

Southwest Fisheries Science Center
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**DETERMINATION OF ENVIRONMENTAL POLLUTANTS
IN GREEN TURTLES (*CHELONIA MYDAS*)
AFFLICTED WITH FIBROPAPILLOMAS IN THE HAWAIIAN ISLANDS**

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NOT FOR PUBLICATION

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PREFACE

Prepared under contract as part of the Southwest Fisheries Science Center Honolulu Laboratory's research program on protected species of marine turtles, this report provides the results of analyses of green turtle (Chelonia mydas) tissues for numerous environmental pollutants that might possibly play a role in the formation of tumors known as fibropapillomas. Based on this study, it would appear that the pollutants tested for may not be a significant factor in the formation of fibropapillomas.

The incidence of these life-threatening tumors on green turtles in the Hawaiian Islands has increased to epidemic proportions during recent years. A similar situation exists among green turtles at certain sites in Florida, the Caribbean, and other selected locations worldwide. The cause of fibropapillomatosis in green turtles remains unknown. The impact of the disease on the afflicted populations can have serious consequences and represents one more threat to the survival of green turtles worldwide. The nature of this disease and its cause must be determined in order to develop a long-term disease management program. The present report by Dr. Alonso Aguirre constitutes progress in that direction which must be followed up with additional studies.

Because this report was prepared by an independent investigator, its statements, findings, conclusions and recommendations do not necessarily reflect the views of the National Marine Fisheries Service, NOAA.

George H. Balazs
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April 1993

EXECUTIVE SUMMARY

Brain, fat, liver, and kidney tissues from 11 juvenile green turtles (*Chelonia mydas*), one pelagic green turtle, and one pelagic loggerhead turtle (*Caretta caretta*) were tested to determine exposure to environmental pollutants. Egg shells and tissues from three green turtle hatchlings were also tested. The tissues and shells analyzed in this study indicated that none contained any of the listed OC, PCB, OP, or carbamate insecticides in concentrations above the stated method of detection limits. Most of the concentrations of selenium and heavy metals were also considered to be below levels reported normal in other animal species. Further research identifying an infectious etiology is recommended.

INTRODUCTION

Green Turtle Fibropapillomatosis (GTFP) is a condition affecting several green turtle (*Chelonia mydas*) populations in epidemic proportions throughout the world. Although a virus has been suggested as the causative agent, the primary etiology is unknown. Chemical pollutants impairing the immune system and stress have been listed among many other possible etiologic agents (Aguirre, 1991, 1992; Balazs and Pooley, 1991).

Pollutant-mediated stress causing morphophysiological changes such as cellular proliferation (skin tumors) is well documented in aquatic organisms (Giam and Ray 1987). This chronic stress results in a reduction of energy available for basic physiologic processes as well as causing alterations in the cellular immune response increasing susceptibility to infectious agents. Papillomas and other tumors in fish have been used as an index to monitor chemical carcinogens in the marine environment; relatively few studies, however, correlate the exposure of sea turtles and their eggs to organic pollutants (Hutchinson and Simmonds, 1991).

The purpose of this study was to determine the involvement of selected environmental pollutants on the etiology of GTFP. Toxicological information was compared to determine baseline data for the accumulation of organochlorines (OCs), polychlorinated biphenyls (PCBs), organophosphates (OPs), n-methyl carbamate compounds, selenium (Se), and heavy metals in selected tissues collected from a discrete population of juvenile green turtles in Kaneohe Bay, Oahu, and elsewhere in the Hawaiian Islands.

MATERIALS AND METHODS

Field Sampling

Tissue specimens for this study were obtained from different sources. Four turtle carcasses were recovered through the National Marine Fisheries Service Hawaiian Sea Turtle Stranding & Salvage Program, from which two turtles were found in a bull pen net at Palaau, Island of Molokai, one was found at Kaneohe Bay, Island of Oahu, and a fourth one was found at Waialae Beach Park, Island of Oahu. Six juvenile green turtles severely affected by GTFP (score tumor = 3) and in poor condition were caught by hand while snorkeling at Ahu-O-Laka (n = 4) and Mark Reef (n = 2), Kaneohe Bay, by NMFS personnel and transported to the Honolulu Laboratory for necropsy. Subcutaneous fat, kidney, liver, and brain tissues were collected for toxicological analysis from these turtles. One clinically normal green turtle held in captivity for five years at the NMFS research facilities because of traumatic amputation of front flippers, served as a control. Tissue samples from one pelagic green turtle and one pelagic loggerhead turtle (*Caretta*) free of GTFP were also tested as

controls (Table 1). These two turtles had been salvaged as the result of incidental mortality in the foreign driftnet fishery. In addition, we tested egg shells and tissues from three green turtle hatchlings collected at French Frigate Shoals, Hawaii, from the same nest resulting from a turtle known to be afflicted by GTFP.

