

**Polycyclic Aromatic Hydrocarbons (PAHs) and n-alkanes in Beaked Sea Snake *Enhydrina schistose* (Daudin, 1803) from the Mandovi estuary, Goa**

**Sambhaji Mote, Ranjeet Kumar, Naik. B.G, Baban S. Ingole\***

CSIR-National Institute of Oceanography, Dona Paula, Goa-403004, India

Corresponding Author:

Dr. Baban Ingole

Biological Oceanography Division, CSIR -National Institute of Oceanography, Dona Paula, Goa, India 403004

Email:baban@nio.org ; Phone: +91 832 2450242; Fax +91 832 2450602.

**Abstract**

An ecotoxicological study were conducted to evaluate the concentrations of PAHs and n-alkanes, in gut, liver and kidney tissues of two individuals of *Enhydrina schistose* (Daudin, 1803), using gas chromatography/mass spectrometry (GC/MS). The concentrations of PAHs (0.10 µg/g) and n-alkanes (8.12 µg/g) were elevated in the gut, and liver (PAHs 0.05 µg/g tissue, n-alkanes 29.16 µg/g tissue). In kidney of both specimen-A and B the PAHs (0.01 and 0.1 µg/g) and n-alkanes (0.22 and 2.06 µg/g) concentration was detected. This was an initial survey (n=2) and the main goal was to know accumulation and distribution of PAHs and n-alkanes in the sea snakes. This study indicates high accumulation of petroleum hydrocarbon in gut, liver and kidney of sea snake. Since, this species also act as pray for sea eagles and some predatory fishes such as tiger shark, there is high possibilities of PAHs being circulated in marine food chain.

**Keywords:** Sea snake; *Enhydrina schistose*; PAHs; n-alkanes; Mandovi estuary; Goa.

## Introduction

As environmental contaminants have been implicated as a possible cause of some declines of herpetofauna, there has been a substantial increase in the amount of reptile ecotoxicology research conducted over the last decade. In general, previous reptile studies have focused on measuring the body burdens of various pollutants from samples collected in the field (Meyers-Schöne 2000). While these data are useful for understanding the historical exposures of given populations, the actual risks and population level effects of pollutants on reptiles is still largely unknown and generally understudied. According to eminent eco-toxicologist “Hopkins”, reptiles are the least studied group of vertebrates compared to birds, mammals, fish and amphibians (Hopkins *et al.* 2001).

Snakes can be the ideal candidate species in monitoring the various contaminants in the wild life, because all the snakes are carnivores and known to accumulate environmental contaminants like chlorinated hydrocarbons (Fleet *et al.* 1972), polychlorinated biphenyls (Bishop *et al.* 2000), polychlorinated dioxin (Sabourin *et al.* 1984) and metals (Hopkins *et al.* 1999; Mohsen Raziie 2011) primarily accumulated from their prey items. The lethal effects of PHC (petroleum hydrocarbon) spills have been more frequently investigated, even phytoplankton and algal-derived hydrocarbons alter the endocrine and reproductive function in invertebrates and fish (Collier *et al.* 1986; Oberdo *et al.* 1999, 2000). However relatively little data is available on effect of PHC exposure in sea snakes. Worldwide decline of sea snake population has been causing a great concern in the scientific community (Gibbons *et al.* 2000). PHC can be used as valuable tool for tracing the eventual fate of oil in marine ecosystem; however, analysis of the total petroleum hydrocarbon (TPH) content is not sufficient to understand exposure of oil spill on marine organisms. A more extensive characterization and a separation of the complex hydrocarbon mixture into individual components is necessary for distinguishing between hydrocarbons of recent biogenic origin and hydrocarbons from fossil fuels. Among the most ubiquitous PHC in estuarine environment, polyaromatic hydrocarbons (PAHs) have become increasingly important because of their potential carcinogenicity, mutagenicity and teratogenicity to aquatic organism (Dhanajayan *et al.* 2012). When an organism is exposed to the PHC, owing to their lipophilic nature, it can easily penetrate biological membrane (Tuvikine 1995; Hamlink *et al.* 1977) and accumulate in the organisms. However, the concentration of these compounds in the tissues of candidate species under evaluation varies until it reaches a steady state level (Naser *et al.* 1992). In fact, tissues of biological organisms are frequently analyzed to describe the route of hydrocarbon uptake and biotransformation (Jeffery *et al.* 1999).

