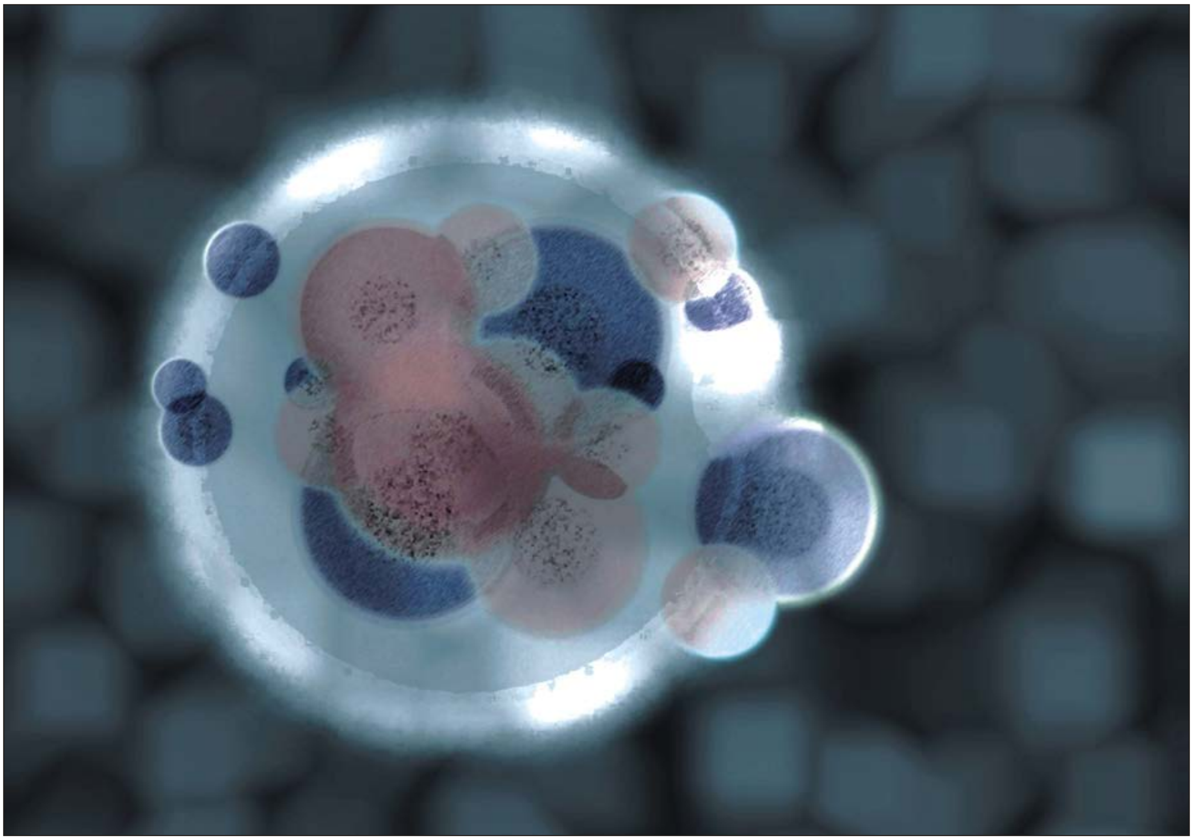

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 7 (6): 925-930, 2007
ISSN 1727-3048

Accumulation of Metals in the Egg Yolk and Liver of Hatchling of Green Turtles *Chelonia mydas* at Ras Al Hadd, Sultanate of Oman

[1]Salim H. Al-Rawahy, [1]Abdulaziz Y. AlKindi, [1]Abdulkadir Elshafie, [1]Mahmoud Ibrahim, [1]Saif N. Al Bahry, [1]Sultan S. Al Siyabi, [2]Mohamed H. Mansour and [3]Ali A. Al Kiyumi
[1]Department of Biology, College of Science, Sultan Qaboos University, P.O. Box 36, Alkhod 123, Sultanate of Oman
[2]College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, Alkhod 123, Sultanate of Oman
[3]Ministry of Regional Municipalities, Environment and Water Resources, P.O. Box 323, Muscat 113, Sultanate of Oman