The seven live turtles were euthanized with a lethal intraperitoneal injection of T-61 Euthanasia Solution (American Hoechst Corp., Somerville, New Jersey) and thorough necropsies were performed according to a protocol previously described (Wolke and George, 1981). A sample of 50-100 g of liver and adipose tissue, and the whole brain with cervical spinal cord specimens were wrapped in acetone-rinsed (and dried) aluminum foil, then labeled and placed in double zip-lock bags. Liver and kidney specimens (50 g) were placed in double zip-lock bags for heavy metal analysis. All specimens were frozen immediately on dry ice and shipped overnight to the California Veterinary Diagnostic Laboratory System (CVDLS) Toxicology Laboratory, University of California, Davis, California.

Laboratory Techniques

Cholinesterase Activity

The quantitative determination of acetylcholinesterase (ChE) activity in brain specimens was performed by using a plate reader. The principle of this method is the measurement of the rate production of thiocholine as described by Ellman et al. (1961). The original Ellman assay was adapted for a 96-well kinetic microplate reader. The activity of ChE was expressed in μM of acetylthiocholine hydrolyzed per gram of brain sample per minute ($\mu\text{M/g/min}$). Specimens were homogenized, diluted with Ph 8.0, phosphate buffer and pipetted into wells of the microplate. DTNB (Ellman reagent) and ATCI (substrate solution) were then added and the A^{405} was measured every 8 seconds for 5 minutes by a microplate reader. The method detection limit (MDL) was 0.1 $\mu\text{M/g/min}$ (Hill, 1988; CVDLS-Toxicology Laboratory, 1992, unpubl. data).

Multiresidue Insecticide Screen

In the laboratory, tissue specimens were prepared for a multiresidue pesticide screen of selected OCs, PCBs, OPs, and carbamates using gel permeation chromatography (GPC). Briefly, 10-g aliquots of selected tissues were homogenized and subsamples of 20-ml aliquots were evaporated to dryness at 40°C with a stream of N_2 after adding 3 drops of 5% decanol in acetone as keeper. Then, 10 ml of hexane:ethyl acetate 7:3 were added, filtered through a 0.45 μ filter and loaded onto GPC. Extraction,

sample cleanup, and separation of OC pesticides from PCBs were performed as previously described (Clark and Krynitsky 1980, 1985).

For OP analysis, 2 μ l of extract and standard were injected on a HP5890 Gas Chromatograph/FPD(P) and a 30M x 0.53mm x 1 μ M DB-17 column. For OC analysis, the extract and the standard were injected on a PE Sigma 2000 Gas Chromatograph/ECD, column 30M x 0.53mm x 0.83 μ M DB-608. For carbamates, 10 μ l of extract and standard were injected on a HP1090 Liquid Chromatograph with Hitachi F-1050 fluorescent detector and dual post column hydrolysis/reaction. Gas chromatography/mass spectrometry was required to completely exclude the presence of p,p'DDD, Gamma chlordane, Lindane, and Heptachlor in several samples, since initial screening for those compounds was inconclusive. The MDL was 0.1 ppm wet weight for carbamate residues and 1 ppm for PCBs. Method detection limits for OCs and OPs are stated in Table 2 (CVDLS-Toxicology Laboratory, 1992, unpubl. data).

Total Selenium

Total Se was analyzed by inductively-coupled plasma (ICP) atomic emission using hydride generation. Liver and kidney samples were digested in nitric acid and then boiled in a mixture of sulfuric and perchloric acids to convert all Se species to selenate. The selenate was reduced to selenite with hydrochloric acid at 95°C. Then, the selenite was reduced by acidic sodium borohydride to hydrogen selenide which was determined by ICP atomic emission at 196.090 nm. The instrument MDL was determined to be 0.007 ppm wet weight (CVDLS-Toxicology Laboratory, 1988, unpubl. data).

Extended Heavy Metal Screen

The preparation of kidney and liver specimens for the analysis of heavy metals was a simple nitric acid digestion of a 1 gram sample diluted to 10 ml of distilled deionized water. Samples were analyzed in an ICP spectrophotometer (Martin et al., 1987). This screen quantitated method detection limits for 16 heavy metals (Table 3).

