple positive relationships with GR seem to be part of the compensatory effect of GR due to the decrease of SOD production against the high and chronic exposure to certain xenobiotics, such as Cd. On the other hand, CAT seems to be not used much, and glutathione detoxification of H_2O_2 may be more important in this species.

Despite the high Cd concentrations found in this population, the authors didn't find significant relationships between any tissue with metallothionein gene expression. These results, along with very high Cd concentrations and a negative relationship with Cu, lead the authors to consider some kind of disruption in *mt* gene expression in these turtles.

Environmental contaminants have been proposed as one of the possible factors contributing to the development of fibropapillomatosis (FP) in sea turtles by reducing immune function (Balazs 1991). Some studies have already found correlations with FP prevalence and pollution, which justifies the belief that there may be a relationship, but more studies are still need to elucidate this possible correlation.

In Brazil, Silva et al. (2016) determined the concentrations of some metals (Ag, Cd, Cu, Fe, Ni, Pb, and Zn) in the blood of 27 *Chelonia mydas*, 14 with FP and 13 without FP, which were capture alive by diving and pound nets at Ubatuba, São Paulo State, Brazil. Green sea turtles were grouped and analyzed according to the severity of tumors, and the levels of metals were compared with parameters of oxidative stress, cholesterol concentration, and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) activity.

The results indicate that the reduced concentration of serum cholesterol observed in green sea turtles afflicted with FP is associated with an inhibition of HMGR activity induced by increased concentrations of Cu and Pb. They also suggest that oxidative stress induced by elevated concentrations of Fe and Pb (higher LPO levels) may be involved in the etiology and development of the disease.

Prioste (2016) also evaluated the concentrations of metals in 233 blood samples and 488 tissues samples of *Chelonia mydas*, along the Brazilian coast and correlated with FP. The results obtained from tissues samples showed that green turtles with signs of fibropapillomatosis present lower As and Se levels in all analyzed organs and higher Pb in the liver, kidneys, and bone tissues. The higher Pb levels are in agreement with the results found by Silva et al. (2016). The studies emphasize that samples obtained from the same population with reduced biological variability (gender and age) provide the most reliable results in terms of biochemical parameters of healthy individuals and disease response.

9.3.2 Tissues

Tissues are widely used as biomarkers. The contaminants found in most tissues of sea turtles (except blood) tend to reflect their foraging sites, and the distribution of metals among organs is influenced by both duration and concentration of exposure.

Since sea turtles are listed from vulnerable to critically endangered (IUCN 2019), it is difficult to obtain proper licenses to collect specimens, leading to studies that rely on carcasses. Sometimes samples are from animals that died in rehabilitation centers or stranded on the beach, which can influence the results when comparing with healthy animals. The advantage of using tissues is that a large mass can be collected and stored for a long time, allowing many analyses to be made (Keller et al. 2014).

In Latin America, majority of the studies were carried out in Brazil and Mexico (eight and seven studies, respectively), and the most analyzed tissues were the liver and kidney, followed by the muscle (Table 9.2). The highest concentrations of nonessential metals in the liver, kidney, and muscle were found in Brazilian *Chelonia mydas* for Hg (Bezerra et al. 2015) and in Mexican *Lepidochelys olivacea* for Pb (Frías-Espericueta et al. 2006) and Cd (Cortés-Gómez et al. 2018b, c).

In Brazil, Barbieri (2009) evaluated the concentrations of metals in the liver and kidney of 30 *Chelonia mydas* (15 adults and 15 juveniles). The animals were found stranded along the Cananéia estuary, in the state of São Paulo. The most striking feature of the study was the organotropism found, with Cd levels being higher in the kidney and Cu in the liver, while Mn, Ni, and Pb concentrations were not significantly different between organs. The same pattern was found by Macêdo et al. (2015) in *C. mydas* from Bahia state. The authors likewise noticed significant differences in the liver between adults and juveniles regarding Cd, Pb, Cu, and Ni levels. Adult livers had higher concentrations of these metals than juveniles. Since Cu and Ni can be classified as essential, it is possible that there is a metabolic regulation of them, whereas Cd and Pb are nonessential metals and its accumulation must be related to aging.

Silva et al. (2014) collected tissue samples from 29 juvenile *Chelonia mydas* found stranded along the southern coast of Brazil (Rio Grande do Sul State). Gonads were histologically analyzed for sex identification, resulting in nonsignificant differences between males and females in relation to metal contamination. A positive correlation was observed between nonessential (Ag, Cd, and Pb) and essential (Cu or Zn) metals in the liver and kidney. Silva et al. suggested that this correlation is likely due to an induced metallothionein synthesis induced by Zn and/or Cu to protect the tissue against the toxic effect of nonessential metals. The authors also found organotropism between metals evaluated with highest levels of Cu and Ag detected in the liver and Pb, Cd, and Zn in the kidney. Silva (2011) sampled tissues of *Chelonia mydas* in the same region and had similar results. The presence of high levels of Cd in the kidney and Hg in the liver is highlighted. Considering the results from both studies, it is clear that the populations of *C. mydas* are under heavy anthropogenic pressures in the coastal regions of Rio Grande do Sul State.

Andreani et al. (2008), like the aforementioned studies, found clear organotropism in tissues of *Chelonia mydas* from Tortuguero National Park, Costa Rica. The concentrations of Fe, Cu, and Mn were greater in the liver, whereas Cd was accumulated preferentially in the kidney. Pb did not show any clear tissue distribution pattern. The authors also evaluated hepatic and renal metallothionein (MT) as a biomarker of environmental metal exposure and compared the results obtained for *Chelonia mydas* with samples from *Caretta caretta* collected in the Mediterranean Sea. Metallothionein concentrations were higher in green than in loggerhead turtles. In addition, positive correlations were found between Cu and Cd concentrations and Cu–MT and Cd–MT in liver and kidney in both species, suggesting a pivotal role of MT in metal storage and detoxification.

They found significant differences between the two species. Green turtles had higher Cu and Cd levels in the liver and kidney and Fe concentrations in the liver. On the other hand, loggerhead turtle had higher Zn and Fe levels in the kidney. Andreani et al. attribute the variability in metal concentrations to differences in food habit. Food is probably the main source of exposure to trace elements. Certainly, feeding mainly on cephalopods results in higher Cd concentrations in loggerhead turtles. By contrast, green sea turtles are herbivorous and feed on macroalgae. Algae have the capacity to accumulate trace metals several thousand times higher than the concentration in sea water, so the foraging habits of this species could influence the high Cu concentration found in the liver. The authors emphasized that besides diet composition, age and gender could be important factors affecting metal accumulation in tissues.

