Olive Ridley as Environmental Bioindicator - A Review

Madhurika Nandi* and Dr.Papiya Ghosh**
Department of Zoology
Bijoy Krishna Girls' College Howrah
5/3,Mahatma Gandhi Rd, Howrah, West Bengal- 711101, India
*nandi.madhurika@gmail.com, **zoo.bkgc@gmail.com

Abstract - The Olive Ridley Sea Turtles are listed as vulnerable under IUCN. Characterization of different molecular parameters rather scaling the correlation of different pollutants to different proteins and genes of this animal is necessary to deduce the path of their sustainability related to reptiles' biodiversity. These turtles show large geographical range of migration and exposed to different stressors i.e. biochemical as well as biophysical marine environment and hence considered as an interesting bio-indicator too. Monitoring of these turtle population for the toxicological effects of different persistent organic pollutants as well as different heavy metals is the fundamental approach for their protection and sustainability.

This review includes the analysis of several toxicological studies that characterizes the pollutants accumulation nature in different tissues of Olive Ridley as well as the nature of excretion, effects of pollutants on different molecular, morphological and developmental parameters.

Olive Ridley were surveyed in several geographical marine habitats like in pelagic Pacific longline that revealed high ingestion rate of black and blue plastic fibres with microbeads, thermoplastics by different methods and most of them accumulated in their large intestine. The amount of plastic ingested were found to be correlated to POP (Persistent Organic Pollutants) in their fats and the result analysis rather review revealed higher ∑DDT (dichlorodiphenyltrichloroethane ethane and metabolites) than ∑PCB(Polychlorinated biphenyl).The POPs were maternally transferred to the hatchlings, resulting in developmental abnormality . Cd, Zn was found to be highest in blood and yolk with presence of Hg, Cu, Ni, in the excreta of egg laying females. Evaluation of the metals in nesting females showed highest renal Cd level in any sea turtle. POP concentrations were unrelated to the amounts of ingested plastic in olive ridleys, suggesting that their exposure to POPs is mainly through prey. Findings suggests that environmental rather marine contamination levels correlates with the animal's biochemical parameters, oxidative stress, diseases etc. Several research outcomes indicate carapace asymmetry is significantly associated with pollutant level in adult Olive Ridley and could be used as biomarker for some metals accumulation. The rate of gene expression of different anti-oxidants like Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) was increased mainly in the liver tissue with heavy metal exposure that is indicative of both their increased adaptability as well as their acceptance as a bio-indicator. P-nitrophenyl acetate esterase activity (EA) and cortisol concentrations in serum of marine Olive Ridley are also found to have immense applications be used as a biomarker as their amount in the serum of the animal was found to be positively correlated to metal and metalloids pollution in their respective marine environment. The malformed embryos tested and confirmed to have higher levels of Hg and the embryos with Schistosomusreflexus syndrome (SR), had higher

DNA methylation than normal. A potential accumulation of Polychlorinated biphenyl (PCB) that is CYP1A substrate was found, providing the Olive Ridley with biotransformation capacity.
Thus, Olive Ridley tends to accumulate organic and inorganic pollutants in their long life and can be harnessed as useful bioindicators. Moreover, usefulness of blood for biomonitoring with respect to pollutants was also established.

Keywords*: *Olive Ridley, Bioindicators, Plastics, Heavy metals, Antioxidant, DNA methylation, Pollutants.