Liver and kidney are main organs of storage and excretion of PHC and its metabolism involves number of reaction, that convert lipid soluble (non-polar) organic compound (e.g. PAHs) to more water soluble to make them easy for excretion. These transformation processes are enzymatic and usually classified in to two types: phase-I and phase-II (Jimenez *et al.* 1990). Phase-I reaction involves enzymes (cytochrome 450 monooxygenase system) which introduce polar group in to xenobiotic molecule through oxidative, reductive and hydrolytic processes (Tuvikene 1995). The phase-II reaction involves conjunction to other molecules or their phase-I metabolites with polar endogenous constituent such as glucuronic acid, sulphate, amino acid or glutathione (Lech *et al.* 1985).

There are many studies that document the detection of petroleum hydrocarbons in the marine environment (Goa coastal water (Fondekar *et al.* 1980), north-west coastal water (Kadam *et al.* 1993), fish and prawn (Mehta *et al.* 1994; Dhanajayan *et al.* 2012; Ansari *et al.* 2012), bivalve in east coast (Mohan and Prakash *et al.* 1998), fresh water and fish (Malik *et al.* 2008), sediment (Ingole *et al.* 1989, 2006; Harji *et al.* 2008), and marine environment of Mumbai (Chouksey *et al.* 2004)). However, there is no information available on evaluation of n-alkanes and PAHs in sea snakes. *E. schistose* lives in the in near shore shallow waters and estuaries, especially in the soft bottom marine environments, where its preferred prey, catfish, occur in high abundances (Voris H K 1985; Aaron Lobo *et al.* 2004)

Present study demonstrates the tissue specific distribution of 15 EPA priority PAHs and 27 n-alkanes compounds in two individuals of *E. schistose*. We used GC-MS for analysis, which is the standard analytical technique for oil chemical fingerprinting, because it can resolve a broad range of petroleum biomarkers and polycyclic aromatic hydrocarbons (PAHs).

## **Materials and Methods**

Two individuals of *E. schistose* were collected from the intertidal region of Caranzalem beach, Mandovi estuarine of Goa. The specimens were partly injured as they were entangled in the shore fishing net “*Rampanni*”. The first individual specimen was collected on 15<sup>th</sup> September 2012 and marked as Specimen A, while second specimen was collected on 27<sup>th</sup> November 2012 and marked as specimen B. Both specimens were immediately transported to the laboratory from the collection site. The morphological characteristics were noted and specimens were stored at -20°C until further analysis. Snake samples were taken out from the deep freezer, thawed and dissection was performed on ice cold condition, using solvent rinsed instruments and glass dishes. Tissues (gut, liver and

kidney) were separated and dissolved in 20 ml dichloromethane/hexane (1:3 v/v) with surrogate internal standard (SIS) 20 ppm-200 $\mu$ l Tetracosane-d<sub>50</sub> for alkane, 5ppm-200 $\mu$ l Chrysene-d<sub>12</sub> for PAHs were spiked and transferred to 5% H<sub>2</sub>O deactivated silica gel column (1 cm i.d. x 9 cm). Elution was performed using 20 ml of dichloromethane/hexane (1:3 v/v) to get the solution containing alkanes and PAH's. The resultant solution was concentrated to 1-2 ml using rotary evaporator. This has been fractionated using fully activated silica gel column chromatography (0.45 cm i.d. x 18 cm) called the second step column chromatography to separate alkenes and PAHs. The extract was then rinsed with 8 ml (the first of 2 ml: 0.4 ml, 0.3 ml, 0.3 ml, 0.5 ml, 0.5 ml, respectively and 6 ml of distilled hexane) and the eluent was collected in pear shaped flask (1). Then, the column was eluted with 14 ml of dichloromethane/hexane (1:3 v/v) and the eluent was collected in another pear shaped flask (2). Both the eluent in the pear shaped flasks (1) and (2) were concentrated to a few milliliters using rotary evaporator followed by gentle nitrogen blow down to remove interfering of H<sub>2</sub> and CO<sub>2</sub>. These two fractions were evaporated to dryness under the nitrogen blow down and rinsed with calculated amount of DCM and transferred to suitable glass vial for further GC-MS analysis. One solvent method blank and one SIS blank were processed together for each batch of samples. The concentration SIS from each analysis was used to estimate and check the recovery. The recovery of SIS ranged from 60% to 120%. Correction factor for recovery were not applied while estimating concentration of analyte. However, samples were taken up for repeat analysis, where recovery estimated is less than 90% or more than 105%.