Abstract: An investigation of the bioaccumulation of heavy metals in green turtles at Ras Al Hadd turtle reserve (Oman) was undertaken. A total of 12 heavy metals in liver of hatchlings and egg yolk of eggs were determined by using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) analytical methods. Random samples collected over 2 year period from different beaches at Ras Al Hadd reserve. The average metal contents varied from not detectable (Sn) to 8.58±1.03 (Zn) $\mu g\ g^{-1}$ wet weight in egg yolk and 0.01±0.00 (Sn) to 23.16±1.84 (Zn) $\mu g\ g^{-1}$ wet weight in liver of hatchlings. The concentration level of metals in egg yolk of freshly laid eggs occurred in the following order: Zn > Cu > Mn > Cd > V > Se > Hg > Cr > Pb > Ni > Co and in liver of untreated hatchlings occurred in the following order: Zn > Cu > Mn > V > Cr > Hg > Se > Cd > Pb > Co > Ni > Sn. Laboratory simulation with experimental metal contamination in sands and waters showed a significant accumulation of Cr, Co, Cu, Pb, Hg, Ni and Se in egg yolk and Cd, Cr, Cu, Pb, Mn, Hg, Ni, Se and V in liver compared with controls. Toxicological studies on the effect of heavy metals are urgently needed for the endangered population of green turtles.

Key words: *Chelonia mydas*, egg yolk, liver, heavy metals

INTRODUCTION

Populations of the green turtles *Chelonia mydas* in the world have declined drastically (Craig *et al*., 2004). The number of female green turtles nesting at Ras Al Hadd, Oman, believed to be in excess of hundred thousand annually, this figure has subsequently declined as a result of habitat destruction, overexploitation and egg harvesting. Conservation and monitoring of the green turtle populations in Oman began recently and according to a recent estimation, the population of female green turtles nesting at Ras Al Hadd beaches and other beaches along Omans coastline annually exceeds 20,000 (AlKindi *et al*., 2005).

Pollution from various human activities may pose threats to the population of green turtles in Oman. Over the last few decades, most cities along Omans Indian Ocean coastline have experienced an accelerated industrial growth. As a consequence, the human population of coastal regions has increased significantly. The Northwest Indian Ocean, including Gulf of Oman and Arabian Sea, is an area of busy maritime traffic connecting the Indian Ocean to the Arabian Gulf, the Gulf of Aden and the Red Sea (Al-Rawahy *et al*., 2006), making one of the most polluted seas of the world. Beach debris along coastlines is the most visible expressions of the negative impact of human activities on our marine environments (Claereboudt, 2004). Waste-water discharges from industries and sewage and surface run-off from high lands emit various kinds of pollutants into the sea. Among these are heavy metals from anthropogenic sources. Activities, such as mining, industrial processing, military waste discharge, shipment and oil transportation are the main source of heavy metal contamination. These heavy metals may accumulate to toxic levels and cause ecological and biological damage (Linde *et al*., 1998). As a consequence, heavy metals are considered to be the most important form of pollution of aquatic environments because of their toxicity and accumulation in the tissues of marine organisms (Emmam Khansari *et al*., 2005). They

Corresponding Author: Salim H. Al-Rawahy, Department of Biology, College of Science, Sultan Qaboos University, P.O. Box 36, Alkhod 123, Sultanate of Oman

can have a negative effect at several levels of organization, from a biochemical response to a change in population size (Dauwe *et al*., 2004). Recent reports have documented that marine pollution have been of great concern and that may have played a role in declining sea turtle populations in the world (Sakai *et al*., 2000; Al-Rawahy *et al*., 2006). Although metal bioaccumulation by marine organisms has been the subject of considerable interest recently, the information on the bioaccumulation and health risks of heavy metals in Indian Ocean is limited. Most research efforts in this area have focused primarily on highly productive and sensitive areas such as salt marshes, estuaries and mangrove ecosystems. Various fish species have been studied with respect to their ability to accumulate certain metals and the effects of that metal exposure can produce on them (Emmam Khansari *et al*., 2005).