I INTRODUCTION

The Olive Ridley turtle (Lepidochelysolivacea) is a pantropical species. Its largest populations are found in the Pacific and Indian Oceans (Marquez et al. 1996; Zug et al. 2006). Like all marine turtles, the Ridley turtle is considered an endangered species (IUCN 2012) and is listed in Appendix I of the Convention of International Trade in Endangered Species (CITES). The main threats that have brought turtles to this precarious situation are, among others, their exploitation for meat, eggs, and shells in certain regions around the world, by-catch, global warming, loss of nesting sites, and environmental pollution (Aridjis 1990; Camacho et al. 2014; Casale and Margaritoulis 2010; MTSG 2007; Wallace and Saba 2009). According to several authors, anthropogenic-induced stressors, such as coastal pollution, can exacerbate other adverse impacts and seriously compromise the ability of sea turtles to adapt to climate change (Poloczanska et al. 2009). In this context, it is necessary to study biological markers for the evaluation of pollution effects in sea turtles. Possible toxic metals (such as Cd and Pb) are contaminants of important environmental relevance due to their high risk of toxicity and persistence (Bjerregaard and Anders 2007). Lead (Pb) and cadmium (Cd) are among the element most studied in aquatic environments, and both have been associated with different pathologies in many vertebrate species, such as bone necrosis, endocrinological alterations, and immune and reproductive problems (Boughammoura et al.2013; Nunes et al. 2014; Rana 2014; Sonne et al. 2009).

II MATERIALS AND METHODS

Polychlorinated Biphenyls and Biotransformation enzymes of 3 species of Sea Turtle from Baja California Peninsula of Mexico

Between 2001 and 2003, samples of liver tissue were collected from stranded wild-born loggerhead, green, and olive ridley turtles from the waters surrounding the Mexican states of BC and Baja California Sur. Turtles were necropsied and a sample of liver tissue was placed in aluminum foil and stored on ice for transport and then stored a -80C until analysis.
Approximately 3 g of liver tissue from four loggerhead, three green, and four olive ridley turtles was spiked with PCB 65 surrogate standard prior to homogenization with anhydrous sodium sulphate, followed by extraction with hexane using an ultrasonic disruptor (550 Sonic Dismembrator; Fisher Scientific) according to EPA method 3550B as described elsewhere (Sapozhnikova et al. 2004). The extract was evaporated to 2 mL and 250 lL were removed for gravimetric lipid determination. The remaining extract was treated by vigorous vortexing with 2 mL of hexane cleaned concentrated sulfuric acid. The acid layer was then back-extracted two times with hexane and the hexane fractions were combined and evaporated to 0.25 mL for gas chromatograph–electron capture detector (GC-ECD) analysis. Quantification of individual

PCB congeners (IUPAC PCB8,18, 28, 44, 52, 66, 77, 81, 101, 105, 114, 118, 123, 126, 128,138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206, and 209) was performed using the relative response factors generated from the calibration curve of standards (Mullin et al. 1984). The total PCB (tPCB) concentration in samples was defined as the sum of these congeners. The subcellular fractions were isolated by sequential centrifugation at 20,000g for 30 min and then at 100,000g for 90 min, both performed at 4C to prevent protein degradation. Due to limited biomass, profiles of CYP isoforms in sea turtles were evaluated by Western blotting using CYPantiserum raised against fish CYP isoforms because antibodies for sea turtle CYP isoforms are not available. Microsomal proteins (50 lg per lane), along with molecular-weight markers (See Blue Plus2; Invitrogen, Carlsbad,CA, USA) were resolved using polyacrylamide gels[sodium dodecyl (SDS)-PAGE, 10% gradient].

P-Nitrophenyl Acetate Esterase activity and Cortisol as Biomarkers of metal pollution in blood of Olive Ridley Turtles

Forty-four apparently healthy nesting female turtles wererandomly selected. Blood samples were carefully taken fromthe dorsal cervical sinus, using a 5-mL syringe with 21″needles. The turtles showed no alteration in their behaviourafter sampling. Before blood extraction, the neck region was carefully cleaned using ethanol. For EA and cortisol analysis, blood samples were immediately transferred into empty tubes and centrifuged at 2000 rpm for 10 min at room temperature to obtain serum, which was transferred into Eppendorf® microtubes and stored at − 20 °C. For metal analysis, the complete blood samples were stored into 1.5 Eppendorf®microtubes at − 20 °C until analyses. Blood samples were pretreated as previously described (Cor-tés-Gómez et al. 2014). Inorganic element concentration was determined using an Inductively Coupled Plasma Optical Emission Spectrophotometer. EA was analysed in an automated clinical chemistry analyser by measuring the hydrolysis of p-nitrofenol acetate to p-nitrophenol as described by Haagen and Brock (1992), with some modifications (Tvarijonaviciute et al. 2012). Cortisol was analysed in an automated chemiluminescent immunoassay.

