

A preliminary estimate of Total Petroleum Hydrocarbons in water and some commercially important Fish species in the Amba Estuary, west coast of India

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Abstract

Amba Estuary, which receives effluent from several industries including a petrochemical complex, opens to the southern limits of the Mumbai Harbor. The study was conducted to find out the level of Total Petroleum Hydrocarbons (TPHs) in water and their bioconcentration in ten commercially important fishes from Amba Estuary during different months. In water high concentration of TPHs (39.7µg/l) was obtained during December (middle of estuary) and minimum value (7.2µg/l) was observed in September (lower estuarine). The maximum concentration of TPHs was found to be in *Trichiurus savala* (3.2µg/g) during December and minimum in *Boleophthalmus sp* (0.4µg/g) during May. Irrespective of the monthly variations, TPHs accumulation in all the species was considerably lower than hazardous levels. Although there was no statistical significance between TPHs and total length/weight, the *Trichiurus savala* recording maximum concentration during all months and it can be used as indicator of hydrocarbon pollution in this region.

Keywords: Total Petroleum Hydrocarbons. Amba Estuary. Fish. Bioconcentration. Bio-indicator.

Introduction

Although petroleum is a naturally occurring organic compound found deep under the earth's crust and toxic to nearly all life forms. One of the major environmental problems today is oil pollution. It has acknowledged considerable scientific attention resulted from the activities related to the petrochemical industry. Hydrocarbons may originate from a variety of sources: municipal and industrial wastes, runoff, accidental spillage of oil and shipping. The annual worldwide oil-seepage rate (NAS, 2003) is estimated to be between 0.2 and 2.0 Mt (60 and 600 million gallons), with a best estimate of 0.6 Mt (180 million gallons) (Kvenvolden and Cooper 2003). These hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Nilanjana and Preethy 2011). TPHs can enter aquatic systems either as components of uncombusted petroleum and petroleum products such as fuel, lubricating oils, or as products of incomplete combustion in exhaust emissions.

Oil and oily discharges from ships symbolize a noteworthy risk to marine ecosystems (Pikkarainen and Lemponen 2005). For the past few decades the problem of environmental pollution due to the oil and petrochemical industry has received substantial scientific awareness. Oil, which does not disband in water, floats on the sea surface as a thin layer when spilled or otherwise discharged because it is less dense than water and inexplicable in it. This thin surface layer tends to split into droplets by wave action and these droplets become dispersed in the volume of sea water underneath the slick (Veerasingam et al. 2011). Such type of oil slicks set pressures on marine habitats; these will accumulate in the tissues of mollusks, mussels, fish and marine mammals (Bernem et al. 2008). Although a significant portion of petroleum hydrocarbons entering the marine environment is removed by evaporation, a portion gets disseminated in water, accumulated in sediment and transferred to biota (Chouksey et al. 2004). The aquatic organisms would consume hydrocarbons through the food chain for a period of time thus bio-accumulate hydrocarbons in their tissues. These components are carcinogens and engage a variety of biological processes and effectual cell mutagens.

Petroleum pollutants tend to accumulate more in the organism than the environment; therefore fish are largely being used for the assessment of the quality of aquatic environment and it can be used as a bio-indicator to assess the contamination levels in environment (Copat et al. 2012; Wang et al. 2014). These animals are ideal for this study because they are at the top of the food web in the aquatic ecosystem; for this reason they accumulate a large fraction of constant organic pollutants present in the environment (Alkindi et al. 1996). Bioconcentration from water via gills, skin and

ingestion of contaminated food are potential routes for accumulation in fish tissue; where the routes depend mainly on feeding preference, general behaviour and trophic level (Bancroft et al. 2008; Ansari et al. 2012; Barja et al. 2013). The Amba Estuary is known to be breeding ground for many fish species and one of the important artisanal fisheries. The area receives effluent from multiple industries including a major petrochemical industry. Published information on the accumulation of TPHs in the Amba Estuary is of the time when there was no much discharge in the area (Mehta et al. 1994). There is no post industrial record of analysis of TPHs in fishes found in the region. Hence, it was necessary to investigate and find out status of TPHs in water and its accumulation, if any in fishes of the region. The present communication reports the concentration of TPHs in water and in the muscle tissue of 10 species of fish, which are commercially exploited for human consumption on monthly basis.