RESULTS

Cholinesterase Activity

Acetylcholinesterase levels were determined in 12 brain samples from green turtles. The brain samples contained the listed ChE activities within expected ranges for most animals. Turtles captured at Kaneohe Bay averaged a ChE activity level of 5.85 ± 1.3 μ M/g/min, compared to turtles recovered through the Stranding Program which levels averaged 3.7 ± 0.5 μ M/g/min and to a pelagic green with 11.7 μ M/g/min. The control turtle presented

ChE levels of 6.2 $\mu\text{M/g/min}$ (Table 1). A one-way analysis of variance demonstrated significant differences among the stranded group and the other turtle groups ($p < 0.05$). The pelagic green had higher values than the other turtles. Also, the group of turtles captured alive in Kaneohe Bay had higher levels than the dead stranded turtles. Similar ChE activity values were recorded between the Kaneohe Bay turtle group and the control turtle.

Multiresidue Insecticide Screen

Extended pesticide screenings were performed for 23 OC, 8 PCB, 43 OP, and 11 carbamate insecticides (Table 2). Liver, kidney, and adipose tissues from the green turtle specimens tested contained none of the organophosphorus, organochlorine, and carbamate insecticides or PCBs in concentrations greater than the stated MDL; that is, the lowest concentration detectable by the test method implemented by the CVDLS-Toxicology Laboratory. Additional testing was required to exclude the presence of heptachlor (6 samples), lindane (6 samples), and p,p'DDD (2 samples).

The egg shells and hatchling tissues contained none of the listed OC insecticides or PCBs in concentrations above the stated MDL (Table 2). Additional testing was required to rule out the presence of gamma chlordane in the hatchling tissues.

Selenium and Heavy Metals

The levels of selenium and 13 heavy metals detected on selected samples of sea turtles are summarized in Table 3. Most tissue samples contained none of the listed metals or selenium in toxic concentrations for most animals. The liver samples collected from the pelagic green and loggerhead turtles, however, contained relatively high selenium levels, 2.53 ppm and 3.39 ppm wet weight respectively (normal values for most terrestrial animal livers are < 1.5 ppm). Four green turtles captured at Kaneohe Bay evidenced hepatic trace levels of thallium (Tl) (0.8-1.0 ppm). All liver and kidney tissues, hatchlings, and egg shells from the turtles analyzed in this study were negative for the presence of trace levels of beryllium, lead, and mercury.

DISCUSSION

Cholinesterase Activity

Measuring the inhibition of ChE activity in brain tissue is a sensitive indicator of acute exposure to organophosphorus or carbamate insecticides, with a reduction of 20% normal activity indicating exposure. The major problem, however, is to determine what is a 'normal' ChE value for a given species (Fairbrother and Bennett, 1988). At Johnston Atoll, Hawaii, erythrocyte ChE was

measured for nine green turtles by identifying changes in cellular Ph (Balazs, 1985). That data set, however, is not comparable with the values obtained in this study since different techniques and tissues were utilized. Comparative studies of fish, birds, and mammals on levels of ChE inhibition have shown remarkable differences in sensitivity to different OP and carbamate compounds (Hall, 1980). For example, amphibians apparently are less sensitive to OPs than other animal groups. More studies in reptilian sensitivity are required to determine the validity of the ChE inhibition test in sea turtles.

During this study, the lower values identified in the stranded turtles when compared to other turtles were considered normal since brain ChE activity is severely depressed by the time of death (Fairbrother and Bennett, 1988). Further studies are required to determine the significance of higher levels of ChE activity shown in the pelagic green when compared to other turtle groups.

Multiresidue Insecticide Screen

No OC, PCB, OP, or carbamate residues were detected in any of the green turtle tissues analyzed. Based on the results obtained, juvenile green turtles in this study were not exposed to any of these pollutants at toxic levels at the time of sample collection.

Organochlorines and PCB's

Several investigators have measured organochlorine and PCB levels of green turtles and their eggs (Thompson et al., 1974; Clark and Krynitsky, 1980, 1985; Rybitski et al., 1993). Trace levels of p,p'DDE and PCBs were detected in 10 green turtle egg yolks from Ascension Island, South Atlantic Ocean (Thompson et al., 1974). DDE and DDT residues averaging 0.025 ppm wet weight were also found in nine clutches (170 eggs) of green turtles nesting on Merritt Island, Florida (Clark and Krynitsky, 1980). Postyearling green turtles collected in Florida presented DDE levels of <10 ppb and PCB levels of 43-80 ppb wet weight (McKim and Johnson, 1983). More recently, Rybitski et al. (1993) determined PCB concentrations from below quantification limits to 58.2 ppb in subcutaneous fat and up to 17.1 ppb in livers of Hawaiian green turtles. DDE levels in adipose tissue ranged below quantification limits to 22.5 ppb and up to 6.31 ppb in liver.