Trace metals (Cd, Ni, Cu, Zn) in blood and eggs of sea turtle Lepidochelysolivacea from a nesting colony in Oaxaca, Mexico

Eggs and blood of L. olivacea were sampled from EB, Oaxaca, Mexico during the nesting season 2005–2006 during the third ‘‘arribada’’ event, between 10 and 14 August 2005.
After biometric measurements, blood and egg samples were collected from each female turtle; blood samples were taken from the dorsal cervical sinus using a sterile plastic syringe and needle in order to collect 5–10 ml that were immediately placed in an acid-washed (Moody and Lindstrom 1977) polyethylene tube. During blood extraction from each individual, careful cleaning of the neck region (with ethanol and deionized water) prior to sampling was practiced. All samples were kept under fresh conditions (4C) and were transported to the laboratory. Once at the lab, these eggs and blood were stored at -20C.
Egg samples were rinsed with deionized water (Milli-Q;18.3 MX/cm) to remove any particulate matter that might have adhered. Next, eggs were weighed and sized and subsequently separated into shell, albumen, and yolk. The separation was carried out quickly to prevent thawing. Blood and pooled samples of eggs were freeze-dried (72 h at -49C and 133 9 10-3 mbar) and then powdered. Powdered samples (0.25 g) were digested with quartz-distilled concentrated nitric acid (5 mL) in microwave equipment (CEM; MDS 2000) under established conditions (MESL 1997). The digested material was finally diluted to25 ml using purified (Chelex-100 resin; Bio-Rad; 100–200mesh) deionized water and stored in

polyethylene container for further analysis. Analyses were made by flame atomic absorption spectrophotometry (FAAS) for Zn; in the case of Cd, Cu, and Ni, graphite furnace atomic absorption spectrophotometry (GFAAS) was used. Metals were quantified in a Varian SpectrAA 220 spectrophotometer equipped with

Relationship between plasma biochemistry values and metal concentration in nesting Olive Ridley

Blood samples were taken from the dorsal cervical sinus, using a 5-mL syringe with 21″ needle. For the biochemical parameters, blood was placed in repose into a tube without anticoagulant (Vacutainer®) for approximately 2 h to separate serum from the red cellular package. Then, serum was transferred into microtubes(Eppendorf®). For metal analysis, the whole blood samples were stored in 1.5 microtubes (Eppendorf®). All samples were keep at − 20 °C until analyses. The biochemical constituents of the serum were measuredusing an automated clinical chemistry analyze The biochemical panel included alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, albumin, cholesterol, glucose, proteins, triglycerides, urea, and P-nitrophenyl acetate esterase activity (EA). Cortisol was analyzed in an automated chemiluminescentimmunoassay. Prior to metal analysis, an acid digestionwas performed using 0.5 g of the sample into 4 mL ofHNO3 (69%) and 1 mL of H2O2 (33%) mixed in specialTeflon reaction tubes in a microwave digestion system36672 Environ SciPollut Res (2018) 25:36671–36679(UltraClave-Microwave Milestone) for 20 min at 220 °C and finally diluted with 25 mL of double deionized water (MilliQ). Metal concentrations were determined using an inductively coupled plasma optical emission spectrophotometer (ICPOES, ICAP 6500 Duo Thermo) following descriptions fromCortés-Gómez et al. (2018). All concentrations are expressed in microgram per gram in wet weight.