Study area

Amba River originates from the Western Ghats as a narrow stream and after a meandering course along a length of over 140 km; opens into the Mumbai harbor. It receives an average of 2100 mm rainfall annually, of which 95% occurs during the southwest monsoon period. As the upstream of the river is narrow, the tidal height is more at the upper segments than at the lower estuary. The estuary is shallow with an average depth of 3m during an average tide but 2.6 km wide near its mouth during spring tide (Kumar and Sarma 1991; Paulinose et al. 2004). The tidal pattern of this estuary is semidiurnal type and currents are entirely governed by tides during non-monsoon season. The flushing time of the estuary is around 6-7 tidal cycles during spring tide and around 23-45 tidal cycles during neap tide (Swamy et al. 1980; Dinesh Kumar et al. 1996). The lower estuary is characterized by high and variable suspended load due to the dispersion of bed sediment in the water column as a result of strong velocity of tidal current in the region. The estuary receives significant amount of waste water from a petrochemical complex and other industries that opens into this area (Ram et al. 2009; Pande and Nayak 2013). Fig1 shows the sampling locations of study area in Amba Estuary.

Materials and Methods

Ten fish species (*Coilia dussumieri*, *Arius maculatus*, *Kathala axillaris*, *Otolithoides biauritus*, *Pellona ditchela*, *Pampus argenteus*, *Boleophthalmus sp*, *Johnius glaucus*, *Trichiurus savala* and *Harpadon nehereus*) were sampled along with water sampling during the month of May 2014, September 2014 and December 2014 at 7 stations (Fig. 1). 1L water sample was collected from 1m depth at each station using an amber coloured glass bottle (IOC-UNESCO 1984). The bottle was pre-

cleaned with n-hexane, rinsed with the ambient seawater before sampling. Seawater was extracted twice with 25 ml n-hexane to transfer TPHs in the organic phase and the organic extract was concentrated after drying. Fluorescence of the extract was measured by using fluorescence spectrophotometer (Shimadzu RF-5301PC) at excitation wavelength 310 nm and emission wavelength of 360 nm. As recommended, each sample was tested for quenching by dilution (IOC-UNESCO 1984; Law et al. 1997). After collection fishes were put in ice box and brought to the laboratory where they were kept at -20° C in deep freezer for further analysis of TPHs. The muscle tissue of each species was dissected and kept in aluminum foil and frozen until the analysis. All dissection apparatus were pre-cleaned and precaution was taken to minimize contamination while handling (FAO 1983). The thawed and homogenized tissue (\approx 10-20 g) was saponified using 3 g KOH in 100 ml methanol under reflux for 90 min and filtered through Whatman filter paper No. 1. The filtrate was extracted twice with 25 ml n-hexane and the organic layer was dried with sodium sulfate and evaporated to 1 ml. The separation of hydrocarbons was done by eluting alkane in n-hexane followed by aromatic fraction using Dichloromethane: n-hexane (7:3) and Dichloromethane in an alumina filled column and the intensity of fluorescence of the aromatic fraction was measured using fluorescence spectrophotometer (Shimadzu RF-5301PC) (IOC-UNESCO 1984) at excitation wavelength 310 nm and emission wavelength of 360 nm. All blanks, standards and samples were measured in a Teflon-capped 1-cm silica fluorescence cell under identical instrumental settings and conditions. The methods for estimation of petroleum hydrocarbons in water and fish were calibrated using Saudi Arabian Mixed (SAM) crude oil (Ehrhardt and Burns 1999; Chouksey et al. 2004; Veerasingam et al. 2011). Appropriate blanks were analyzed with each set of samples. Standard reference material (Chrysene) was used for quality assurance/ quality control. To authenticate the result, recovery for spiked samples was estimated and the recovery ranged from 95% to 97%. Precision of the method was 5%. The detection limit (3σ) of the method was $0.2\mu\text{g/l}$ and $0.1\mu\text{g/g}$. All samples were analyzed in triplicates and the averages of the values were reported along with standard deviations. The chemicals used for all analyses were of analytical and HPLC grade (Spectrochem, India and Merck, Germany). Results were expressed as $\mu\text{g/l}$ for water and $\mu\text{g/g}$ (wet weight) for tissue samples. The results were treated using STATISTICA 7.0 software for ANOVA test from which the correlation coefficient between total length, total weight and TPHs of the studied fish species was obtained.

