Bull. Environ. Contam. Toxicol. (1997) 58:326-333

Bulletin of Environmental Contamination and Toxicology

Contamination in Marine Turtle *(Dermochelys coriaca)* Egg Shells of Playon de Mexiquillo, Michoacan, Mexico

G. F. Vazquez, M. C. Reyes, G. Fernandez, J. E. C. Aguayo, V. K. Sharma*

Instituto de Ciencias del Mar y Limnologia, UNAM, Cd. Universitaria, D. F., Apartado Postal 70-305, C.P. 04510, Mexico

Received: 26 January 1996/Accepted: 29 October 1996

Concern for the decreasing population sizes of marine turtles around the world is growing. Potential contamination within habitats of marine turtles, and human activities, such as poaching, modification of nesting sites, and capture of adult turtles, may be responsible for their decreasing populations (Shabica 1981; Gramentz 1988; Hutchinson and Simmonds 1991; Benin et al. 1995). Little is known about the baseline levels and physiological effects of environmental contaminants on marine turtle populations (Witkowski and Frazier 1982; Bishop et al. 1991). Responding to this concern, the Mexican government has designated areas along the Mexican coastline to preserve marine turtle nesting habitats. "Playon de Mexiquillo", Michocan, Mexico is one of the coastal preservation areas located near the mouth of Rio la Manzanilla which flows between Sierra Madre del Sur and the Pacific Ocean (Fig. 1). Samples of seawater, sand , and marine turtle egg *(Dermochelys Coriaca)* shells were collected monthly from October, 1992- March, 1993. Contaminants investigated were oil and grease, and metals (cadmium, copper, zinc, nickel, and lead). Seawater samples were collected where the turtles lay eggs in the preservation area and sand samples were taken from the area surrounding the eggs.

MATERIALS AND METHODS

The detailed procedure for sample collections is given elsewhere (Vazquez et al. 1993, 1994). In brief, the water samples were taken in Van Dorn bottles previously soaked in aqua regia. The site of water samples collection is shown in Fig. 1. All samples were filtered through 0.45 μm filters (Millipore Corporation) and stored in 1000-mL plastic bottles containing 1 mL of concentrated HNO_3 to acidify the samples to pH 1.6-2.0. The sand samples were taken with an acid-washed plastic spatula from the central portion of a Smith-McIntyre grab sample within 4 cm of the surface to avoid contamination, placed in plastic bags, and stored frozen (-8°C) with one to two centimeter strata analyzed. Sand

*Present address: Center for Coastal Studies, Texas A&M University–Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX 78412
Correspondence to: G. F. Vazquez