Gardner et al. (2006) also found higher levels of some metals in *Chelonia mydas* compared to *C. caretta*, *L. olivacea*, and *E. imbricata* that died by incidental fisheries in the northwestern coast of Mexico. Metal concentrations in the liver of sea turtles from Baja California did not vary among the species. In the kidney, *C. mydas* had greater Zn concentrations as compared to the other species and greater Ni concentrations than *L. olivacea*. In adipose, the concentration of Zn in *C. mydas* was also greater than *L. olivacea*. The authors also attributed the higher exposure of these elements in *Chelonia mydas* to their food habits.

To verify the influence of anthropogenic activities in coastal areas, Bezerra et al. (2015) compared Hg concentrations in tissues of *Chelonia mydas* from two foraging grounds in the northeast coast of Brazil (Ceará and Bahia states). In Ceará, the study area is a nearly pristine region, while in Bahia it is located on an industrial site with chemical and petrochemical activities. They also evaluated the food items from both areas. The results showed significant differences among the liver and muscle. Hg concentration was higher in the liver and lower in the muscle; the kidney and scutes had median values. Comparing the two areas, they found similar food preferences in specimens from both areas, but liver Hg concentrations were significantly higher in green turtles from Bahia compared to Ceará. These variations in the amount of Hg reflect the influence of local Hg backgrounds in food items, since in Bahia the foraging ground is in a highly industrialized area suggesting that the turtles are exposed to Hg burdens from locally anthropogenic activities.

In Mexico, Talavera-Saenz et al. (2007) compared metal concentrations in the *C. mydas* kidney and liver with plant and algae species found in their stomach contents and with the same species of food items collected inside a sea turtle refuge area. The results showed concentrations of Cd and Zn in flora from the sea turtle stomach contents were greater than the same species of marine plants collected in the bay. For both metals, the concentrations in sea turtle liver were not significantly different from the stomach contents. The authors concluded that sea turtles residing in Estero Banderitas are feeding in areas outside of the bay, most likely in coastal regions with high upwelling. Additionally, the similar levels of metals between liver and stomach contents could be explained by the fact that the liver reflects the concentration of metal in the food and the analyses in this organ may provide a better indication of recent environmental exposure. Metal levels that were similar or higher in

Table 9.2 Metal levels (mean ± SD, $\mu g.g^{-1}$ dry weight) in tissues of sea turtles at different areas in Latin America

						Essential metals						Nonessential metals				
Tissue	Study site[a]	Sp	*n*	CCL	Method	Fe	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	Source
Liver	Mexico – BC	*Cc*	5	61.4[b]	AAS	301	33.94	1.29	0.35	–	69.14	–	1.75	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.15 ± 0.02	–	Kampalath et al. (2006)
	Brazil – BA	*Cm*	10	35.6	ICP-OES/ ICP-MS	4542 ± 2783	36.7 ± 9.3	8.73 ± 2.45	0.79 ± 0.34	16.8 ± 7.8	132 ± 22	29.8 ± 26.5	18.8 ± 10.6	1.34 ± 0.61	0.53 ± 0.45	Macêdo et al. (2015)
	Brazil – BA	*Cm*	25	36.4	CV-AAS	–	–	–	–	–	–	–	–	9.82 ± 7.11	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	12	40.7	ICP-MS	–	–	–	–	4.14 ± 3.2	29.5 ± 14.0	2.97 ± 3.03	6.40 ± 6.01	0.42 ± 0.32	0.22 ± 0.21	Prioste (2016)
	Brazil – CE	*Cm*	15	40.42	CV-AAS	–	–	–	–	–	–	–	–	0.70 ± 1.02	–	Bezerra et al. (2013)
	Brazil – CE	*Cm*	16	35.2	CV-AAS	–	–	–	–	–	–	–	–	4.75 ± 1.98	–	Bezerra et al. (2015)
	Brazil – ES	*Cm*	30	40.7	ICP-MS	–	–	–	–	2.47 ± 1.65	28.7 ± 10.2	3.05 ± 5.0	3.99 ± 2.26	0.104 ± 0.07	0.15 ± 0.11	Prioste (2016)
	Brazil – RS	*Cm*	15	36.65	FAAS/ CV-AAS	–	–	–	–	–	–	–	10.49	0.74	0.18	Silva (2011)
	Brazil – RS	*Cm*	29	39	FAAS	–	100.9	–	–	–	45	–	5.9	–	4.5	Silva et al. (2014)
	Brazil – SP	*Cm*	30	J	FAAS	–	20.7 ± 2.46	4.81 ± 0.9	0.13 ± 0.04	–	–	–	0.279 ± 0.14	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	30	A	FAAS	–	39.9 ± 1.94	4.32 ± 0.71	0.28 ± 0.08	–	–	–	0.957 ± 0.31	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	41	40.7	ICP-MS	–	–	–	–	3.54 ± 2.54	33.2 ± 12.2	2.71 ± 2.90	5.96 ± 3.57	0.144 ± 0.14	0.19 ± 0.14	Prioste (2016)
	Costa Rica – LI	*Cm*	34	A	FAAS/ GFAAS	2482	100	8.92	–	–	82.5	–	10.6	–	0.07	Andreani et al. (2008)
	Mexico – BC	*Cm*	11	62.13	AAS	14.35	60.04	0.06	0.01	–	62.91	–	3.30	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Cm*	8	62 b	FAAS	350[c]	76.52[c]	0.24[c]	0.00[c]	–	90.95[c]	–	16.92[c]	–	0.00[c]	Talavera-Saenz et al. (2007)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.091 ± 0.05	–	Kampalath et al. (2006)
	Brazil – BA	*Ei*	16	33.6	ICP-OES/ ICP-MS	5566 ± 1441	21.8 ± 9.2	7.97 ± 1.69	0.75 ± 0.39	29.5 ± 4.80	144 ± 21.0	30.3 ± 11.8	20.1 ± 5.43	1.36 ± 0.61	0.27 ± 0.19	Macêdo et al. (2015)
	Mexico – BC	*Ei*	1	51.2[b]	AAS	71.88	2.47	0.74	2.48	NA	25.89	NA	0.49	–	BDL	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1[b]	AAS	731	36.73	0.1	0.58	–	47.14	–	17.89	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.21 ± 0.28	–	Kampalath et al. (2006)