Results and Discussion

The distribution of TPHs in water samples of the Amba Estuary varied between 7.2 to $39.7\mu\text{g/l}$. TPHs concentration for the three months ranges from 15.7 to $36.3\mu\text{g/l}$ in May, from 7.2 to $14.1\mu\text{g/l}$

in September and from 17.4 to 39.7 $\mu\text{g/l}$ in December (Fig. 2). The highest concentration of TPHs 39.7 $\mu\text{g/l}$ was recorded during December in the middle of the estuary (station 4) whereas lowest value 7.2 $\mu\text{g/l}$ was found in September in the lower estuarine region (station 2). The TPHs found in ten commercially important fish species along Amba Estuary varied between 0.4-3.2 $\mu\text{g/g}$ wet wt. The results of the length and weight measurements and concentration of TPHs in the tissues are presented in (Tables 1-3). During the December average TPHs minimum values were in *Boleophthalmus sp* 1.0 $\mu\text{g/g}$ and the maximum in *Trichiurus savala* 3.2 $\mu\text{g/g}$. whereas the result of the samples of May showed wide variability in the average TPHs concentration in tissues. The minimum values were 0.4 $\mu\text{g/g}$ in *Boleophthalmus sp* and maximum of 2.5 $\mu\text{g/g}$ in *Trichiurus savala*, during September. The minimum observed TPHs value was 0.8 $\mu\text{g/g}$ in the tissue of *Boleophthalmus sp* and the maximum in *Trichiurus savala* 3.1 $\mu\text{g/g}$, (Fig. 3) respectively.

High concentrations of TPHs recorded during December in the study region might be due to effluent from the industries including a petrochemical complex situated on the bank of Amba Estuary. Apart from effluent discharge, the estuary also experience exchange of water from adjacent Mumbai harbor due to high tidal influence and strong currents (Dinesh Kumar et al. 1997). Mumbai harbor receives pollutants from several sources and frequent accidental spillage of petroleum products (Soniya et al. 2014). The low concentration levels during the September indicated flushing out of the pollutant due to high incoming fresh waters and river discharges during the September effectively into the sea. In the case month of the May, the evaporation of TPHs is due to high temperatures and low atmospheric pressures at considerable extent.

Values obtained in the present study are much higher than the results reported previously in the same estuary (Chouksey et al. 2004). Elevated TPHs concentration observed in Amba Estuary waters may be attributed to the increased land-based sources, such as industrial effluents originating from nearby industries; marine based sources and increased ship traffic in the Estuary. According to FAO (1982) seawater containing hydrocarbon levels of less than 2.5 $\mu\text{g/l}$ are considered to be the natural background level in a region. The overall TPHs in the Amba Estuarine waters, although higher than the background level; do not indicate much contamination when compared with the values reported for selected coastal areas in India and around the world (Table 4).

While the concentration of most of the species fluctuated in the three months, the average value recorded in the December was on higher side. It was sensible that most fish species acquire a higher concentration of TPHs in the December as compared to the May and September. The values of TPHs in the present study were distinctly higher than the reported values 0.28-1.38 $\mu\text{g/g}$ Shriadah (2001) from Arabian Gulf fish and Veerasingam et al. (2011) Tamilnadu coast 0.52-2.05 $\mu\text{g/g}$, but it's

comparatively lower than the background values. In recent studies like North and Central Arabian Sea 0.47-3.77 $\mu\text{g/g}$ (Avg. 3.67 $\mu\text{g/g}$) (Sen Gupta et al. 1993), off Mumbai coast 1.8 and 10.8 ppm (wet wt) (Chouksey et al. 2004), Arabian Gulf 2.1-7.4 $\mu\text{g/g}$ (Waqar Ashraf and Mian 2010) and Bay of Bengal 1.45-17.56 $\mu\text{g/g}$ (Avg. 13.70 $\mu\text{g/g}$) Ansari et al. (2012) has showed same trend. This may be attributed to high concentration of TPHs in water during same month, thus accumulating in the fishes in proportional to the availability in the surrounding water. This indicated an important fact that TPHs concentration not only varied between the tissues of different fish species but it also varied within the same species depending on the month. The increased hydrocarbon concentration in December is probably due to the active intake during the winter season and as a result, large amounts are stored. Another logical reason for greater concentrations of TPHs in the winter month would be reduced metabolism, due to reduced temperatures. El-Deeb and El-Ebiary (1998) have reported slow movement of fish in winter, particularly demersal species, near the bottom provides a positive condition for the accumulation of hydrocarbons in their muscle tissue. Also changes which take place in the environmental conditions of the habitat contribute to the monthly variations in the hydrocarbons in fish tissue (Shriadah 1999; 2001).