III Review on Results

Polychlorinated Biphenyls and Biotransformation enzymes of 3 species of Sea Turtle from Baja California Peninsula of Mexico

In each sample, the number of congeners detected ranged from 7 to 17. Several PCB congeners (28, 81, 195, and209) were not detected in any sample and were removed from further analysis. Three congeners that were detected in all samples—105, 138, and 153—constituted 33–62% of the tPCB concentration in each sample, with 153 as the most abundant congener (Table 2). Mean tPCB concentrations were 18.1, 10.5, and 15.2 for loggerhead, green, and olive ridley turtles, respectively. However, no significant differences were found in the tPCB concentrations among species ($p = 0.451$). Pentachlorinated biphenyls also contributed considerably to tPCBs.

Hepatic microsomal protein showed a high degree of variability in the patterns of bands among individuals; some turtles exhibited a singlet band, whereas others displayed doublets. No correlations were observed when comparing CYP expression with tPCB or PCB metabolic groups.

p-Nitrophenyl Acetate Esterase activity and Cortisol as Biomarkers of metal pollution in blood of Olive Ridley Turtles

The detected concentrations of EA, cortisol, and chemical elements in blood are given in Table 2. All of the samples analysed exhibited values above the detectable limit for all elements, except cortisol, which was below DL in many experimental cases as reported. Zn was the metal

with highest concentrations in blood, whereas Pb was the element with lowest concentrations ions

Trace metals (Cd, Ni, Cu, Zn) in blood and eggs of sea turtle Lepidochelysolivacea from a nesting colony in Oaxaca, Mexico

The metal concentrations in the analyzed materials in L. olivacea from EB were reported as follows: forZn, yolk ; for Cu, eggshell; for Cd, blo; for Ni, eggshell showed maximum level and concentrations in other parts are given in the following figure .
The multiple comparisons of means of metals evidence various significant (p\0.05) differences: The Cu and Ni mean concentrations were highest in the eggshell than the other tissues; Zn levels were different among shell, albumen, and yolk (Fig. 1). The Cd concentration was higher in blood and eggshell, whereas Zn concentration was higher in yolk.
For the correlation between the weights of eggs and the concentration of metals analyzed in each fraction of eggs, only a defined pattern was evidenced and significant (p\0.05); Ni in albumen had a negative tendency with the natural logarithm of weight of eggs (Fig. 4a); clearly, it indicates that when the egg size is increased, the Ni content in albumen is reduced. On the other hand, in the case of load of metal in the blood of the nesting females versus the metal levels found in the fractions of eggs, direct linear relationships were not significantly correlated (p\0.05);only Ni in eggshell versus the natural logarithm of Ni in blood showed a significant (p\0.05) correlation (Fig. 4b), which indicates that when Ni is increased in the female turtles, the concentration of Ni is increased in the eggshell.