	Mexico – OA	*Lo*	7	–	ICP-OES	9914 ± 8669	46.08 ± 19.9	–	0.24 ± 0.20	40.08 ± 27.1	230 ± 160	14.8 ± 10.8	301 ± 221	–	0.81 ± 0.53	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	13	–	ICP-OES	8681 ± 5604	45.34 ± 30	–	0.24 ± 0.16	44.93 ± 15.7	188 ± 42	24.2 ± 14.3	390 ± 200	–	0.28 ± 0.20	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	13	65.50	ICP-OES	–	66.81 ± 42.2	13.7 ± 5.46	0.032 ± 0.24	33.67 ± 9.8	190 ± 4.01	13.6 ± 6.16	338 ± 149	–	0.44 ± 0.32	Cortés-Gómez et al. (2014)[d]
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	53.06 ± 33.8	–	0.20 ± 0.16	37.5 ± 19.1	–	48.9 ± 40.8	302 ± 187	–	0.81 ± 0.40	Cortés-Gómez et al. 2018c)[d]
	Mexico – SI	*Lo*	7	80.0	FAAS	–	33.4	–	–	–	–	–	13.12 ± 1.5	–	13.3 ± 2.1	Frías-Espericueta et al. (2006)
Kidney	Mexico – BC	*Cc*	5	61.4[b]	AAS	237	4.35	6.0	0.04	–	32.47	–	73.11	–	0.03	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.09 ± 0.05	–	Kampalath et al. (2006)
	Brazil – BA	*Cm*	10	35.6	ICP-OES/ ICP-MS	435 ± 232	13.6 ± 6.53	6.05 ± 2.81	1.92 ± 1.41	13.4 ± 6.3	151 ± 21	1205 ± 1054	54.5 ± 21.2	0.36 ± 0.14	0.15 ± 0.14	Macêdo et al. (2015)
	Brazil – BA	*Cm*	25	36.4	CV-AAS	–	–	–	–	–	–	–	–	4.29 ± 2.82	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	12	40.8	ICP-MS	–	–	–	–	2.03 ± 1.41	30.1 ± 19.3	2.5 ± 2.27	17.28 ± 20.8	0.23 ± 0.21	0.071 ± 0.07	Prioste (2016)
	Brazil – CE	*Cm*	16	35.2	CV-AAS	–	–	–	–	–	–	–	–	3.86 ± 3.02	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	17	39.42	CV-AAS	–	–	–	–	–	–	–	–	0.42 ± 0.36	–	Bezerra et al. (2013)
	Brazil – ES	*Cm*	30	40.8	ICP-MS	–	–	–	–	1.47 ± 1.17	28.6 ± 16.5	2.38 ± 3.63	13.4 ± 6.93	0.043 ± 0.03	0.11 ± 0.08	Prioste (2016)
	Brazil – RS	*Cm*	15	36.65	FAAS/ CV-AAS	–	–	–	–	–	–	–	33.45	0.46	0.17	Silva (2011)
	Brazil – RS	*Cm*	29	39	FAAS	–	12.2	–	–	–	54.3	–	28.3	–	5.4	Silva et al. (2014)
	Brazil – SP	*Cm*	30	J	FAAS	–	12.55 ± 1.04	3.82 ± 0.73	0.089 ± 0.01	–	–	–	1.0 ± 0.32	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	30	A	FAAS	–	13.72 ± 1.15	4.17 ± 0.86	0.19 ± 0.02	–	–	–	2.18 ± 0.27	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	41	40.8	ICP-MS	–	–	–	–	1.49 ± 0.85	30.8 ± 21.7	2.2 ± 2.67	19.5 ± 17	0.054 ± 0.06	0.10 ± 0.09	Prioste (2016)
	Costa Rica – LI	*Cm*	34	A	FAAS/ GFAAS	300	8.34	5.75	–	–	77.4	–	39.2	–	0.044	Andreani et al. (2008)
	Mexico – BC	*Cm*	11	65.2[b]	AAS	44.09	5.67	0.31	1.15	–	128	–	121	–	0.01	Gardner et al. (2006)
	Mexico – BS	*Cm*	8	62[c]	FAAS	93.16[c]	5.83[c]	1.51[c]	3.19[c]	–	189[c]	–	110[c]	–	0.05[c]	Talavera-Saenz et al. (2007)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.08 ± 0.08	–	Kampalath et al. (2006)

(continued)

Table 9.2 (continued)

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals						Nonessential metals				Source
						Fe	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	
	Brazil – BA	*Ei*	16	33.6	ICP-OES/ ICP-MS	309 ± 145	7.03 ± 2.95	5.28 ± 1.88	0.72 ± 0.39	11.0 ± 1.9	121 ± 30	1271 ± 480	76.2 ± 38.1	0.57 ± 0.42	0.07 ± 0.09	Macêdo et al. (2015)
	Mexico – BC	*Ei*	1	51.2[b]	AAS	362	3.89	7.62	1.61	–	82.45	–	4.20	–	BDL	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1 a	AAS	–	4.86	5.31	0.02	–	6.68	–	60.03	–	0.03	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.14 ± 0.19	–	Kampalath et al. (2006)
	Mexico – OA	*Lo*	7	–	ICP-OES	66.48 ± 22.5	2.86 ± 0.67	–	0.05 ± 0.05	3.99 ± 1.31	88.2 ± 27.0	1.47 ± 0.69	209 ± 141	–	0.08 ± 0.02	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	13	65.50	ICP-OES	–	3.80 ± 1.84	10.1 ± 3.21	0.18 ± 0.10	4.47 ± 2.09	108 ± 60.6	3.69 ± 2.30	404 ± 29.2	–	0.16 ± 0.08	Cortés-Gómez et al. (2014)[d]
	Mexico – OA	*Lo*	13	–	ICP-OES	273 ± 230	4.39 ± 1.63	–	0.08 ± 0.02	6.24 ± 3.91	114 ± 39.9	4.02 ± 2.06	603 ± 276	–	0.05 ± 0.05	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	3.75 ± 1.60	–	0.05 ± 0.02	4.28 ± 1.34	–	3.21 ± 3.48	316 ± 222	–	0.10 ± 0.08	Cortés-Gómez et al. (2018c)[d]
	Mexico – SI	*Lo*	7	80.0	FAAS	–	17.15	–	–	–	–	–	15.84 ± 1.2	–	13.4 ± 1.9	Frías-Espericueta et al. (2006)
Muscle	Mexico – BC	*Cc*	5	61.4 a	AAS	77.44	0.41	0.84	0.01	–	31.11	–	0.1	–	0.01	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.02 ± 0.01	–	Kampalath et al. (2006)
	Brazil – BA	*Cm*	25	36.4	CV-AAS	–	–	–	–	–	–	–	–	1.84 ± 1.93	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	12	40.9	ICP-MS	–	–	–	–	3.96 ± 5.36	8.0 ± 2.06	5.49 ± 6.18	0.06 ± 0.05	0.027 ± 0.04	0.008 ± 0.011	Prioste (2016)
	Brazil – CE	*Cm*	16	35.2	CV-AAS	–	–	–	–	–	–	–	–	1.73 ± 0.71	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	18	37.68	CV-AAS	–	–	–	–	–	–	–	–	0.20 ± 0.18	–	Bezerra et al. (2013)
	Brazil – ES	*Cm*	30	40.9	ICP-MS	–	–	–	–	1.42 ± 1.34	8.99 ± 5.66	4.48 ± 7.30	0.07 ± 0.09	0.006 ± 0.009	0.005 ± 0.007	Prioste (2016)
	Brazil – RS	*Cm*	15	36.65	FAAS/ CV-AAS	–	–	–	–	–	–	–	0.21	0.23	0.11	Silva (2011)
	Brazil – RS	*Cm*	29	39	FAAS	–	1.2	–	–	–	16.6	–	0.4	–	4.2	Silva et al. (2014)
	Brazil – SP	*Cm*	42	40.9	ICP-MS	–	–	–	–	1.69 ± 1.23	9.75 ± 4.07	3.64 ± 5.73	0.07 ± 0.05	0.006 ± 0.008	0.004 ± 0.006	Prioste (2016)
	Mexico – BC	*Cm*	11	65.2 a	AAS	20.99	0.03	0.003	0.03	–	38.26	–	0.01	–	0.01	Gardner et al. (2006)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.02 ± 0.02	–	Kampalath et al. (2006)