Preliminary investigations on the effects of heavy metal contaminants on some turtle species, including marine and freshwater turtle species, have been reported from various countries (Godley *et al*., 1999; Sakai *et al*., 2000; Anan *et al*., 2002; Storelli and Marcotrigiano, 2003; Fujihara *et al*., 2003), but limited studies on the effects of marine pollution on green turtle populations in Oman have been conducted. The present study was conducted at Ras Al Hadd turtle reserve, in the Sultanate of Oman with the following aims: (i) assess the presence of 12 heavy metals in green turtle egg yolk and liver of hatchlings (ii) to assess the uptake of these metals by eggs and hatchlings using experimentally contaminated sand and water.

MATERIALS AND METHODS

Study site: The study site, Ras Al-Hadd Reserve, is located on the Gulf of Oman in the Arabian Sea between 22°32` N-59°45` E and 22°14` N-59°48` E. The coastline of the reserve consists of twenty beaches of different length ranging from 50 to 53 km. The coast is characterized by moderate to high energy wave action and is mostly surrounded by rocky hills. The high energy waves build a curb of sand that can reach up to 2 m high.

Collection and preparation of eggs and hatchlings: Green turtle eggs were collected from randomly selected nests in the tidal zone during the peak egg-laying seasons (May-August) of 2003 and 2004. Fifteen eggs were collected from each clutch, immediately after oviposition and transported to laboratory using cool box. Egg yolks were separated and stored in plastic vials at -15°C until analysis. With the appropriate permits from the Ministry of Regional Municipalities, Environment and Water Resources (Oman), green turtle hatchlings were collected between May and December of 2004 and 2005, from 14 nests that emerged during morning hours at least 18 m from the tidal zone, as they otherwise lost to predators or desiccation. Hatchlings were transported to the laboratory and, those required for chemical analysis were sacrificed within 48 h of collection. Livers were removed, sealed in plastic bags and stored at -15°C until analysis.

Uptake monitoring experiments: Uptake of Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Sn, V or Zn metals in eggs and hatchlings was determined by keeping them in experimentally contaminated sand samples and waters. Fifteen eggs collected from at least 3 different clutches were incubated for 15 days in 10 kg of sand obtained from Ras Al Hadd beaches. All sand samples were washed three times with distilled water, sun-dried for 6 h and re-wetted prior to incubation with 1.0 L of solution containing 250 mM of one of the above metals. Eight hatchlings from different nests were kept for 15 days in an aerated water tank (100×70×50 cm) spiked with 100 mM of one of the above metals. About 75 g of food material (from Arasco Co., Saudi Arabia) was supplied and the content of the tank was changed every 3 days. Each treatment for eggs and hatchlings was repeated for each of the 12 metals listed above, making 12 experiments for eggs and 12 experiments for hatchlings. No heavy metals were added on control treatments (experiment 13). Egg yolks and livers were obtained immediately after the termination of treatments and stored in plastic bags at -15°C until analysis. Analysis was performed for Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Sn, V or Zn as per treatment.