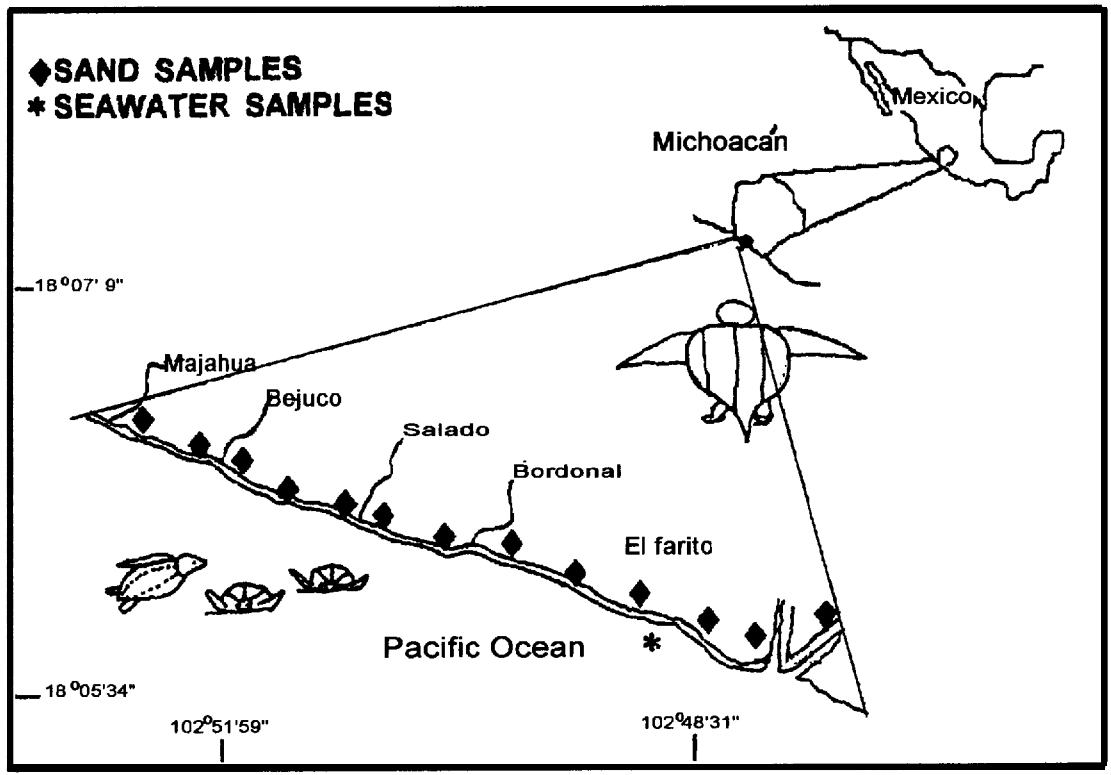

Figure 1. Collection sites for samples from Playon Mexiquillo, Michoacan, Mexico.

samples represent composite samples of 13 sites (Fig 1). Three samples were taken every month from October to March, 1993, approximately ten days apart, for water and sand analyses. Turtle eggs were sampled in the field and egg shells were collected after the eggs had hatched, a period lasting 50-75 days. Eggs collected via acid-cleaned tongs were held under three different conditions prior to contaminant analyses. In one, designated as "natural", eggs were kept at the preservation area, a secured place at the "Playon de Mexiquillo" (Fig. 1). Eggs brought to UNAM laboratory were subjected to one of two preserving procedures prior to contaminant analyses. In one procedure, eggs were kept in containers (glass for oil and grease and plastic for metal samples) and in the other, an artificial environment of beach sand was created. In both procedures, eggs were kept at temperatures of 24-31 °C. The latter procedure is designated as "in situ" and the former as "container", respectively. Eggs in all three different conditions were hatched for the same period of time and success rate was ~50%. In each of the three conditions, sample sizes consisted of ten egg shells, five for metals and five for oil and grease. Five egg shells weighted ~20 g.

The glassware utilized was soaked in aqua regia, rinsed with Mini Q water and kept at 110°C prior to use. The reagents utilized were of high purity and standards were prepared from commercially available solutions (Tritisol, Merck). The extraction of metals was performed by the microwave extraction technique (Nakashima et al. 1988) and is described briefly here. For metals analyses in seawater, 50 mL of the seawater sample was placed in an acid-digestion vessel with a Teflon cap. Then 5 mL of doubly distilled HNO_3 and 0.5 mL suprapure HCl acid were added. The vessel was put into a microwave (MDS-2000 CEM) for 90 min. For sand samples, 10 mL of Mini Q water, 5 mL of HNO_3, 4 mL of HF, and 1 mL of HCl were added to 0.5 g of sand and then digested in the microwave for 90 min. Prior to analysis, 2.0 g of boric acid was added into the digested samples. A certified sediment reference material, IAEA/SOIL-7 (International Laboratory of Marine Radioactivity, Mosee Oceanography, Inc, MC 98000, Monoco) was regularly submitted to the same digestion procedure and analyzed for metals. The coefficients of variation for recoveries of metals were 8.1%, 4.1%, 2.8%, 5.6% and 5.6% for Cd, Cu, Zn, Ni, and Pb, respectively. For metals in egg shell samples, ten egg shells were subjected to the same extraction procedure as was used for the sand samples.

Metal concentrations (Cd, Cu, Zn, Ni, and Pb) were determined on a Perkin Elmer model 2380 with a graphite furnace Model MGA400. Standard dilutions were prepared by dissolving metals in 1:1 solutions of nitric and hydrochloric acids. Three replicates were prepared and the precision of metals analyses was estimated to be ±7%. Detection limits (µg/L) in our instrument were: Cd (0.02), Cu(0.25), Ni(0.80), Zn(0.30), and

Pb(0.15). Oil and grease analyses of the samples were done by Soxhlet extraction method (APHA 1992).

RESULTS AND DISCUSSION

Mean concentrations of oil and grease in seawater and sand samples are given in Table 1. In general, oil and grease had little variation in sand, while seawater values had much higher concentrations in January and March. The higher values were unexpected and probably related to increased recreational activities such as boating in January and March. However, similar higher values of oil and grease were not found in February which suggests that many other processes can also influence the distribution of oil and grease. These processes may include wind-driven discharge from "Lazaro Cardenas" industrial port which is sixty miles from the study area. In addition, surface input from the Balsas River which is highly contaminated with hydrocarbon compounds may also contribute to the increased oil and grease concentrations (Reimnitz and Gutierrez 1970). At present, the authors are not certain as to which process was responsible for the high values in January and March. Oil and grease concentrations were higher in seawater than in sand. This was expected as seawater tends to wash off oil and grease components from the sand.