	Mexico – BC	*Ei*	1	51.2 a	AAS	258	3.68	1.78	BDL	–	102	–	1.02	–	0.38	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1 a	AAS	93.09	1.28	0.77	0.01	–	85.78	–	0.48	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.05 ± 0.04	–	Kampalath et al. (2006)
	Mexico – SI	*Lo*	7	80.0	FAAS	–	15.5	–	–	–	–	–	2.48 ± 0.4	–	8.9 ± 1.0	Frías-Espericueta et al. (2006)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	4.16 ± 1.04	–	0.31 ± 0.52	40.1 ± 16.14	–	19.7 ± 9.37	3.64 ± 1.56	–	0.20 ± 0.15	Cortés-Gómez et al. (2018c)[d]
Adipose	Mexico – BC	*Cc*	5	61.4 a	AAS	1.33	0.69	1.82	0.17	–	12.66	–	0.5	NA	BDL	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.008 ± 0.01	–	Kampalath et al. (2006)
	Costa Rica – LI	*Cm*	34	A	FAAS/ GFAAS	45.2	0.446	0.826	–	–	62.1	–	0.113	–	0.063	Andreani et al. (2008)
	Mexico – BC	*Cm*	11	65.2[b]	AAS	2.63	0.01	0.003	0.02	–	49.82	–	0.002	NA	0.03	Gardner et al. (2006)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.004 ± 0.003	–	Kampalath et al. (2006)
	Mexico – BC	*Ei*	1	51.2[b]	AAS	11.14	0.72	2.53	BDL	–	42.39	–	0.43	NA	BDL	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1[b]	AAS	27.91	0.83	2.1	0.03	–	3.7	–	0.69	NA	BDL	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.03 ± 0.06	–	Kampalath et al. (2006)
Bone	Brazil – BA	*Cm*	10	35.6	ICP-OES/ ICP-MS	44.7 ± 33.6	1.26 ± 1.37	4.68 ± 1.63	3.52 ± 1.43	2.97 ± 0.45	196 ± 34	7422 ± 667	< 0.060	< 0.128	0.98 ± 0.61	Macêdo et al. (2015)
	Brazil – CE	*Cm*	10	40.4	ICP-MS	–	–	–	–	1.0 ± 0.63	51.3 ± 11.1	6.58 ± 6.07	0.08 ± 0.07	0.019 ± 0.013	1.06 ± 1.13	Prioste (2016)
	Brazil – ES	*Cm*	23	40.4	ICP-MS	–	–	–	–	0.98 ± 0.84	56.3 ± 27.8	2.94 ± 4.15	0.11 ± 0.06	0.011 ± 0.007	0.6 ± 0.47	Prioste (2016)
	Brazil – SP	*Cm*	40	40.4	ICP-MS	–	–	–	–	0.69 ± 0.61	57.3 ± 28.0	11.9 ± 15.2	0.14 ± 0.15	0.007 ± 0.006	0.6 ± 0.49	Prioste (2016)
	Brazil – BA	*Ei*	16	33.6	ICP-OES/ ICP-MS	41.5 ± 17.8	0.73 ± 0.73	7.10 ± 2.12	1.71 ± 0.3	1.65 ± 0.41	215 ± 18	7006 ± 421	0.56 ± 0.63	< 0.128	0.64 ± 0.48	Macêdo et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	2.0 ± 5.0	–	0.25 ± 0.12	1.62 ± 05	–	0.75 ± 0.62	1.0 ± 0.37	–	1.25 ± 0.87	Cortés-Gómez et al. (2018c)[d]
Salt gland	Brazil – CE	*Cm*	8	39.6	ICP-MS	–	–	–	–	1.5 ± 1.07	11.8 ± 2.08	206.3 ± 578	1.04 ± 0.68	0.032 ± 0.05	0.11 ± 0.30	Prioste (2016)
	Brazil – ES	*Cm*	29	39.6	ICP-MS	–	–	–	–	1.2 ± 1.06	13.8 ± 7.48	1.71 ± 2.83	0.91 ± 0.43	0.008 ± 0.013	0.95 ± 2.39	Prioste (2016)
	Brazil – SP	*Cm*	39	39.6	ICP-MS	–	–	–	–	1.31 ± 0.72	15.3 ± 6.39	1.54 ± 1.96	0.98 ± 0.64	0.009 ± 0.010	0.008 ± 0.007	Prioste (2016)

(continued)

Table 9.2 (continued)