Chemical analyses: Egg yolk and liver samples were analyzed for Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Sn, V and Zn, using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) type Perkin Elmar 3300 DV ICP (USA). Approximately 1 g of yolk or liver tissue was digested with 5 mL of concentrated HNO_3 and 1 mL of 30% H_2O_2 in a Teflon bomb. All digestion bombs were securely closed and placed on the rotar of the microwave oven (Milestone 1200 MDR, USA). They were heated from room temperature to 200°C over a period of 5 min, then held at 200°C for another 20 min. Samples were left to cool, filtered through a glass microfilter into a 25 mL volumetric filter and analyzed by ICP-OES. Blanks and Certified Reference Materials (CRMs) were obtained from the Marine Biological Association in the United Kingdom, the Marine Environment Laboratory (MEL-IAEA) in Monaco and the Laboratory of the Government Chemists (LGC) in the United Kingdom. In-house reference materials were also used in situations where certified reference materials were not available.

Data analyses: Statistical analysis of data was performed using a factorial design Analysis Of Variance (ANOVA) using SPSS package. All data were presented as means±SE.

RESULTS

The concentration level of metals in egg yolk was as follows, in descending order: Zn > Cu > Mn > Cd > V > Se > Hg > Pb > Cr > Ni > Co and in liver of hatchlings occurred in the following order: Zn > Cu > Mn > V > Pb > Cr > Hg > Se > Cd > Co > Ni > Sn. The level of Sn in freshly laid eggs was not detectable (Table 1). The levels of all metals were significantly higher in liver tissue than in egg yolk, with Cr, Pb, Mn and Zn nearly four- to five-fold higher and the levels of Co, Cu, Hg, Se and V were nearly twice as high in liver compared with egg yolk.

Table 2 shows the accumulation of heavy metals in egg yolk of eggs incubated in experimentally contaminated sands for 15 days and liver of hatchlings kept in experimentally contaminated waters for 15 days. The concentration level of metals in egg yolk of eggs incubated in contaminated sands occurred in the following order: Zn > Cu > Mn > Se > Hg > Pb > Cr > Cd > Ni > V > Co > Sn and in liver of hatchlings kept in contaminated waters occurred in the following order: Zn > Cu > Mn > Cd > Hg > V > Se > Pb > Sn > Cr > Ni > Co.

Table 3 shows differences in egg yolk metal concentrations among three categories: (i) freshly laid eggs, (ii) eggs incubated in contaminated sands and (iii) eggs incubated in control sand. The table also shows

Table 1: The concentration of heavy metals in egg yolk of freshly laid eggs and liver of untreated hatchlings of green turtles from Ras Al Hadd turtle reserve area

Metal	Concentration ($\mu g\ g^{-1}$) Egg yolk*	Liver⁺	p-value
Cd	0.16±0.03	0.21±0.03	<0.05
Cr	0.07±0.00	0.25±0.03	<0.01
Co	0.05±0.00	0.11±0.01	<0.01
Cu	1.09±0.09	2.21±0.32	<0.01
Pb	0.08±0.01	0.27±0.02	<0.01
Mn	0.30±0.02	1.54±0.17	<0.01
Hg	0.10±0.01	0.22±0.03	<0.01
Ni	0.06±0.00	0.09±0.01	<0.05
Se	0.12±0.02	0.22±0.02	<0.01
Sn	ND	0.01±0.00	-
V	0.15±0.02	0.36±0.03	<0.01
Zn	8.58±1.03	23.15±1.84	<0.01

*n = 75; ⁺n = 50; ND: Not detectable

Table 2: The concentration of heavy metals in egg yolks of treated eggs and liver of treated hatchlings of green turtles