Organochlorine and PCB compounds elicit many biologic effects including death, birth defects, tumors, and a wasting syndrome. These compounds are known to bioaccumulate and biomagnify within the food chain. According to the studies mentioned above and confirmed by this study, OC and PCB residues exist at extremely low concentrations in this turtle species. In

addition to species, sex, age, nutritional status, and exposure, the dietary habits of the species may explain these findings. Green turtles feed primarily on sea grasses and algae making them less susceptible to bioconcentrate these pesticides.

Organophosphates and Carbamates

To the best of our knowledge, no studies are available in sea turtles related to organophosphate and carbamate toxicity. Many of these compounds are complex mixtures with components that are metabolized selectively by certain species and their quantification is difficult and has not been standardized. In addition to inhibition of ChE activity and delayed neurotoxicity, these compounds have other sublethal effects in wildlife including impaired reproduction in birds and reduced tolerance to cold stress (Smith, 1987). These compounds have a relatively low environmental persistence and their monitoring is very difficult. Brain ChE activity values, detection of residues in the gastrointestinal tract, and die-offs characterized by acute poisoning in a defined geographic area can confirm exposure to these pesticides.

Selenium

Selenium is nutritionally important as an essential trace element of many animals, but is toxic at slightly higher concentrations. Total Se background levels in sea turtles are unknown and most concentrations in this study were considered to be below levels reported as 'normal' for other animal species. Accumulation of Se in marine animals is highly variable, it ranges from 0.05 in crustaceans to 30 ppm wet weight in some fish species. Levels in kidneys collected from marine birds have been recorded at 1.2-10.2 ppm wet weight. Levels of this magnitude are sufficient to impair reproduction in shorebirds (Eisler, 1985).

Marine mammal tissues contain extremely high Se concentrations. For example, hepatic levels in seals range from 6.1 ppm in pups to 170 ppm wet weight in adults. High hepatic concentrations of maternal California sea lions (*Zalophus californianus*) were not reflected in livers of pups (Eisler, 1985). This trend was not observed in the green turtles sampled. The pelagic specimens had significantly ($p < 0.05$) higher Se levels than the turtles recruited at near shore environments. Further research of sea turtles and Se in marine ecosystems is necessary.

Heavy Metals

Sea turtles and their eggs have been analyzed for traces of heavy metals (Balazs, 1986; Hillestad et al., 1974; Stoneburner et al., 1980; Witkowski and Frazier, 1982; Davenport and Wrench, 1990; Hutchinson and Simmonds, 1991). Concentrations of 13 heavy metals in eggs of loggerhead turtles provided useful information

on feeding ecology of this species (Stoneburner et al., 1980). In most cases, however, it was difficult to interpret the significance of these trace metals due to the lack of baseline data. The effects of these elements in sea turtles are unknown.

Thallium levels of Kaneohe Bay turtles were relatively high when compared to other animal groups. These levels may be nontoxic to sea turtles, and reflect exposure from their food source or the aquatic environment. Most cases reporting toxicity in wildlife and humans occur as acute poisonings. Chronic cases have not been well documented with the most consistently reported effect in terrestrial mammals being alopecia. The available data indicate that Tl acute toxicity to salt-water aquatic life occurs at concentrations as low as 2.13 ppm. No information was available concerning chronic toxicity of Tl to sensitive saltwater aquatic life (U.S. Environmental Protection Agency, 1980).

Further research on other contaminants such as airplane pollutants, oil, and dioxin is recommended. Reported sublethal effects of oil ingestion include metabolic production of potential carcinogenic compounds, immunosuppression, diminished salt gland function, and hormonal and behavioral abnormalities (Hall et al., 1983; Hutchinson and Simmonds, 1991). In the absence of background information in the literature related to environmental pollutants in terrestrial or aquatic reptiles, data from other species indicated that the presence of these and other compounds represent an increased risk for sea turtles.