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals						Nonessential metals				Source
						Fe	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	
Spleen	Brazil – CE	*Cm*	10	40.8	ICP-MS	–	–	–	–	1.27 ± 1.16	29.6 ± 16.8	0.88 ± 0.74	1.14 ± 1.16	0.041 ± 0.05	0.023 ± 0.014	Prioste (2016)
	Brazil – ES	*Cm*	30	40.8	ICP-MS	–	–	–	–	1.57 ± 1.93	22.5 ± 12.2	1.68 ± 2.44	0.91 ± 1.26	0.006 ± 0.01	0.019 ± 0.011	Prioste (2016)
	Brazil – SP	*Cm*	40	40.8	ICP-MS	–	–	–	–	1.46 ± 0.8	19.8 ± 3.64	1.57 ± 1.69	0.80 ± 0.48	0.009 ± 0.009	0.022 ± 0.02	Prioste (2016)
Heart	Mexico – SI	Lo	7	80.0	FAAS	–	44.9 ± 4	–	–	–	–	–	11.0	–	10.1 ± 1.1	Frías-Espericueta et al. (2006)
Brain	Mexico – OA	Lo	17	27.98	ICP-OES	–	13.6 ± 38.8	–	0.8 ± 0.8	29.2 ± 68	–	6.0 ± 17.6	3.2 ± 6.4	–	0.4 ± 0.4	Cortés-Gómez et al. (2018c)[d]

As arsenic, *Cd* cadmium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Se* selenium, *Zn* zinc, *Cc Caretta caretta*, *Cm Chelonia mydas*, *Cma Chelonia mydas agassizii*, *Dc Dermochelys coriacea*, *Ei Eretmochelys imbricata*, *Lo Lepidochelys olivacea*, *AAS* atomic absorption spectrophotometry, *CV-AAS* cold vapor atomic absorption spectrometry, *CVAFS* cold vapor atomic fluorescence spectrometry, *FAAS* flame atomic absorption spectrophotometry, *GFAAS* graphite furnace atomic absorption spectroscopy, *ICP-OES* inductively coupled plasma- optical emission spectrophotometry, *ICP-MS* inductively coupled plasma- mass spectrometry, *J* juvenile, *A* adult

[a] Country-state/province/department abbreviation

[b] Original publication in straight carapace long (SCL), transform into CCL using the following formulas: $Lo = (SCL - 9.244)/0.818$ (Whiting et al. 2007); $Cc = 1.388 + 1.053$ (SCL) (Bjorndal et al. 2000); $Cm = -0.028 + 1.051$ (SCL) (Bjorndal and Bolten 1989) and $Ei = (SCL - 0.449)/0.935$ (Wabnitz and Pauly 2008)

[c] Median, *BDL* below detection limits

[d] Results originally published in wet weight transformed into dry weight using the humidity percentage reported by Frías-Espericueta et al. (2006) (liver, 75.5%; kidney, 62.7%; muscle, 80.8%), Garcia-Fernandez et al. (2009) (bone, 20%; brain, 75%), and Adballah and Abd-Allah (2011) (adipose, 20%)

the liver and kidney but lower than in the stomach contents may indicate metabolic processing of these metals and/or accumulation over time.

Cortés-Gómez et al. (2018c) developed a method to use carapace morphologies from photographs to quantify developmental instability (DI) and examined relationships between inorganic elements and asymmetry of the carapace. They compared the concentrations of 16 elements in tissues (liver, kidney, muscle, brain, bone, blood, and egg components) of stranded dead *Lepidochelys olivacea* from Mexico. The results suggested that individuals with more asymmetric carapaces seem to be more susceptible to accumulate the organic elements. They also found negative significant relationships between the DI of adult females and the concentration of metals in their eggs. However, the authors highlight that more studies are needed to validate the use of carapace asymmetry as a biomarker.

9.3.3 Eggs and Hatchlings

Egg samples provide many advantages for monitoring pollutants. It can be collected in a nonlethal manner if unhatched eggs and/or dead hatchlings are sampled after the live hatchlings have emerged or if the eggshell can be found in remaining nest, instead of sacrificing a fresh egg that has the potential to develop. Eggs are usually abundant, easy to collect, and more accessible for sampling compared to capturing juveniles or adults at sea (Keller et al. 2014).

In reptiles, the ovulation and the supply of albumen and eggshell for all the eggs to be laid during the season happen progressively throughout the nesting season (Palmer et al. 1993). Thus, the contents of sea turtle eggs represent the diet, nutrients, and chemical compounds ingested by adult females in their foraging sites and during the breeding season (Miller 1997). Eggs also constitute a potential tool for monitoring the excretion route or maternal transfer and are good indicators of the metal load of nesting colonies.

During incubation, contaminants could also be transferred from the nest environment into the eggs. Indeed, during incubation, the number of permeable open pores on eggshell of turtles increases due to water or gas exchange between eggs and nest environment, facilitating the transfer of contaminants from nest material into eggs (Hewavisenthi and Parmenter 2001; Canas and Anderson 2002). Permeability of eggshells to soil contaminants should also be considered as a way of contamination that could affect hatching success.

Early life stages of oviparous organisms seem to exhibit higher sensitivity to chemical contaminants than adults (Russell et al. 1999). In reptiles, ovo exposure to toxic elements has been shown to impact the development of the embryo, resulting in hatchlings deformities, disorientation, and lower fitness, thus increasing the risk of predation and negatively affecting migration to feeding sites (Bishop et al. 1991, 1998).

In Latin America few studies were found regarding the contamination of eggs, unhatched eggs, and hatchlings (Table 9.3). In these studies, some authors analyzed eggs contents individually (Cortés-Gómez et al. 2018c; Páez-Osuna et al. 2010a, b, 2011; Dyc et al. 2015) and others used the whole egg (Guirlet et al. 2008; Ross et al. 2016; Roe et al. 2011) or only the eggshells found in the nest after the hatching (Simões 2016; Vazquez et al. 1997). The eggs were collected at the time of oviposition (Guirlet et al. 2008; Páez-Osuna et al. 2010a, b, 2011; Dyc et al. 2015), within 12 hours of oviposition (Ross et al. 2016) or 2 days after hatchling ceased (unhatched eggs) (Roe et al. 2011) and from the oviduct of dead animals (Cortés-Gómez et al. 2018c).

The highest concentration of Cu, Cd, and Se in the yolk and eggshell were found in eggs collected directly from the oviduct of dead *L. olivacea* in Mexico (Cortés-Gómez et al. 2018c). The highest Pb levels in the yolk and albumen were also detected in *L. olivacea* from the same area, however, in a different study (Páez-Osuna et al. 2010a). In the eggshell the highest Pb concentrations were found in *D. coriacea* from Mexico (Vazquez et al. 1997).