GC-MS of the samples were performed using a Shimadzu QP-2010 gas chromatograph interfaced with GCMS-QP2010 mass spectrometer. The gas chromatograph was equipped with a 20 m length. 0.1 mm i.d. 0.1  $\mu$ m film thickness Rtx-5 MS capillary column coated with 5% phenyl 95% methyl siloxane. The GC conditions were; oven temperature hold at 70°C for 1 minute, rise to 140°C at rate of 10°C minutes-hold 0, then rise to at 300°C at rate 6°C hold 30 minutes. Other settings of GC/MS; interface & injection temperature as 300°C, ultra-pure 'He' as carrier gas with a flow rate of 0.5 ml min<sup>-1</sup>, split less injection mode, scan range of 40-650 amu, and electron ionization mode with 70 eV. Quantification and identification of individual hydrocarbon compounds were achieved using standard method of external reference standard technique. Detection of hopanes and steranes was done using ions at *m/z* 191 and *m/z* 217, respectively (Zaghden *et al.* 2005; Harji *et al.* 2008). Reproducibility of the whole analytical procedure was checked through ten numbers of analysis of 5ppm alkanes, hopanes and PAHs mix standards. Relative standard deviation of the individual alkanes, hopanes and PAHs standard concentration were less than 10%, 15% and 5% respectively.

## Results

The concentration of PAHs among the different organ tissues of the two individuals of *E. schistose* (Daudin, 1803) are listed in table 1 and fig. 2. The total PAH in the gut region was found to be higher as compared to liver tissues of both the specimen. The kidney of the both specimen detected trace concentration of PAHs, the total PAHs of kidney tissue was compared with specimen-B, it was found to be higher by 33.3% than specimen-A. Acenaphthene (C<sub>12</sub>H<sub>10</sub>) and benzo(a)anthracene (C<sub>18</sub>H<sub>12</sub>) were detected in liver and gut tissues of Specimen-A and only acenaphthylene (C<sub>12</sub>H<sub>8</sub>) was detected in gut of specimen-B. Out of 15 PAHs compound, 12 were detected in liver tissue of specimen-A. Whereas only 3 compounds (Naphthalene, phenanthrene and dibenz(a,h)anthracene) were detected in liver of specimen-B. Naphthalene and anthracene were detected in kidney tissues of both the specimens (Table 1). Out of 15 PAHs, the predominant PAH in the gut was found to be Pyrene (56.56%) and naphthalene (84.57%) was found to be the predominant in liver tissue.

The concentration of n-alkanes among the different organ tissues of two individuals of *E. schistose* are listed in table 2. However the concentration of n-alkanes in liver tissue of specimen-A was higher compared to specimen-B (Fig.3). The kidney of specimen-B suggest the higher concentration of total n-alkanes than the kidney of specimen-A. The total 27 n-alkanes compounds were analyzed, kidney of specimen-A detected C<sub>14</sub>, C<sub>15</sub>, C<sub>16</sub>, C<sub>17</sub>, C<sub>19</sub> and C<sub>28</sub> while the kidney of specimen-B detected C<sub>14</sub>, C<sub>16</sub>, C<sub>17</sub>, C<sub>19</sub> to C<sub>33</sub> and C<sub>37</sub> which is found in gut and liver tissues of specimen-A. However it is absent in Specimen-B. Out of all the 27 n-alkanes compounds, n-Heptacosane (C<sub>27</sub>) was found to be predominant in the gut and liver.

## Discussion

Being carnivores, reptiles can easily magnify the contaminant through food chains as they have high food conversion efficiencies and can convert substantial amount of ingested energy into biomass (Hopkins 1999). As the gut content of both the sea snake specimen has high concentration of PAHs, therefore the snake specimens investigated here must have consumed contaminated prey items which led to the increased accumulation of hydrocarbons.

As shown in table 2, the distribution of short chain n-alkanes (C<sub>13</sub> to C<sub>22</sub>) demonstrated the predominance of even carbon n-alkanes C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub> and C<sub>20</sub>. The predominance of these short chain even n-alkanes indicates the contribution of marine microorganisms and petroleum products (Grimalt *et al.* 1987; Garg *et al.* 2004 and Harji *et al.* 2008). Pristane and phytane are isoprenoid hydrocarbons originating from geologic alteration of phytol and other isoprenoidyl products and are not primary

constituents of most terrestrial biota (Peters *et al.* 1993; Gao *et al.* 2007). They are usually present in most petroleum as major constituents and are considered as good indicators of petroleum contamination (Volkman *et al.* 1986; Readman *et al.* 2002; Zaghdan *et al.* 2005). The presence of trace petroleum biomarkers viz., hopanes and steranes (results not shown) in alkane fraction authenticate hydrocarbon found in the tissue of sea snakes is most likely from petroleum source.