		Metal Conc. ($\mu g\ g^{-1}$ wet wt.)			Control, Exp. 13 ($\mu g\ g^{-1}$ wet wt.)	
Exp No.	Metal added	Egg yolk*	Liver⁺	Metal	Egg yolk*	Liver⁺
1	Cd	0.22±0.03	1.49±0.09	Cd	0.23±0.02[a]	0.39±0.02[b]
2	Cr	0.23±0.02	0.38±0.03	Cr	0.10±0.01[a]	0.27±0.02
3	Co	0.07±0.00	0.12±0.01	Co	0.06±0.00	0.14±0.01[b]
4	Cu	1.41±0.12	2.72±0.20	Cu	1.16±0.08	2.43±0.16
5	Pb	0.24±0.02	0.52±0.03	Pb	0.11±0.01[a]	0.30±0.02
6	Mn	0.39±0.04	1.79±0.11	Mn	0.33±0.02	1.66±0.08
7	Hg	0.33±0.02	0.92±0.06	Hg	0.11±0.01	0.29±0.02
8	Ni	0.19±0.01	0.26±0.02	Ni	0.08±0.01	0.77±0.04
9	Se	0.36±0.02	0.61±0.03	Se	0.12±0.01	0.21±0.01
10	Sn	0.01±0.00	0.40±0.02	Sn	0.01±0.00	0.01±0.00
11	V	0.17±0.03	0.87±0.04	V	0.18±0.02[a]	0.37±0.02
12	Zn	10.11±1.55	25.34±2.07	Zn	9.49±1.36	22.24±1.83

*n = 15; ⁺n = 8; [a]Significant difference with freshly laid eggs at 0.05 level; [b]Significant difference with untreated hatchlings at 0.05 level

Table 3: Statistical significance in the levels of metals among yolks of three egg categories and livers of three hatchling categories

	Egg yolk			Liver		
Metal	Freshly laid vs contaminated* eggs	Control vs contaminated* eggs	All three categories	Untreated vs contaminated* hatchlings	Contaminated* vs control hatchlings	All three categories
Cd	NS	p<0.05	p<0.05	p<0.01	p<0.01	p<0.01
Cr	p<0.01	p<0.05	p<0.05	p<0.01	p<0.05	NS
Co	p<0.05	NS	NS	NS	NS	NS
Cu	p<0.05	p<0.05	NS	NS	NS	NS
Pb	p<0.01	p<0.01	p<0.05	p<0.01	p<0.05	NS
Mn	NS	p<0.05	NS	NS	NS	NS
Hg	p<0.01	p<0.01	p<0.05	p<0.01	p<0.01	p<0.01
Ni	p<0.05	p<0.01	p<0.05	p<0.01	p<0.01	p<0.01
Se	p<0.01	p<0.01	p<0.05	p<0.01	p<0.01	p<0.05
Sn	-	NS	NS	p<0.01	p<0.01	p<0.01
V	NS	p<0.01	p<0.05	p<0.01	p<0.01	p<0.05
Zn	NS	NS	NS	NS	NS	NS

*Contaminated: means eggs and hatchlings were kept in contaminated sands and waters, respectively differences in liver metal concentrations among three categories: (i) untreated hatchlings (ii) hatchlings kept in contaminated waters and (iii) hatchlings kept in control water.