CONCLUSIONS

Reproductive failure, hormonal imbalance, low population recruitment, and suppression of the immune system are known results of exposure to persistent environmental pollutants. The possibility that these compounds may have deleterious effects of disease in green turtles is a consideration, particularly in the case of GTFP. Research in other species with fibropapillomas has suggested on numerous occasions that chemical pollution may activate latent viruses or indirectly increase their virulence (Gamache and Horrocks, 1992). An immune-mediated component to the disease has been suggested (Balazs and Pooley, 1991; Hutchinson and Simmonds, 1991) implicating many pollutants as possible immunosuppressants. Based on this study, however, environmental contaminants play a relatively minor role in the etiology of GTFP in this discrete green turtle population in the Hawaiian Islands. Further research on infectious and parasitic agents is recommended.

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Table 1.--Size, weight, sex, and brain cholinesterase activity levels for 12 green turtles (*Chelonia mydas*) sampled from the Hawaiian Islands.

TURTLE NO.	LOCATION	SCL ¹ (cm)	WGT ² (kg)	SEX	LEVEL ³ (μ M/g/min)
035	Palaau, Molokai	50.8	15.5	F	3.6
036	Palaau, Molokai	58.6	22.7	F	3.5
037	Mark Reef, Kaneohe	46.0	10.0	M	5.4
043	Control, Captive	52.3	16.4	F	6.2
044	Waialae Beach Park	49.5	16.4	F	4.5
045	Kaneohe Bay, Oahu	69.0	42.3	F	3.2
046	Ahu-O-Laka, Kaneohe	56.1	18.2	F	7.9
047	Ahu-O-Laka, Kaneohe	52.3	16.8	F	3.7
048	Mark Reef, Kaneohe	71.3	43.6	M	5.4
049	Ahu-O-Laka, Kaneohe	52.9	20.0	F	5.9
050	Ahu-O-Laka, Kaneohe	66.9	37.5	M	6.8
051	Pelagic green	28.7	3.2	M	11.7

¹SCL(cm)- Straight Carapace length (centimeters)

²WGT(kg)- Weight (kilograms)

³The method detection limit was 0.1 μ M/g/min

Table 2.--Organochlorines, polychlorinated biphenyls (PCBs), carbamates, and organophosphates with method of detection limits (MDL) in parts per million (ppm) wet weight analyzed in selected green turtle (*Chelonia mydas*) tissues from the Hawaiian Islands.

ORGANOCHLORINES	MDL (ppm)
Aldrin	0.02
BHC	0.02
Chlordane	0.25
p,p'DDD	0.1
o,p'DDD	0.1
p,p'DDE	0.1
o,p'DDE	0.1
p,p'DDT	0.1
o,p'DDT	0.1
Dicofol	0.1
Dieldrin	0.02
Endosulfan I	0.02
Endosulfan II	0.02
Endrin	0.02
Gamma Chlordane	0.02
HCB	0.02
Heptachlor	0.05
Heptachlor Epox	0.02
Lindane	0.05
Methoxychlor	0.04
Mirex	0.04
Oxychlordane	0.05
Toxaphene	0.5

PCBs	MDL (ppm)
Aroclor 1016	1.0
Aroclor 1221	1.0
Aroclor 1232	1.0
Aroclor 1242	1.0
Aroclor 1248	1.0
Aroclor 1254	1.0
Aroclor 1260	1.0
Aroclor 1262	1.0

METHYL CARBAMATES	MDL (ppm)
Aldicarb	0.1
Bendiocarb	0.1
Carbaryl	0.1
Carbofuran	0.1
Methiocarb	0.1
Methomyl	0.1
Mexacarbate	0.1
Oxamyl	0.1
Propoxur	0.1
Aldicarb sulfon	0.1
3-Hydroxy carbo	0.1

Table 2.--Continued.

ORGANOPHOSPHATES	MDL (ppm)
Acephate	0.025
Azinphos-Methyl	0.025
Carbophenothion	0.025
Chlorfenvinphos	0.02
Chlorpyrifos	0.02
Coumaphos	0.025
Crotoxyphos	0.025
Cruformate	0.025
DDVP	0.025
DEF	0.01
Demeton	0.03
Diazinon	0.01
Dicrotophos	0.025
Dimethoate	0.02
Dioxathion	0.05
Disulfoton	0.02
EPN	0.025
Ethion	0.01
Ethoprop	0.025
Fenamiphos	0.025
Fensulfothion	0.025
Fenthion	0.025
Fonophos	0.025
Isofenphos	0.025
Malathion	0.01
Merphos	0.02
Methamidaphos	0.025
Methidathion	0.02
Methyl Parathion	0.01