Elevated levels of PHC was observed in gut>liver> kidney, it might be due to the fact that virtually all xenobiotic substances are absorbed from the gastrointestinal tract which passes through the liver before entering the central circulation via hepatic portal circulation (Vandenberghe 1996). Tissue specific distribution of PAHs and n-alkanes are not well known in sea snake but the studies on biotransformation mechanisms in other marine organism suggest that biotransforming enzymes are widely distributed throughout the body and liver is the primary biotransforming organ. Thus the total PAHs (Fig.2) and total n-alkanes (Fig.3) have been detected in significant concentration in liver tissues of both the specimens, which may be due to lipophilic nature of PHC and will be stored for varying times in the tissues with higher concentration in liver. The result shows that concentration of n-alkanes was appreciably high in specimen-B (29.164 µg/g of wet tissue) liver tissue as compared to Specimen-A (6.055 µg/g of wet tissue) liver tissue. This was perhaps due to the digestion and metabolic activities of snake, as gut region of specimen-B had fully digested prey whereas, gut of specimen-A had undigested prey (catfish *sp.*?) as shown in fig.4. The metabolism of PHC involves number of reaction that convert lipophilic organic compound PAHs to water soluble substance which facilitate easy excretion. Kidney has been studied in the various reptilian groups as target organ for xenobiotics, especially in turtle and crocodilians (McClellan-Gren *et al.* 2006). In the present study, out of 15 PAHs compounds only naphthalene and anthracene were detected in kidney tissues of both specimens. The petroleum hydrocarbon is absorbed in the body via diverse routes and removed immediately by the liver through the first-pass metabolism. When the quantity of hydrocarbon ingested exceeds the capacity of the liver to metabolize, it is absorbed in the blood at gastrointestinal tract and finally reaches kidney (Janssen *et al.* 1988). According to Nagelkerke (1996), toxic substances are mixed in the blood and passes through kidney thus reducing their concentration in body tissue. However, there is a paucity of literature on the toxic impacts of oil contamination in sea snakes and further study is required for underline the reasoning for significant accumulation of PAHs and n-alkenes in various tissues. Although the results described in this study shows elevated PAHs and n-alkanes in different organs of sea snake, it need to be treated with prudence.

## Conclusion

Even though, no major accidental oil spill is reported in last two years in and around mouth of Mandovi river of Goa, daily routine activities like casino's, ferry boat, larger number of fishing trawlers, ore carriers are moving in the river. Moreover, tar balls are occurring frequently along the Goa coast and all these above activities are responsible for increased level of oil contamination in the estuarine environment. Our study suggested elevated accumulation of PHC in the gut, liver and kidney of two individual sea snakes (n=2). Thus, sea snake being a carnivorous, and act as prey for sea eagles and some predatory fishes such as tiger shark, there is higher possibility of PAHs being circulated in the marine food chain. However, further study is needed to understand the mechanism of detoxification of petroleum hydrocarbon in sea snakes and also to take requisite action in order to control the anthropogenic activities that are responsible for PHC contamination in the coastal environment.

**Acknowledgements:** The authors are grateful to the Director, CSIR-National Institute of Oceanography, for encouragement and facilities. Our thanks to Dr. Aninda Muzumdar, Sr. Scientist NIO for allowing the wet lab and GC/MS facilities We acknowledge the help of Dr. Aaron Lobo of Deutsche Gesellschaft für Internationale Zusammenarbeit Germany, for confirming the taxonomic identification of sea snake. We also thank Prof. Ramola Antao for correcting the English grammar. We also thanks to anonymous reviewers for suggestions and comments. This is the contribution number .....of CSIR-NIO, Goa.

## References

- Ansari ZA, D'silva C, Badesab S (2012) Total petroleum hydrocarbon in the tissues of some commercially important fishes of the Bay of Bengal. *Mar Pollut Bull* 64:2564–2568
- Bishop CA, Rouse JD (2000) Chlorinated hydrocarbon concentrations in plasma of the Lake Erie water snake (*Erodia sipedon insularum*) and northern water snake (*Nerodia sipedon sipedon*) from the Great Lakes basin in 1998. *Arch Environ Con Tox* 39:500–505
- Chouksey MK, Kadam AN, Zingde MD (2004) Petroleum hydrocarbon residues in the marine environment of Bassein-Mumbai. *Mar Pollut Bull* 49:637–647
- Collier TK, Stein JE, Wallace RJ, Varanasi U (1986) Xenobiotic metabolizing enzymes in spawning English sole (*Parophrys vetulus*) exposed to organic solvent extracts of marine sediments from contaminated and reference areas. *Comp Biochem Phys* 84:291–298
- Dhananjayan V, Muralidhran S (2012) Polyaromatic hydrocarbon in various species of fishes from Mumbai Harbour, India and dietary intake concentration to human. *International Journal of Oceanography*. Article ID 645178, 1-6
- Fleet RR, Clark DR, Plapp FW (1972) Residues of DDT and dieldrin in snakes from two texas agroecosystems. *BioScience* 22:664-665