Table 1. Mean concentrations and ranges of oil and grease (mg kg^{-1} dry wt) in seawater and sand samples from Playon de Mexiquillo, Michocan, Mexico in 1992-1993. Sample Size, N = 3 each month.

Month	Seawater			Sand		
	Mean	Min.	Max.	Mean	Min.	Max,
October	6.1	3.4	11.0	0.43	0.23	0.54
November	11.3	9.0	13.6	0.30	0.06	0.53
December	8.5	6.5	10.6	0.41	0.04	0.60
January	159	86.5	220	0.25	0.09	0.57
February	13.7	4.0	28.2	0.35	0.03	0.85
March	94.5	3.2	188	0.55	0.03	0.91

Metal concentrations in seawater and sand are given in Table 2. The results were statistically analyzed by performing a "t-test" at a 95% confidence level. There was little variation in the concentrations of metals within either seawater or sand. A "t-test" showed that in seawater, metal levels decreased in the order: Ni > Pb ≈ Zn > Cd > Cu and in the sand the decreasing order was: Ni ≈ Zn > Pb ≈ Cd ≈ Cu. In most instances, the concentrations of metals were significantly higher in sand than in water.

This is related to adsorption of metals to the surfaces of particles (Muller and Sigg 1990).

Table 2. Mean concentrations and ranges of metals (mg kg^{-1} dry wt) in seawater and sand samples from Playon de Mexiquillo, Michocan, Mexico in 1992-1993. Sample Size, N = 3 for each month.

Month	Seawater Mean	Seawater Min.	Seawater Max.		Sand Mean	Sand Min.	Sand Max.
				Cadmium			
October	0.04	0.01	0.12		49.0	3.99	134
November	0.03	0.01	0.05		4.00	2.00	4.99
December	0.03	0.01	0.08		8.00	2.00	5.99
January	0.03	0.01	0.06		36.9	5.99	132
February	0.01	0.01	0.02		25.2	3.00	82.8
March	0.02	0.01	0.07		11.9	0.01	26.0
				Copper			
October	0.03	0.01	0.07		2.99	13.0	7.31
November	0.04	0.03	0.05		5.95	7.00	8.49
December	0.04	0.06	0.13		2.99	18.1	10.5
January	0.06	0.03	0.09		0.99	70.0	21.0
February	0.07	0.06	0.08		3.93	19.0	8.48
March	0.05	0.04	0.07		0.99	17.0	6.66
				Zinc			
October	0.35	0.04	0.58		45.0	41.0	92.1
November	0.70	0.06	1.33		41.0	37.0	45.0
December	0.12	0.06	0.22		53.8	22.0	76.9
January	0.17	0.04	0.24		53.5	9.00	82.9
February	0.07	0.06	0.10		72.4	29.0	99.7
March	0.16	0.09	0.30		61.6	34.0	104
				Nickel			
October	0.48	0.31	0.64		0.10	109	44.3
November	0.54	0.46	0.61		67.9	77.0	72.5
December	0.52	0.43	0.75		0.20	113	70.6
January	0.61	0.39	0.65		0.10	97.0	67.4
February	0.50	0.43	0.54		0.20	89.8	48.4
March	0.60	0.45	0.66		58.9	115	82.4
				Lead			
October	0.41	0.13	0.36		31.0	4.00	58.9
November	0.35	0.17	0.52		27.5	20.0	35.0
December	0.50	0.40	0.72		15.8	4.00	21.0
January	0.22	0.05	0.35		19.2	5.00	32.0
February	0.30	0.19	0.43		24.4	9.00	42.0
March	0.30	0.19	0.41		26.0	5.99	65.9

The contaminant concentrations in the three different samples of turtle egg shells are given in Table 3. In all three samples, no seasonal trend was found and, therefore, they are presented in ranges and means. It is interesting that similar concentrations of contaminants were found in all three samples of egg shells. Mean contaminant concentrations in seawater and sand, with respect to season, are compared with mean concentrations in turtle egg shells (see Table 4). A "t-test" of comparing means at a 95% confidence level showed that the oil and grease concentrations in the seawater were significantly higher than concentrations in sand and egg shells while concentrations in sand and egg shells were not significantly different. Cadmium, Ni and Zn concentrations in the egg shells were significantly lower than sand concentrations and Cu and Pb concentrations were not significantly different. Metal concentrations in seawater were higher than those in sand and egg shells. It appears that turtle egg shells receive oil and grease contamination from seawater while metal contamination is from sand.