The highest Hg levels in yolk, albumen, and eggshell were found in *E. imbricata* from Guadeloupe Islands (Dyc et al. 2015), *C. mydas* from Guadeloupe Islands (Dyc et al. 2015), and *E. imbricata* from Brazil (Simões 2016), respectively.

Considering the whole egg, the highest concentrations of Cu, Mn, and Cd were from *D. coriacea* collected in Mexico (Roe et al. 2011), while the highest concentrations of nonessential metals (Pb and Hg) were found in eggs of *D. coriacea* from French Guiana (Guirlet et al. 2008).

Vazquez et al. (1997) collected egg samples from *Dermochelys coriacea* in the field and held them under three different conditions prior to contaminant analyses. In “natural” condition eggs were kept at a preservation area (Playon de Mexiquillo); in “container” and “artificial” conditions, eggs were brought to the laboratory and kept in plastic containers or in an artificial environment of beach sand, respectively. In the last cases, eggs were kept at temperatures of 24–31 °C. The authors also analyzed metal concentrations in seawater and sand from the nesting area. They found significantly higher levels of metals in the sand than seawater and similar concentrations of contaminants in all three samples of eggshells. So, they concluded that the beach sand might be responsible for the eggshell contaminations.

In French Guiana, Guirlet et al. (2008) evaluated the levels of some metals in blood and eggs of *Dermochelys coriacea* and sampled multiple clutches from each female to assess the variations of trace element concentrations, according to the number of nesting events (time).

The results found in blood show that time had no effect on Hg, Cd, Se, and Zn concentrations indicating that concentrations remain constant throughout the nesting season, whereas Cu concentrations in blood decreased significantly with time and Pb concentrations increased. The decrease of Cu during nesting season could be a result of an important maternal transfer to albumen combined with a low dietary intake and insufficient reserves of this metal in the liver and kidney, resulting in Cu limitation at the end of the nesting period. In contrast, the increasing trend that occurred in Pb is likely to suggest Pb mobilization from bones associated with Ca requirement for egg formation and eggshell secretion.

On the other hand, in eggs, no fluctuation had been observed for trace elements concentrations between the different clutches laid suggesting a constant maternal transfer to egg along the nesting season. Moreover, the amount of elements transferred

Table 9.3 Metal levels (mean ± SD, μg.g^{-1} wet weight) in eggs and hatchlings of sea turtles at different areas in Latin America

						Essential metals				Nonessential metals				
Tissue	Study site[a]	Sp	*n*	CCL	Method	Cu	Ni	Se	Zn	As	Cd	Hg	Pb	Source
Yolk	Guadeloupe Islands	*Cm*	12	–	ICP-MS/ DMA	0.59	–	0.17	–	–	<0.003	0.0020	0.010	Dyc et al. (2015)
	Guadeloupe Islands	*Ei*	4	–	ICP-MS/ DMA	0.49	–	1.37	–	–	<0.003	0.017	0.017	Dyc et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	1.2 ± 0.4	BDL	2.8 ± 1.1	–	0.2 ± 0.07	0.2 ± 0.09	–	0.03 ± 0.03	Cortés-Gómez et al. (2018c)
	Mexico – OA	*Lo*	25	66.4	GFAAS/ CV-AAS	0.82 ± 0.55	1.23 ± 0.22	–	27.11 ± 1.51	–	0.09 ± 0.03	0.010 ± 0.003	0.30 ± 0.03	Páez-Osuna et al. (2010a, b, 2011)[b]
Albumen	Guadeloupe Islands	*Cm*	12	–	ICP-MS/ DMA	0.85	–	0.27	–	–	<0.014	0.018	0.019	Dyc et al. (2015)
	Guadeloupe Islands	*Ei*	3	–	ICP-MS/ DMA	0.86	–	4.6	–	–	<0.014	0.013	<0.019	Dyc et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	0.2 ± 0.2	BDL	0.8 ± 0.5	–	0.1 ± 0.1	0.02 ± 0.05	–	0.01 ± 0.0	Cortés-Gómez et al. (2018c)
	Mexico – OA	*Lo*	25	66.4	GFAAS/ CV-AAS	0.09 ± 0.07	0.10 ± 0.10	–	0.90 ± 0.16	–	0.005 ± 0.002	0.00003 ± 0.00002	0.029 ± 0.005	Páez-Osuna et al. (2010a, b, 2011)[b]
Eggshell	Guadeloupe Islands	*Cm*	12	–	ICP-MS/ DMA	1.63	–	0.15	–	–	0.083	0.0018	0.006	Dyc et al. (2015)
	Mexico – MI	*Dc*	5	–	GFAAS	–	3.11	–	4.87	–	–	–	5.69	Vazquez et al. (1997)[b]
	Mexico – MI	*Dc*	5	–	GFAAS	–	2.75	–	4.18	–	–	–	3.11	Vazquez et al. (1997)[b, c]
	Mexico – MI	*Dc*	5	–	GFAAS	–	4.3	–	5.61	–	–	–	2.53	Vazquez et al. (1997)[b, d]
	Brazil – PE	*Ei*	20	–	XRF	0.10 ± 0.07	1.92 ± 2.03	–	–	–	–	0.006 ± 0.006	0.016 ± 0.015	Simões (2016)[b]
	Guadeloupe Islands	*Ei*	3	–	ICP-MS/ DMA	2.64	–	1.39	–	–	0.07	0.0033	<0.0076	Dyc et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	8.6 ± 2.3	0.02 ± 0.0	6.6 ± 3.3	–	BDL	0.4 ± 0.7	–	BDL	Cortés-Gómez et al. (2018c)
	Mexico – OA	*Lo*	25	66.4	GFAAS/ CV-AAS	3.06 ± 1.06	19.88 ± 5.28	–	5.08 ± 0.61	–	0.19 ± 0.03	0.0035 ± 0.0001	0.43 ± 0.08	Páez-Osuna et al. (2010a, b, 2011)[b]

(continued)

Table 9.3 (continued)