- Fondekar SP, Topgi RS, Noronha RJ (1980) Distribution of petroleum hydrocarbons in Goa coastal waters. *Indian J Mar Sci* 9(4):286-288
- Gao X, Chen S, Xie X, Long A, Ma F (2007) Non-aromatic hydrocarbons in surface sediments near the Pearl River estuary in the South China Sea. *Environ Pollut* 148:40-47
- Garg A, Bhosale NB (2004) Abundance of macroalgal OM in biofilms: Evidence from n-Alkane Biomarkers. *Biofouling* 20:155-165
- Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville T, Metts BS, Greene JL, Millis T, Leiden Y, Poppy S, Winne CT (2000) The global decline of reptiles, déjà vu amphibians. *Bioscience* 50 (8): 653-666
- Grimalt J, Albaiges J (1987) Sources and Occurrence of C<sub>12</sub>-C<sub>22</sub> n-alkane distributions with even carbon number preference in sedimentary environments. *Geochimica Et Cosmochimica Acta* 51:1379-1384
- Hamelin JL, Spacie A (1977) Fish and chemical of the processes of the accumulation. *Annu Rev Pharmacol* 17:167-177
- Harji R, Bhosale N, Yvenat (2008) Sources of hydrocarbons in sediments of the Mandovi estuary and the Marmugoa harbour, west coast of India. *Environ Int* 34: 959-965
- Hopkins WA, Rowe CL, Cogndon JD (1999) Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. *Environ Toxicol Chem* 18: 1258-1263
- Hopkins WA, Roe JH, Snodgrass JW, Jackson BP, Kling DE, Rowe CL, and Congdon JD (2001) Nondestructive indices of trace element exposure in Squamate reptiles. *Environ Pollut* 115:1-7
- Ingole SA, Dhaktode SS, Kadam AN (1989) Determination of petroleum hydrocarbons in sediment samples from Bombay harbour, Dharamtar creek and Amba river estuary. *Indian J Environ Prot* 9:118-123
- Ingole BS, Sivadas S, Goltekar R, Clemente S, Nanajkar M, Sawant R, D'Silva C, Sarkar A, Ansari Z (2006) Ecotoxicological effect of grounded MV River Princess on the intertidal benthic organisms off Goa. *Environ. Int.* 32:284 – 291
- Janssen S, Van der Geest S, Meijer S, Uges DRA (1988) Impairment of organ function after oral ingestion of refined petrol. *Intens Care Med* 14: 238-40
- Jeffery P (1999) Toxicological Profile for Total petroleum hydrocarbons (TPH). Agency for Toxic Substances and Disease Registry 1-136
- Jimenez BD, Stegeman JJ (1990) Detoxication enzymes as indicator of environmental stress on fish, biological Indicators of stress in fish. *Am Fish Soc Symp* 8:67-79
- Kadam AN, Bhangale VP (1993) Petroleum hydrocarbons in northwest coastal waters of India. *Indian J Mar Sci* 22: 227-228
- Lech JJ, Vodcnik MJ (1985) Biotransformation. In: Rand G M & Petrocelli S R (eds.), *Fundamentals of aquatic toxicology*. Taylor and Frances:526-557
- Lobo A, Pandav B, Vasudevan K (2004) Weight –Length relationship in two species of marine snakes along the west of India, Western India. *Hamadryad* 29:89 – 93
- Malik A, Ojha P, Singh KP (2008) Distribution of polycyclic aromatic hydrocarbons in edible fish from Gomti river, India. *B Environ Contam Tox* 80:134-138