DISCUSSION

Data presented here provide strong evidence that eggs and hatchlings of green turtles are able to accumulate various metals from their environments. Several of these metals (e.g., Mn, Co, Cu and Zn) are known to be essential for life and play important role in body enzyme chemistry (Kannan *et al*., 2006). The concentration of Zn was the highest among all metals analyzed in egg yolk of freshly laid eggs and liver of untreated hatchlings and the Sn was the lowest among all metals. In egg yolk of freshly laid eggs the data of Mg and Zn were lower and that of Cd, Co, Cu, Pb, Hg and Ni were higher compared with the result reported by Sakai *et al.* (2000) from Haya-Jima Island, Japan. The source of freshly laid egg contamination must be from the oviduct during egg retention. In a study conducted by AlKindi *et al.* (2005), freshly laid eggs of turtles taken from nests immediately after oviposition were contaminated with heavy metals in yolks, albumin and egg shells. Eggs of various marine species in the North Sea were also found to accumulate environmental pollutants (Latif *et al*., 2001). Commercial shrimp eggs in Indian were contaminated with significant higher levels of various heavy metals, most probably originated from mothers during egg incubation (Clark, 2001). It has been reported that a small quantity of Hg is transferred from female fish eggs during oogenesis (Niimi, 1983). Recently, bioaccumulation of Se in eggs from maternal source was observed in 20 fish species of Belews Lake (Lemly, 2002). Henny *et al*. (2003) analyzed and found accumulation of 16 trace elements in eggs of the western pond turtle (*Clemmys marmorata*) in Oregon. In a study conducted by Bunger and Gibbons (1998), contamination of 6 heavy metals was found in egg contents of yellow-belly slider (*Trachemys scripta*) in Savanna river. Mobilization of various environmental contaminants from females into eggs has also been observed in terrestrial reptiles, e.g., snake (Cañas and Anderson, 2002) and birds (Ek *et al*., 2004; Dauwe *et al*., 2004; Hui, 2002; Jeng *et al*., 1997). Sea turtles are known to cover large stretches of ocean between nesting seasons during which they may accumulate heavy metals in their tissues. There are three possible routes for metal to enter a turtle: food, drinking water and skin. The diffusion of metals through the skin may be less important in turtles but generally the greater amount enters the turtle via food and drinking. Metals that enter the body in food or drinking are carried by blood bound to proteins, where they move first into liver (Edwards *et al*., 2001) and can bioaccumulate over time to reach sublethal, or even lethal, levels and then gradually into other tissues including reproductive organs unless they are excreted or detoxified (Fent, 1996). The excretion mechanisms are sequestration in carapace (Sakai *et al*., 2000), through the digestive track (Fent, 1996) or through salt gland. Females turtles can also excrete metal in their eggs (Edwards *et al*., 2001) and metal excreted to eggs derive both from stored body burdens and food choice of the female during egg formation (Burger and Gochfeld, 1991).

In this study, we were able to demonstrate the accumulation of Cr, Co, Cu, Pb, Hg, Ni and Se in egg yolk from contaminated sands. Statistically, the levels of these metals were different between background (freshly laid eggs and control treatment) and eggs incubated in contaminated sands, which mean that the excess levels of these metals compared to their background are depended on concentration in contaminated sands. Mechanisms of metal uptake by turtle eggs during incubation are unclear and high accumulation of these metals in yolk indicates that protective barrier, if any, is inadequate. For Cd, Mn, V and Zn, however, there were no significant differences in egg yolk accumulation of metals between background levels and eggs incubated in contaminated sands, suggesting that there is a protective barrier for the uptake of these metals. Furthermore, the levels of all metal concentration, except Cd, were significantly higher in liver of untreated hatchlings compared with egg yolk of freshly laid eggs, suggesting that these accumulations were during egg incubation period. However, the pattern of metal accumulation in liver of hatchlings did not parallel the uptake that found in egg yolk of eggs incubated in contaminated sands. In the other words, concentrations of Cd, Co and Mn in egg yolk of eggs incubated in contaminated sands did not differ from egg yolk of control eggs, but significantly higher in liver of hatchlings compared with freshly laid eggs. These results may suggest that exposure period may play significant role in some metal uptake by eggs.

In this investigation we were also able to provide evidence that the uptake of metals by hatchlings was from surrounding water environment. The levels of Cd, Cr, Pb, Hg, Ni, Se, Sn and V were significantly higher in hatchlings kept in contaminated water tanks compared with control and untreated hatchlings. Prior to this study, reports of normal concentration ranges of heavy metals in liver of green turtle hatchlings were not available. Earlier studies that report the levels of metals were for adult turtles. Various marine organisms were found to accumulate higher levels of pollutions, including heavy metals (Edwards *et al*., 2001; Al-Yousuf *et al*., 2000; Sakai *et al*., 1995 and 2000), hydrocarbons (Davis *et al*.,

2002) and organochlorine pesticides (Sankar *et al.*, 2006). Although the contents and effects of various heavy metals in marine organisms are well documented, information on mobilization from eggs to hatchlings is relatively rare. Toxicological studies on the effect of metals studied here, as well as other pollutants, are urgently needed for the endangered population of sea turtles. The information available in literature related to body burdens, transformation, detoxification and excretion of heavy metals is inadequate.