ORGANOPHOSPHATES	MDL (ppm)
Mevinphos	0.01
Monocrotophos	0.025
Naled	0.04
Parathion	0.01
Phorate	0.01
Phosalone	0.025
Phosmet	0.05
Phosphamidon	0.05
Profenophos	0.025
Propetamphos	0.025
Ronnel	0.025
Terbufos	0.025
Tetrachlorvinphos	0.025
Triazophos	0.025

Table 3.--Selenium and heavy metal concentrations in parts per million (ppm) wet weight detected in liver (L) and kidney (K) tissues of green turtles (*Chelonia mydas*), Hawaiian Islands.

HEAVY METALS (MDL) ¹	TURTLE IDENTIFICATION NUMBERS																											
	035		036		037		043		044		045		046		047		048		049		050		051		052	060	061	
	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	GTH ²	TES ³	
Selenium 0.007	0.576	0.159	0.385	0.194	1.36	0.957	0.999	0.700	0.136	0.223	0.335	0.186	0.844	0.445	0.899	0.390	0.316	0.231	0.657	0.283	0.457	0.208	2.53	1.58	3.39	NT ⁴	NT	
Thallium 0.7	(-) ⁵	--	--	--	--	--	--	--	--	--	--	--	1.0	--	--	--	1.0	--	0.8	--	0.9	--	--	--	1.1	--	--	
Arsenic 0.6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6.4	6.8	0.9	--	--	
Molybdenum 0.1	0.3	--	--	0.1	0.2	0.3	0.1	0.1	--	--	--	0.1	0.4	0.2	0.2	0.2	0.2	0.3	0.6	0.3	0.2	0.2	--	0.2	0.2	--	--	
Zinc 0.03	26.5	12.7	39.5	18.4	45.8	38.1	37.8	31.7	19.7	17.3	19.6	12.5	40.6	26.3	33.6	21.6	34.2	27.1	41.9	22.7	25.1	20.0	18.1	19.1	15.1	12.1	6.25	
Cadmium 0.07	5.44	4.77	4.51	9.84	25.6	70.2	3.10	22.0	0.39	8.85	1.80	9.96	26.0	47.4	11.2	33.9	13.6	47.7	13.8	36.7	5.04	15.9	1.12	4.72	0.96	--	0.2	
Nickel 0.4	--	--	--	0.8	--	1.0	--	--	--	--	--	--	--	0.7	--	0.5	--	0.8	--	--	--	--	--	--	--	--	--	
Manganese 0.03	2.79	0.48	1.65	1.23	2.04	1.24	2.13	0.82	0.15	0.59	0.78	0.66	1.32	1.12	1.56	1.09	1.76	1.39	1.24	1.12	1.39	1.21	2.34	0.56	1.59	1.12	0.31	
Iron 0.2	1730	38.8	1620	126	2260	12.7	765	16.3	155	179	1330	23	2450	9.9	446	8.8	1250	10.5	1740	13.0	1290	14.9	101	66.5	92.8	128	14.1	
Chromium 0.2	--	--	--	--	0.2	--	0.5	0.4	--	--	--	--	0.2	--	--	--	--	--	--	--	0.2	--	--	--	--	--	0.4	
Aluminum 1.0	3.0	2.0	5.0	2.0	1.0	1.0	1.0	1.0	--	--	4.0	1.0	3.0	2.0	1.0	1.0	2.0	1.0	1.0	--	2.0	1.0	--	1.0	--	--	3.0	
Vanadium 0.2	0.2	--	0.2	--	0.7	0.7	0.3	--	--	--	0.9	--	1.2	--	0.4	--	0.3	--	1.5	0.3	0.9	--	0.8	2.5	--	--	--	
Copper 0.2	91.4	1.5	134	1.1	173	3.3	116	10.5	1.3	1.5	33.9	6.9	106	4.4	86.7	2.3	189	3.4	149	2.2	35.6	1.8	20.2	4.7	2.8	2.2	14.3	
Barium 0.07	0.58	0.82	0.61	1.58	0.75	1.01	0.70	0.88	0.58	0.76	0.74	1.0	0.82	0.90	0.69	0.98	0.63	0.86	0.78	0.94	0.66	0.91	0.63	0.98	0.83	0.35	1.0	

¹MDL-method detection limits; ²GTH - green turtle hatchlings; ³TES- turtle egg shells; ⁴NT- not tested; ⁵(---) concentrations below MDL