- McClellan-Green P, Celander M, Oberdorster E (2006) Hepatic, renal, and adrenal toxicology, In: Gardner SC, Oberdorster E (ed) Toxicology of reptiles, CRC Press, Taylor & Francis Group:123-148
- Mehta P, Kadam. AN, Gajbhiye SN, Desai BN (1994) Petroleum hydrocarbon concentration in selected species of fish and prawn from northwest coast of India. Indian J Mar Sci 23:123-125
- Meyers-Schöne L (2000) Ecological risk assessment of reptiles. In: Sparling DW, Linder G, Bishop CA. (ed) Ecotoxicology of Amphibians and Reptiles, Chapter 14b. SETAC Technical Publications Series, Society of Environmental Toxicology and Chemistry. SETAC Press, Pensacola:793- 810
- Mohan PC, Prakash.RR (1998) Concentration of petroleum hydrocarbons in bivalve *Mytilopsis sallei* and in the harbour waters of Visakhapatnam, east coast of India. Indian J Mar Sci 27:496–498
- Mohsen Rezaie-Atagholipour (2012) Metal concentrations in selected tissues and main prey species of the annulated sea snake (*Hydrophis cyanocinctus*) in the Hara Protected Area, northeastern coast of the Persian Gulf, Iran. Mar Pollut Bull 64:416–421
- Nagelkerke JF (1996) Nephrotoxicology: Toxicological pathalaogy and biochemical toxicology. In: Raymond J, Heisink M, John Devis, Mannfred A, Hollinger (ed) Toxicology-principles and applications, CRC. Press:424-756
- Oberdo rster E, Wilmot FA, Cottam DM, MilnerMJ, McLachlan JA (1999) Interaction of PAHs and PCBs with ecdysone-dependent gene expression and cell proliferation. Toxicol Appl Pharm, 160:101–108
- Oberdo rster E, Brouwer M, Hoexum-Brouwer T, Manning S, McLachlan JA (2000) Long-term pyrene exposure of grass shrimp, *Palaeomonetes pugio*, affects molting and reproduction of exposed males and the offspring of exposed females. Environ Health Persp 108:641–646
- Peter KE, Moldowan JM (1993) The Biomarker Guide: Interpreting molecular fossils inpetroleum and ancient sediments, Prentice Hall, Englewood Cliffs, New Jersey 363
- Readman JW, Fillmann G, Tolosa I, Bartocci J, Villeneuve JP, Catinni C, Mee LD (2002) Petroleum and PAH contamination of the Black Sea. Mar Pollut Bull 44:48–62
- Sabourin TD, Stickle WB, Michot TC, Villars CE, Garton DW, Mushinsky HR (1984) Organochlorine residue levels in Mississippi River water snakes in southern Louisiana. B Environ Contam Tox 32:460-468
- Tuvikene A (1995) Responses of fish to Polycyclic Aromatic Hydrocarbons (PAHs). Ann zool fenn 32:295-309
- Vanderbrghe J (1996) Hepatotoxicology: Mechanism of liver toxicity and methodological aspect. In: Raymond J, Heisink M, John Devis, Mannfred A. Hollinger (ed) Toxicology-Principles and Applications,CRC. Press:407-423
- Volkman JK, Maxwell JR (1986) Acyclic isoprenoids as biological markers. In: Johns, RB (ed) Biological markers in the sedimentary record, Elsevier, Amsterdam:1-42
- Voris HK (1985) Population Size Estimates for a Marine Snake (*Enhydra shistosa*) in Malaysia. Copeia 4: 955-961
- Zaghden H, Kallel M, Louati A, Elleuch B, Oudot J, Saliot A (2005) Hydrocarbons in surface sediments from the Sfax coastal zone, (Tunisia) Mediterranean Sea. Mar Pollut Bull 50:1287–129

**Figure caption**

**Fig.1** Location of Caranzalem beach, Panaji, Goa.

**Fig.2** Total PAHs concentration in the different tissues of *E. schistose*.

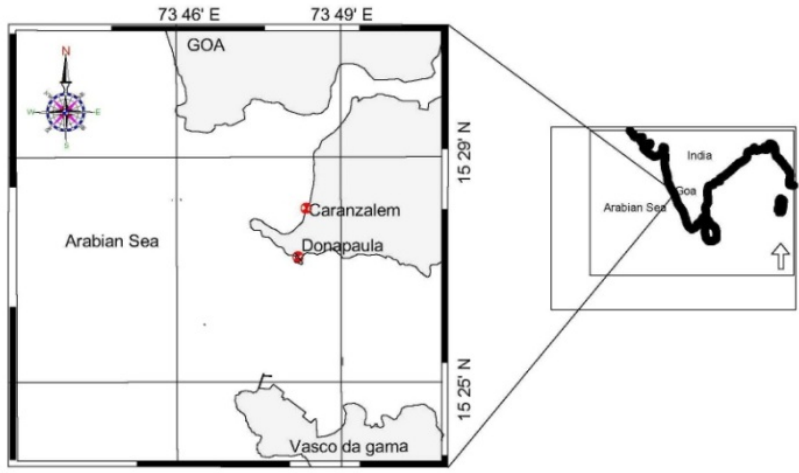
**Fig.3** Total n-alkanes concentration in the different tissues of *E. schistose*.