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals				Nonessential metals				Source
						Cu	Ni	Se	Zn	As	Cd	Hg	Pb	
Whole	Panama	*Cm*	31	–	ICP-MS/ ICP-OES	0.5 ± 0.1	–	–	14.0 ± 3.0	0.12 ± 0.04	0.09 ± 0.04	0.006 ± 0.002	0.003 ± 0.002	Ross et al. (2016)
Egg	Costa Rica – G	*Dc*	38	–	ICP-AES/ ICP-OES	25.9	1.9	–	14.2	–	1.6	–	–	Roe et al. (2011)
	French Guiana – SLM	*Dc*	76	160	ICP-MS/ DMA	0.63 ± 0.10	–	1.44 ± 0.38	14.16 ± 2.23	–	0.024 ± 0.001	0.012 ± 0.003	0.036 ± 0.001	Guirlet et al. (2008)
	Panama	*Lo*	30	–	ICP-MS/ ICP-OES	0.6 ± 0.2	–	–	16.0 ± 2.0	0.12 ± 0.06	0.07 ± 0.02	0.009 ± 0.005	0.004 ± 0.002	Ross et al. (2016)
Hatchlings	Costa Rica – G	*Dc*	38	–	ICP-AES/ ICP-OES	4.2	BDL	–	22.5	–	BDL	–	–	Roe et al. (2011)

As arsenic, *Cd* cadmium, *Cu* copper, *Hg* mercury, *Ni* nickel, *Pb* lead, *Se* selenium, *Zn* zinc, *Cm Chelonia mydas*, *Dc Dermochelys coriacea*, *Ei Eretmochelys imbricata*, *Lo Lepidochelys olivacea*, *CV-AAS* cold vapor atomic absorption spectrometry, *DMA* direct mercury analysis, *FAAS* flame atomic absorption spectrophotometry, *GFAAS* graphite furnace atomic absorption spectroscopy, *ICP-AES* inductively coupled plasma- atomic emission spectrometry, *ICP-OES* inductively coupled plasma- optical emission spectrophotometry, *ICP-MS* inductively coupled plasma- mass spectrometry, *XRF* X-ray fluorescence spectrometer, *BDL* below detection limits

[a] Country-state/province/department abbreviation

[b] Results originally published in dry weight transformed into wet weight using the humidity percentage reported by Páez-Osuma et al. (2010a, b, 2011) (albumen, 97.3%; yolk, 62.5%; eggshell, 59.0%)

[c] Eggs incubated in a container (plastic)

[d] Eggs incubated in an artificial environment of beach sand

by the females to eggs were in the order of Zn > Se > Cu > Pb > Cd > Hg. The authors concluded that females transfer a higher burden of essential elements than toxic elements, during the nesting season. This result is in concordance with data reported by Páez-Osuna et al. (2010a, b, 2011) for Mexican *Lepidochelys olivacea* from a nesting colony at Oaxaca State.

In this study, maternal transfer of trace metals via egg-laying, in terms of metal burden in the whole body, was 21.5%, 7.8%, 3.4%, 2.0%, 0.5%, and 0.2% for Ni, Cu, Zn, Hg, Pb, and Cd, respectively. The excretion rates of trace metals through egg-laying followed the same pattern (Ni > Cu > Zn > Hg > Pb > Cd). It indicates that egg-laying is not a major route for transferring nonessential metals (perhaps with the exception of Ni), but essential metals are transferred at a higher rate, possibly as a source mechanism for the hatchlings.

Considering the proportions of each egg fraction (albumen, 4.9%; yolk, 80.9%; eggshell, 14.2% in dry weight) and concentrations of each metal in each case, the authors observed that the highest percentage (or load) of metals was incorporated in the yolk, except of Ni. This result confirms the importance of yolk in the accumulation of heavy metals in sea turtles hatchlings. Additionally, the concentration of essential metals in yolk is important because they contribute to the physiological processes and good development of the embryo. For example, Cu and Zn are required for normal growth, metabolism, and structure and function of many proteins vital for cell function. Thus, a maternal transfer of metals to eggs is necessary for successful development of the embryos.

As mentioned earlier in this chapter, sea turtles are an important food source in many coastal communities in Latin America; for this reason Ross et al. (2016) evaluated the overall health risks from heavy metal intake through *Chelonia mydas* and *Lepidochelys olivacea* egg consumption along the Pacific coast of Panama. The authors used the average body weights of human consumers according to age, sex, and socioeconomic factors to calculate the consumption rates and correlated with metal levels found in eggs. The results suggest that, except in cases of consistent extreme consumption, heavy metal exposure through sea turtle egg consumption alone is unlikely to pose a threat to those who regularly eat green turtle and/or olive ridley eggs in Panama. However, average consumption rates may contribute substantially to lifetime Cd intake.

In Guadeloupe Islands, Dyc et al. (2015) investigated the effects of contaminants on sea turtles performing a screening-level risk assessment, using hazard quotients (HQ) as a measure of the ratio between measured concentrations, and predicted no effect concentrations. For this, the authors collected eggs from *C. mydas* and *E. imbricata* during the oviposition.

The HQ results indicated that Se, Cd, and Hg exposure may represent a threat for developing marine turtle embryos ($HQ_{worst} > 1$ for Se and Hg and HQ_{best} and $HQ_{worst} > 1$ for Cd, for both species). Se may reduce the embryo viability and Hg could induce embryo deformities and/or reduce the survival of green and hawksbill turtle embryo. However, the authors argument that if Guadeloupian sea turtles are tolerant, the levels found may be harmless, which may be most likely considering the higher hatchling rate of both species. Due to species-specific difference in toxicological responses, screening-level risk assessments may inaccurately estimate the effects of chemical pollutants in sea turtles, and improved risk assessments would rely on more detailed toxicological data being generated.

Investigations of the impact of metal contaminants on hatchlings and unhatched eggs have been conducted in only one publication, in Costa Rica. In this study, Roe et al. (2011) sampled two clutches for each female *D. coriacea*, the first and the fourth nest of the season. Two days after hatching, unhatched eggs and hatchlings were collected to determine metal levels. They also measured 20 hatchlings from each nest during emergence to determine the body condition index. The results of this study indicate that leatherback embryos accumulate a suite of essential metals including Cu, Fe, Mn, and Zn, as well as nonessential metals Cd and Ni. In part, such concentrations reflect the maternal transfer of metals to eggs but also may be influenced by exposure in the nest environment, a contamination route that the authors did not measure.

Regarding differences between clutches, egg trace element concentrations did not vary between nests laid earlier and later in the season. Roe et al. found little evidence that metal levels in leatherback eggs had any significant influence on clutch success, hatchling size, or hatchling body condition. Therefore, the authors concluded that maternal genotype, maternal health, or nest environment may have a more profound influence on clutch viability in leatherbacks than environmental contaminants at current exposure levels in the eastern Pacific Ocean.

Further investigations into the effects of contaminants in sea turtle hatchlings and eggs may be a useful tool in ecotoxicology as a reduction in hatchling success may have big effects on population dynamics.