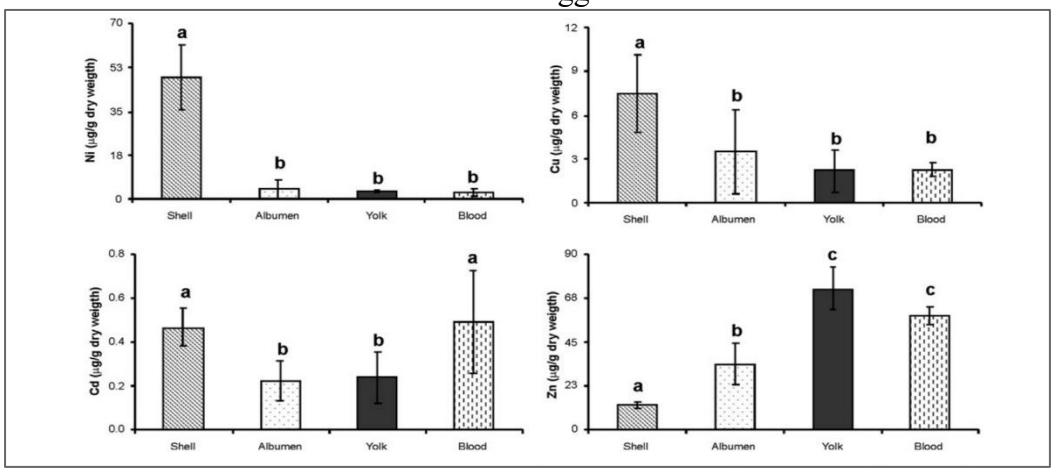

Figure 1: Figure Caption Missing

Relationship between plasma biochemistry values and metal concentration in nesting Olive Ridley

All inorganic elements in whole blood as included from several experimental study (n = 100) were above the detection limit (<0.01 µg g−1). Zinc presented the highest concentration with a mean ± standard deviation of 7.7 ± 2.4 µg g−1; it was followed by Se with 6.72 ± 3.0, As 1.27 ± 0.9, Sr 1.02 ± 0.45, Cu 0.52 ± 0.2, Mn 0.41 ± 0.12, Cr 0.17 ± 0.07, Cd 0.12 ± 0.05,Ni 0.07 ± 0.07, Ti 0.03 ± 0.08, and finally Pb, with 0.02 ±0.01 µg g−1.Strontium had four positive relationships with ALT, AST, urea, and albumin;titanium also had four positive relationships with creatinine,urea, cholesterol, and cortisol; lead had two positive relation-ships (AST and glucose) and one negative (EA); cadmium had two negatives with creatinine and glucose; arsenic had two positives (AST and urea) and two negatives (glucose and cholesterol); selenium had two positives (creatinine and glucose) and two negatives (urea and EA); zinc had three negatives (AST, albumin, and creatinine) and one positive (EA); copper one positive

(cholesterol) and one negative (ALT); and, finally, chrome had only one negative relationship with cholesterol.

IV CONCLUSIONS

In conclusion, PCB levels in loggerhead, green, and olive ridley turtles from the BC region of Mexico are generally lower than those reported for turtles from more polluted regions of the globe. The presence of dioxinlike congeners and relatively high TEQs along with a lack of CYP1A expression suggest a potential mechanism of accumulation of group 1 congeners. Future studies might clarify the contributions of CYP and GST isoforms toward the biotransformation and accumulation of PCBs in sea turtles. Different results of the presentreviewed works indicate that some inorganic elements could have a significant effect on p-nitrophenyl acetate esterase activity and on cortisol in Olive Ridley turtles and could be considered as useful biomarkers related to the differential contamination in this species. The lack of studies regarding concentrations of understudied elements in this species, such as Ti and Sr in marine turtles, and the weak but insignificant correlations detected between EA and Cd, Pb, and Ti, and those between cortical and Sr, As, and Se, highlight the necessity for further research in this field to identify the biological responses to each one of these elements.Results confirm the importance of this component in the accumulation of trace metals in marine turtles. On the basis of the nesting season, the excretion rates of trace metals through egg-laying were estimated; Cd was 0.2%, whereas Cu, Zn, and Ni were 5.9, 4.1, and 20.1%,respectively. 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. Evidences from reviewed articles
suggests several associations between metal concentrations and biochemical parameters.