Metal contamination of coastal regions has been one of the major pollution issues and because of the recent infrastructure layout of the area, Ras Al Hadd now has the substantial development of industrial and ecotourism activities which may result in increasing input of chemical contaminants. It is necessary to establish principles for the disposal of wastes in a whole area that result in the least coastal environmental destruction. There is a need for monitoring chemical pollutants and the ecotoxicology effects in an effort to conserve the population of green turtles and other marine and coastal organisms. Protection and conservation of coastal and marine area-of species and habitats-should be integrated into spatial development strategies for larger areas, under the umbrella of integrated coastal and ocean management (Cicin-Sain and Belfiore, 2005).

In conclusion, this study has shown that both egg yolk of green turtle eggs and liver of green turtle hatchlings are able to accumulate various heavy metals from the environment. Information on mobilization of these metals is not adequate. Further studies are needed for the accumulation of pollutants in green turtle eggs, hatchlings and adults.

ACKNOWLEDGMENT

This study was funded by Sultan Qaboos University (IG/SCI/BIOL/04/02). The authors are grateful to the Ministry of Regional Municipalities, Environmental and Water Resources, Sultanate of Oman, for their valuable assistance support through out this study.

REFERENCES

AlKindi, A.Y., I.Y. Mahmoud, S.N. Al Bahry, I.S. Al Amry, S.S. Al Siyabi and A. Elshafie, 2005. Comparative analysis of elements from eggshells taken immediately after oviposition and after hatching using x-ray microanalysis and image mapping. 25th Annual Symposium on Sea Turtles Biology and Conservation, USA.

Al-Rawahy, S.H., A.Y. Al-Kindi and I. Mahmood, 2006. Heavy metals accumulation in liver of hatchlings and egg yolk of green turtles *Chelonia mydas*. The 26th Annual Symposium on Sea Turtle Biology and Conservation. Island of Crete.

Al-Yousuf, M.H., M.S. El-Shahawi and S.M. Al-Ghais, 2000. Trace metals in liver, skin and muscle of *Lethrium lentjan* fish species in relation to body length and sex. Sci. Total Environ., 256: 87-94.

Anan, Y., T. Kunito, H. Sakai and S. Tanabe, 2002. Subcellular distribution of trace elements in the liver of sea turtles. Mar. Pollut. Bull., 45: 224-229.

Burger, J. and M. Gochfeld, 1991. Cadmium and lead in common terns (Aves: *Sterna hirundo*): Relationship between levels in parents and eggs. Environ. Monit. Assess., 16: 253-258.

Burger, J. and J.W. Gibbons, 1998. Trace elements in egg contents and egg shells of slider turtle (*Trachemys scripta*) from the Savannah River Site. Arch. Environ. Contam. Toxicol., 34: 382-386.

Cañas, J.E. and T.A. Anderson, 2002. Organochlorine contaminants in eggs: The influence of contaminated nest material. Chemosphere, 47: 585-589.

Cicin-Sain, B. and S. Belfior, 2005. Linking marine protected areas to integrated coastal and ocean management: A review of theory and practice. Ocean Coastal Manage., 48: 847-868.

Claereboudt, M.R., 2004. Shore litter along sandy beaches of the Gulf of Oman. Mar. Pollut. Bull., 49: 770-777.

Clark, R.B., 2001. Marine Pollution. Oxford University Press, UK.

Craig, P., D. Parker, R. Brainard, M. Rice and G. Balazs, 2004. Migration of green turtles in the central South Pacific. Biol. Conserv., 116: 433-438.

Dauwe, T., E. Janssens, B. Kempenaers and M. Eens, 2004. The effect of heavy metal exposure on egg size, eggshell thickness and the number of spermatozoa in blue tit *Parus caeruleus* eggs. Environ. Pollut., 129: 125-129.