Table 3. Mean concentrations and ranges of contaminants (mg kg^{-1} dry w-t) in turtle egg shells. Sample size, N = 5.

	in situ			container			natural		
Contamin.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Oil & Grease	1.63	0.01	10.5	0.79	0.02	4.82	0.75	0.07	5.08
Copper	12.9	9.09	5.94	8.72	5.63	11.9	8.38	5.62	11.3
Cadmium	1.04	0.01	5.92	0.71	0.01	1.25	0.51	0.01	1.25
Zinc	11.9	0.01	60.0	10.2	0.94	50.3	13.7	5.93	20.0
Nickel	7.60	0.01	19.7	6.73	5.62	11.9	10.5	5.62	11.3
Lead	13.9	0.62	144	7.59	2.19	20.3	6.19	0.31	17.8

Table 4. Mean concentrations and ranges of contaminants (mg kg^{-1} dry wt) in seawater, sand, and turtle egg shells from Playon de Mexiquillo, Michocan, Mexico in 1992-1993.

Contaminant	Seawater	Sand	Turtle egg shells
Oil & Grease	48.8±65.8	0.40±2.06	1.34±3.4
Cadmium	0.03±0.03	23.2±41.5	0.90±0.61
Copper	0.06±0.03	10.8±15.3	8.90±1.26
Zinc	0.26±0.29	58.9±26.8	11.9±10.0
Nickel	0.54±0.13	63.6±38.6	7.90±5.11
Lead	0.35±0.18	23.2±18.3	11.6±26.0

In summary, high levels of oil and grease in seawater and metals in sand may be responsible for measured levels of contaminant concentrations in turtle egg shells. The contaminant concentrations in turtle tissues are needed to fully understand the impact of contamination present in seawater and sand on the reproduction of marine turtles in Playon de Mexiquillo, Michocan, Mexico.

Acknowledgments. This project was supported by the UNAM (project 132). V. K. Sharma gratefully acknowledges the support by the Center for Coastal Studies, TAMU-CC, Texas.

REFERENCES

American Public Health Association (1992) Standard methods for the examination of water and wastewater, 18th ed., Washington, DC.

Bishop C, Brooks RJ, Carey JH, Ng, P, Norstrom RJ, Lean DRS (1991) The case for a cause-effect between environmental contamination and development in eggs of the common snapping turtle (Chelydra S. Serpentine) from Ontario, Canada. J Toxicol Environ Health 33:5121-547

Benin J, DesGranges JL, Bishop CA, Rodrigue J, Gendron A, Elliott JE (1995) Comparative study of contaminants in the mudpuppy (Amphibia) and the common snapping turtle (Reptilia), St. Lawrence River, Canada. Arch Environ Contam Toxicol 28:184-194

Gramentz D (1988) Involvement of loggerhead turtle with the plastic, metal, and hydrocarbon pollution in the central Mediterranean. Mar Pollut Bull 19:11-13

Hutchinson L, Simmonds M (1991) A review of the effects of pollution on marine turtles. A Greenpeace Ecotoxicology Project London. Greenpeace Int p27

Muller B, Sigg L (1990) Interaction of trace metals with natural particle surfaces: Comparison between adsorption experiments and field measurements. Aquat Sci 52:75-92

Nakashima S, Sturgeon RE, Willie SN, Berman SS (1988) Acid digestion of marine samples for trace elements analysis using microwave heating. Analyst 113:158-163

Reimnitz E, Gutierrez EM (1970) Rapid changes in the head of the Rio Balsas, Submarine Canyon System, Mexico. Mar Geol 8:245-248

Shabica VS (1981) Planning for protection of sea turtle habitat. In:Bjorndal AK (ed) Biology on Conservation of Sea Turtles, Washington DC, p519-522

Vazquez FG, Delgado HD, Huerta CJD, Aguilera LG, Sharma, VK (1993) Trace and heavy metals in San Andres lagoon, Tamaulipas, Mexico water. Environ Int 19:71-77

Vazquez FG, Aguilera LG, Sharma, VK (1994) Metals in sediments of San Andres lagoon, Tamaulipas, Mexico. Bull Environ Contam Toxicol 52: 382-387

Witkowski SA, Frazier JG (1982) Heavy metals in sea turtles. Mar Pollut Bull 7:254-255