V REFERENCES

[1] Marquez MR, Peñafores C, Vasconcelos JC (1996) Olive ridley turtles (Lepidochelysolivacea) show signs of recovery at La Escobilla,Oaxaca. Mar TurtNewsl 73:5–7
[2] Zug GR, Chaloupka M, Balazs GH (2006) Age and growth in Olive Ridley sea turtles (Lepidochelysolivacea) from the North-central Pacific: a skeletochronological analysis. Mar Ecol 27:263–270
[3] Aridjis H (1990) Mexico proclaims total ban on harvest of turtles and eggs. Mar Turtle Newslett 50:13
[4] Camacho M, Boada LD, Oros J, Lopez P, Zumbado M, Almeida-Gonzalez M, Luzardo OP (2014) Monitoring organic and inorganic pollutants in juvenile live sea turtles: results from a study of Cheloniamydas and Eretmochelysimbricata in Cape Verde. SciTotal Environ 481:303–310
[5] MTSG (2007) Lepidochelysolivacea, Marine Turtle Specialist Group Red list assessment
[6] Poloczanska ES, Limpus CJ, Hays GC (2009) Vulnerability of marine turtles to climate change. In: Sims DW (ed) Advances in marine biology, vol 56. Academic Press, Burlington, pp 151–211
[7] Bjerregaard P, Andersen O (2007) Ecotoxicology of metals—sources, transport, and effects in the ecosystem. In: Nordberg GF, Fowler BA, Nordberg M, Friberg L (eds) Handbook on the toxicology of metals, 3rd edn., pp 251–280

[8] Rana SV (2014) Perspectives in endocrine toxicity of heavy metals-are view. Biol Trace Elem Res 160:1–14
[9] Alonso-Aguirre A, Gardner SC, Marsh JC, Delgado SG, Limpus CJ, Nichols WJ (2006) Hazards associated with the consumption of sea turtle meat and eggs: a review for health care workers and the general public. EcoHealth 3:141–153
[10] Baggot JD (1986) Principios de farmacologı´a cli´nicaveterinaria. Editorial Acribia, Zaragoza
[11] Bjorndal KA (1997) Foraging ecology and nutrition of sea turtles. In: Lutz P, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Raton, pp 199–231
[12] Bjorndal K, Bolten A, Bennet R, Jacobson ER, Wronski TJ, Valeski JJ, Eliazar PJ (1998) Age and growth in sea turtles: limitations of skeletochronology for demographic studies. Copeia 1:23–30
[13] Gardner SC, Fitzgerald SL, Acosta-Vargas B, Me´ndez L (2006)Heavy metal accumulation in four species of sea turtles from the Baja California peninsula, Mexico. BioMetals 19:91–99
[14] Godley BJ, Thompson DR, Furness RW (1999) Do heavy metal concentrations pose a threat to marine turtles from the Mediterranean Sea? Mar Pollut Bull 38(6):497–502
[15] MESL (1997) Marine Environmental Studies Laboratory, International Atomic Energy Agency, Inorganic Laboratory, Standard Operating Procedures. Monaco
[16] Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall,Englewood Cliffs
[17] Cortés-Gómez AA, Fuentes-Mascorro G, Romero D (2014) Metals and
[18] metalloids in whole blood and tissues of olive Ridley turtles
[19] (Lepidochelysolivacea) from La Escobilla Beach (Oaxaca,
[20] Mexico). Mar Pollut Bull 89:367–375. https://doi.org/10.1016/j.
[21] marpolbul.2014.09.035 Environ SciPollut Res (2018) 25:36671–36679 36677
[22] Cortés-Gómez AA, Romero D, Girondot M (2017a) The current situation of inorganic elements in marine turtles: a general review and metaanalysis.EnvironPollut 229:567585.https://doi.org/10.1016/j.envpol.2017.06.077
[23] Cortés-Gómez AA, Tvarijonaviciute A, Teles M, Cuenca R, Fuentes-Mascorro G, Romero D (2017b): P-Nitrophenyl acetate esterase activity and cortisol as biomarkers of metal pollution in blood of olive Ridley turtles (Lepidochelysolivacea). Arch environ ContamToxicoldoi:https://doi.org/10.1007/s00244-017-0464-z
[24] Cortés-Gómez AA, Morcillo P, Guardiola FA, Espinosa C, Esteban MA,Cuesta A, Girondot M, Romero D (2018) Molecular oxidative stress markers in olive ridley turtles (Lepidochelysolivacea) and their relation to metal concentrations in wild populations. Environ Pollut233:156–167. https://doi.org/10.1016/j.envpol.2017.10.046