Davis, H.K., C.F. Moffat and N.J. Shepherd, 2002. Experimental tainting of marine fish by three chemically dispersed petroleum products, with comparisons to the *Braer* oil spill. Spill Sci. Technol. Bull., 7: 257-278.

Edwards, J.W., K.S. Edyvane, V.A. Boxall, M. Hamann and K.L. Soole, 2001. Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia. Mar. Pollut. Bull., 42: 389-396.

Ek, K.H., S. Rauch, G.M. Morrison and P. Lindberg, 2004. Platinum group elements in raptor eggs, faeces, blood, liver and kidney. Sci. Total Environ., 334-335: 149-159.

Emmam Khansari, F., M. Ghazi-Khansari and M. Abdollahi, 2005. Heavy metals content of canned tuna fish. Food Chem., 93: 293-296.

Fent, K., 1996. Ecotoxicology of organotin compounds. Criti. Rev. Toxicol., 26: 1-117.

Fujihara, J., T. Kunito, R. Kubota and S. Tanabe, 2003. Arsenic accumulation in livers of pinnipeds, seabirds and sea turtles: Subcellular distribution and interaction between arsenobetaine and glycine betaine. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 136: 287-296.

Godley, B.J., D.R. Thompson and R.W. Furness, 1999. Do heavy metal concentrations pose a threat to marine turtles from the Mediterranean Sea?. Mar. Pollut. Bull., 38: 497-502.

Henny, C.J., K.F. Beal, R.B. Bury and R. Goggans, 2003. Organochloride pesticide, PCBs, trace elements and metals in western pond turtle eggs for Oregon. Northwest Sci., 77: 46-53.

Hui, C.A., 2002. Concentration of chromium, manganese and lead in air and in avian eggs. Environ. Pollut., 120: 201-206.

Jeng, S.L., S.J. Lee, Y.F. Liu, S.C. Yang and P.P. Liou, 1997. Effect of lead ingestion on concentration of lead in tissues and eggs of laying Tsaiya ducks in Taiwan. Poul. Sci., 76: 13-16.

Kannan, K., T. Agusa, E. Perrotta, N.J. Thomas and S. Tanabe, 2006. Comparison of trace element concentrations in livers of diseased, emaciated and non-diseased southern sea otters from the California coast. Chemosphere, 65: 2160-2167.

Latif, M.A., R.A. Bodaly, T.A. Johnston and R.J.P. Fudge, 2001. Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walleye (*Stizostedion vitreum*). Environ. Pollut., 111: 139-148.

Lemly, A.D., 2002. Symptoms and implications of selenium toxicity in fish: The Belews Lake case example. Aquat. Toxicol., 57: 39-49.

Linde, A.R., S. Sanchez-Galan, J.I. Izquierdo P. Arribas, E. Maranon and E. Garcya-Vazquez, 1998. Broun Trout as biomonitor of heavy metal pollution: Effect of age on the reliability of the assessment. Ecotoxicol. Environ. Saf., 40: 120-125.

Niimi, A.J., 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. Can. J. Fish. Aquat. Sci., 40: 306-178.

Storelli, M.M. and G.O. Marcotrigiano, 2003. Heavy metal residues in tissues of marine turtles. Mar. Pollut. Bull., 46: 397-400.

Sakai, H., H. Ichihashi, H. Suganuma and R. Tatsukawa, 1995. Heavy metal monitoring in sea turtles using eggs. Mar. Pollut. Bull., 30: 347-353.

Sakai, H., K. Saeki, H. Ichihashi, H. Suganuma, S. Tanabe and R. Tatsukawa, 2000. Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar. Pollut. Bull., 40: 701-701.

Sankar, T.V., A.A. Zynudheen, R. Anandan and P.G. Viswanathan Nair, 2006. Distribution of organochloride pesticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India. Chemosphere, 65: 583-590.