**Fig.4** The half-digested fish (catfish sp.?) being removed from the gut of *E. schistose*.

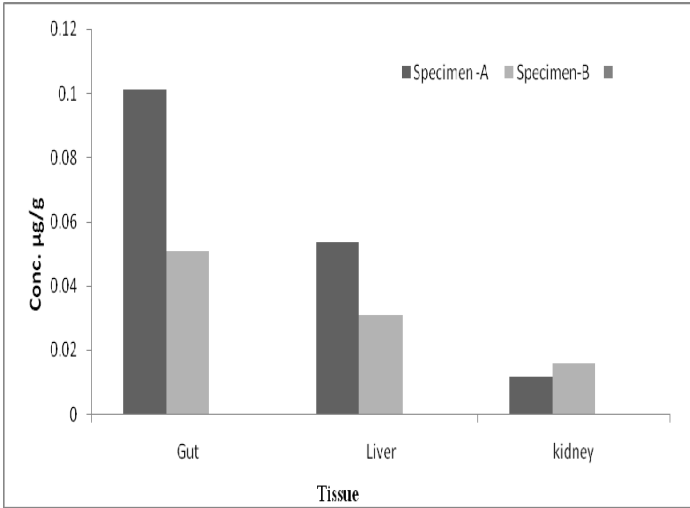
**Table caption**

**Table 1-** PAH concentration in tissues of sea snake (*E. schistose*)

**Table 2-** n-alkanes concentration in tissues of sea snake (*E. schistose*)



**Fig.1** Location of Caranzalem beach, Panaji, Goa



**Fig.2** Total PAHs concentration in the different tissues of *E. schistose*



**Fig.3** Total n-alkane concentration in the different tissues of *E. schistose*



**Fig.4** The half-digested fish (catfish sp.?) being removed from the gut of *E. schistose*

Table 1 PAH concentration in tissues of <i>E. schistose</i>							
Sr.No.	Compounds	Specimen-A (Conc. in µg/g of tissue)			Specimen-B (Conc. in µg/g of tissue)		
		Gut	Liver	Kidney	Gut	Liver	Kidney
1	Naphthalene (C <sub>10</sub> H <sub>8</sub> )	0.0284	0.0455	0.0100	0.0440	0.0307	0.0140
2	Acenaphthylene (C <sub>12</sub> H <sub>8</sub> )	nd	nd	nd	0.0002	nd	nd
3	Acenaphthene (C <sub>12</sub> H <sub>10</sub> )	0.0001	0.0002	nd	nd	nd	nd
4	Fluorene (C <sub>13</sub> H <sub>10</sub> )	nd	nd	nd	nd	nd	nd
5	Anthracene (C <sub>14</sub> H <sub>10</sub> )	0.0018	0.0010	0.0020	0.0005	nd	0.0020
6	Phenanthrene (C <sub>14</sub> H <sub>10</sub> )	0.0018	0.0010	nd	0.0007	0.0003	nd
7	Fluoranthrene (C <sub>16</sub> H <sub>10</sub> )	0.0098	0.0013	nd	0.0013	nd	nd
8	Pyrene (C <sub>16</sub> H <sub>10</sub> )	0.0573	0.0038	nd	0.0038	nd	nd
9	Chrysene (C <sub>18</sub> H <sub>12</sub> )	0.0001	0.0002	nd	0.0002	nd	nd
10	Benzo(a)anthracene (C <sub>18</sub> H <sub>12</sub> )	0.0002	0.0002	nd	nd	nd	nd
11	Benzo(a)fluoranthene (C <sub>20</sub> H <sub>12</sub> )	0.0003	0.0002	nd	0.0002	nd	nd
12	Benzo(a)pyrene (C <sub>22</sub> H <sub>12</sub> )	0.0002	0.0002	nd	nd	nd	nd
13	Benzo(ghi)pyrene (C <sub>20</sub> H <sub>12</sub> )	0.0012	0.0001	nd	0.0002	nd	nd
14	Dibenz(a,h)anthracene (C <sub>22</sub> H <sub>14</sub> )	nd	0.0002	nd	nd	0.0002	nd
15	Indeno(1,2,3-cd)pyrene (C <sub>22</sub> H <sub>12</sub> )	nd	nd	nd	nd	nd	nd
	Total PAHs	0.1013	0.0538	0.0120	0.0510	0.0312	0.0160