9.3.4 Carapace

Scutes from the carapace have increasingly been used as reliable tissue to evaluate metal contents because they can be collected noninvasively and nonlethally, either in juvenile or adult animals. Heavy metals are known to bind with keratin and studies have revealed that elements such as mercury maintain a strong association with keratin following prolonged exposure to UV radiation and extreme temperatures. The keratinized carapace scutes could therefore provide a reliable and temporarily robust measure of determining heavy metal concentration in sea turtles.

A recent study on *C. caretta* found significant positive correlations between scute mercury levels and mercury concentrations in the liver, muscle, kidney, and spinal cord (Day et al. 2005). Another study on *C. caretta* accidentally caught in fishing nets off the coast of Japan found significant positive correlations between carapace concentrations and whole-body burdens for zinc, manganese, and mercury (Sakai et al. 2000).

In Latin America, all studies that used carapace as a biomarker were made in Brazil. The samples were collected from alive and dead animals that were trapped in fishing artifacts, found stranded or nesting on the beach, or died during rehabilitation. The only metal analyzed was mercury and the species sample were *Chelonia mydas* and *Caretta caretta* from Bahia and Ceará states (Table 9.4).

Comparing the studies, juveniles of *C. mydas* from Bahia and adults of *Caretta caretta* from Ceará had the highest Hg levels found (Bezerra et al. 2015; Rodriguez 2017). On the other hand, the lowest levels were observed in adults of *C. mydas* from Ceará (Bezerra et al. 2012) and adults of *Caretta caretta* from Bahia (Rodriguez et al. 2018).

Bezerra et al. (2012, 2015) observed highest levels of mercury in juvenile *C. mydas*, with a significant negative correlation between size of the animal (CCL) and Hg levels. The authors related this correlation factor to the change in eating habits between juvenile and adults. When *C. mydas* are recruiting to coastal habitats, their diet changes from an omnivorous to herbivorous diet which results in feeding mostly on benthic algae and seagrass. However, juveniles that feed on a more omnivorous diet are exposed to higher levels of organic mercury than when feeding on benthic plants.

The differences in Hg levels found in adults of *C. caretta* from Ceará (Rodriguez 2017) and Bahia (Rodriguez et al. 2018) could be explained by the differences in the regions sampled. Ceará coast is characterized as nearly pristine regarding Hg contamination because of low industrial development. On the other hand, the northern coast of Bahia is under influence by a petrochemical industrial complex and an extensive industrial development. As a result, this area has been receiving a large input of heavy metal contaminants in the last several decades, which potentially enhances the exposure of marine species, threatening biodiversity and human safety.

Table 9.4 Mercury levels (mean ± SD, $\mu g.g^{-1}$ dry weight) in the carapace of sea turtles at different areas in Brazil

Study Site[a]	Sp	*n*	CCL	Capture	Method	Hg	Source
Brazil-BA	*Cc*	8	97.5	AL	CV-AAS	0.86 ± 0.95	Rodriguez et al. (2018)
Brazil- BA	*Cc*	76	99 ± 5	DN	CV-AAS	1.71 ± 2.63	Rodriguez (2017)
Brazil- CE	*Cc*	8	95.0	AL	CV-AAS	2.07 ± 1.69	Rodriguez et al. (2018)
Brazil- CE	*Cc*	6	76 ± 17	DN	CV-AAS	3.46 ± 2.38	Rodriguez (2017)
Brazil- BA	*Cm*	8	45.1	AL	CV-AAS	0.19 ± 4.71	Rodriguez et al. (2018)
Brazil- BA	*Cm*	25	36.4	FS/DR	CV-AAS	3.91 ± 3.39	Bezerra et al. (2015)
Brazil- CE	*Cm*	8	44.3	AL	CV-AAS	1.59 ± 1.83	Rodriguez et al. (2018)
Brazil- CE	*Cm*	25	50.5	AFW/DB	CV-AAS	0.154	Bezerra et al. (2012)
Brazil- CE	*Cm*	10	43.4	FS/DR	CV-AAS	0.42 ± 0.37	Bezerra et al. (2013)
Brazil- CE	*Cm*	16	35.2	FS/DR	CV-AAS	3.54 ± 2.75	Bezerra et al. (2015)

Hg mercury, *Cc Caretta caretta*, *Cm Chelonia mydas*, *AFW* alive animals trapped in fish weirs, *AL* alive (unknown capture method), *DB* dead in the beach, *DN* during nesting, *DR* died during rehabilitation, *FR* found stranded on the beach (dead), *CV-AAS* cold vapor atomic absorption spectrometry

[a] Country and state abbreviation

9.4 Conclusion

This chapter presented metal contamination data from Latin American sea turtles, and in spite of all the studies and the importance of nesting and foraging sites in Latin America to the development and reproduction of these animals, there is still a lack of information for several countries. The differences among methods to quantify metals difficult a precise comparison among studies, once some methods are more sensitive. Additionally, the results in the publications do not follow a pattern regarding metal values units. The non-reported moisture percentage could difficult the conversion of values and the results may be over- or underestimated.

The studies described in this chapter show that the differences in the accumulation rate vary depending on several factors, including geographical location, species, sizes, and type of tissue, along with environmental differences between species including pelagic or sedentary life strategy, trophic levels, food items, and growth rates (Guirlet et al. 2008; Andreani et al. 2008; Gardner et al. 2006; Talavera-Saenz et al. 2007). It is important to consider the mobility that some elements have in sick, moribund, or stranded turtles, which could result in a concentration variance between debilitated and healthy turtles (Camacho et al. 2014). Further, it is known that global factors are more relevant than local factors in the distribution of some metals (Fraga et al. 2018), so it is important to know the migration routes of sea turtles to investigate all the areas they inhabit, improving our understanding about the toxicological effects of contaminants and how they might impact sea turtles during key periods of life.

Although there are some studies that evaluated the interactions of heavy metals with biochemical and physiological processes, it is necessary to generate baseline information of health parameters, biochemical reference intervals, and pollution levels for each species and regional populations. The levels in environments with low anthropogenic impact could enable comparison with populations in polluted areas and will help to determine the impact of these contaminants on sea turtle's health, reproduction, and survival.

Additionally, few studies evaluated the variability of heavy metal levels in sea turtles' eggs and hatchlings and their relationship with maternal transfer. Future studies should focus on the influence of pollution in embryo development, reproduction success, and hatchling fitness.

The understanding of the action mechanisms allows a more precise risk assessment, helping to predict and prevent wildlife damage, and being essential in guiding regulatory decisions for the development of national conservation plans.

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