Table 2 n-alkanes concentration in tissues of <i>E. schistose</i>							
Sr.No	Compounds	Specimen-A (Conc. in µg/g of tissue)			Specimen-B (Conc. in µg/g of tissue)		
		Gut	Liver	Kidney	Gut	Liver	Kidney
1	n-Tridecane (C <sub>13</sub> H <sub>28</sub> )	0.0511	0.0388	nd	0.0464	0.0653	nd
2	n-Tetradecane (C <sub>14</sub> H <sub>30</sub> )	0.0766	0.0663	0.0200	0.0709	0.0957	0.0400
3	n-Pentadecane (C <sub>15</sub> H <sub>32</sub> )	0.1050	0.0979	0.0200	0.0944	0.1297	nd
4	n-Hexadecane (C <sub>16</sub> H <sub>34</sub> )	0.1309	0.1451	0.0200	0.1106	0.1554	0.0800
5	n-Heptadecane (C <sub>17</sub> H <sub>36</sub> )	0.1547	0.2716	0.0800	0.1266	0.1810	0.0600
6	Pristane (C <sub>19</sub> H <sub>40</sub> )	0.1541	0.1958	0.0600	0.1198	0.1720	0.0600
7	n-Octadecane (C <sub>18</sub> H <sub>38</sub> )	0.1828	0.3589	nd	0.1446	0.2082	nd
8	Phytane (C <sub>20</sub> H <sub>42</sub> )	0.1671	0.2417	nd	0.1330	0.1952	0.0400
9	n-Nonadecane (C <sub>19</sub> H <sub>40</sub> )	0.1910	0.3995	nd	0.1573	0.2218	0.0400
10	n-Eicosane (C <sub>20</sub> H <sub>42</sub> )	0.2101	0.4841	nd	0.1782	0.2441	0.0600
11	n-Heneicosane (C <sub>21</sub> H <sub>44</sub> )	0.2252	0.5042	nd	0.1887	0.2557	0.0400
12	n-Docosane (C <sub>22</sub> H <sub>46</sub> )	0.2363	0.4548	nd	0.2009	0.2684	0.0200
13	n-Tricosane (C <sub>23</sub> H <sub>48</sub> )	0.2678	0.5051	nd	0.2150	0.2882	0.0400
14	n-Tetracosane (C <sub>24</sub> H <sub>50</sub> )	0.3354	0.7944	nd	0.2461	0.3169	0.0800
15	n-Pentacosane (C <sub>25</sub> H <sub>52</sub> )	0.4681	1.7083	nd	0.2942	0.3449	0.0600
16	n-Pentylheneicosane (C <sub>26</sub> H <sub>54</sub> )	0.5839	2.5861	nd	0.3218	0.3681	0.2400
17	n-Heptacosane (C <sub>27</sub> H <sub>56</sub> )	0.6755	3.4802	nd	0.3469	0.3633	0.2800
18	n-Octacosane (C <sub>28</sub> H <sub>58</sub> )	0.6542	3.3981	0.0200	0.3183	0.3448	0.2000
19	n-Nonacosane (C <sub>29</sub> H <sub>60</sub> )	0.6568	3.3846	nd	0.3198	0.3366	0.1800
20	n-Triacontane (C <sub>30</sub> H <sub>62</sub> )	0.5422	2.7478	nd	0.2716	0.2967	0.1800
21	n-Hentriacontane (C <sub>31</sub> H <sub>64</sub> )	0.4952	2.4936	nd	0.2575	0.2847	0.1600
22	n-Dotriacontane (C <sub>32</sub> H <sub>66</sub> )	0.3984	1.8336	nd	0.2028	0.2410	0.1200
23	n-Tritriacontane (C <sub>33</sub> H <sub>68</sub> )	0.3316	1.1832	nd	0.1886	0.2474	0.0800
24	n-Tettratriacontane (C <sub>34</sub> H <sub>70</sub> )	0.2454	0.7560	nd	0.1582	0.2158	nd
25	n-Pentatriacontane (C <sub>35</sub> H <sub>72</sub> )	0.2098	0.5435	nd	0.1542	0.2143	nd
26	n-Hexatriacontane (C <sub>36</sub> H <sub>74</sub> )	0.2010	0.2886	nd	0.1572	0.0000	nd
27	n-Heptatriacontane (C <sub>37</sub> H <sub>76</sub> )	0.1722	0.2027	nd	0.0000	0.0000	nd
	Total n-alkenes	8.1224	29.1644	0.2200	5.0237	6.0553	2.0600