Moore and Dwyer (1974) estimated that fish larvae may be ten times more prone to soluble aromatic hydrocarbons (ranging from 0.1 $\mu\text{g/g}$ to 1.0 $\mu\text{g/g}$) than adult fish. TPHs and total length and total weight of the studied fish species were checked for their correlation coefficient (Table 5). No significant relationship found between TPHs, and length and weight of the fish that indicate no ability for these fish to concentrate hydrocarbons in their tissues. The similar phenomena were observed in marine fish from Arabian Sea, Gulf of Aden and Bay of Bengal (Durrani and Siddiqui 1990; Al- Shwafi 2008; Ansari et al. 2012). In the present study concentration of TPHs was recorded maximum in *Trichiurus savala*. According to the results this species can be used as a good biological indicator for TPHs pollution in water. Marine fishes are found to accumulate TPHs in the range 0.1–10 $\mu\text{g/g}$ (wet wt) whereas; fish from areas contaminated by TPHs can have concentrations of 10–1000 times higher (Hellou et al. 1990). Though the concentration of TPHs in water is noticeably higher than that in the background, there is no substantiation for its elevated concentration in fish of Amba Estuary. Moreover, TPHs residue levels in all fish samples analyzed in this study are considerably lower than hazardous levels (GESAMP 1977, 2007; Hellou et al. 1990).

Conclusion

This study represents distribution assessment of TPHs in water and fish samples from Amba Estuary, West coast of India. The highest TPHs values were obtained in water samples during the month of December (39.7 $\mu\text{g/l}$) and lowest in September (7.2 $\mu\text{g/l}$). High TPHs values that was recorded during

the month of December (3.2µg/g) in *Trichiurus savala* whereas lowest value was recorded in May (0.4µg/g) in *Boleophthalmus sp.* It might be attributed to land and marine-based anthropogenic sources. Although the concentration of TPHs in Amba Estuary waters is markedly higher than that in the background, there is no evidence for its increase in fish found in the region. In the public health view point the values are lower than the hazardous level. At present, as the Amba Estuary area is in rapid activities of a petrochemical complex, mining, other large scale industries, ship transportation, fishing harbours and industrial effluent from factories there is a need for further detailed assessment of TPHs and continuous pollution monitoring studies should be conducted to improve the aquatic environment.

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Table 1 Range and mean values of TPHs ($\mu\text{g/g}$; wet wt) in different fish species of Amba Estuary collected during May, 2014 (average atmospheric temperature 32.2°C)

Fish species	n	Length (cm)	Weight (g)	TPHs range	TPHs mean
<i>Coilia dussumieri</i>	15	12.5-18.6	15.6-26.4	0.2-1.0	0.5
<i>Arius maculatus</i>	5	17.0-30.5	36.3-76.4	0.6-1.3	0.9
<i>Kathala axillaris</i>	3	12.0-18.8	20.0-35.5	0.5-2.8	2.2
<i>Otolithoides biauritus</i>	6	26.0-29.5	35.5-88.6	0.3-1.4	0.8
<i>Pellona ditchela</i>	3	12.0-14.8	18.9-24.6	0.6-1.8	1.2
<i>Pampus argenteus</i>	3	14.0-16.5	40.8-61.8	0.9-2.2	1.6
<i>Boleophthalmus</i>	5	12.4-15.4	18.4-25.4	0.2-0.6	0.4
<i>Johnius glaucus</i>	8	10.4-14.2	16.6-23.5	0.2-0.8	0.5
<i>Trichiurus savala</i>	5	45.5-52.8	82.4-110.5	1.5-2.9	2.5
<i>Harpadon nehereus</i>	17	10.5-15.8	14.8-21.4	0.6-1.5	0.9

Table 2 Range and mean values of TPHs ($\mu\text{g/g}$; wet wt) in different fish species of Amba Estuary collected during September, 2014 (average atmospheric temperature 29.7°C)

Fish species	n	Length (cm)	Weight (g)	TPHs range	TPHs mean
<i>Coilia dussumieri</i>	19	13.8-19.4	16.4-28.5	1.1-2.7	1.7
<i>Arius maculatus</i>	7	18.4- 28.6	40.5-68.4	1.4-2.1	1.7
<i>Kathala axillaris</i>	4	16.0-19.4	26.8-49.5	0.8-2.7	2.4
<i>Otolithoides biauritus</i>	8	24.5-30.4	45.0-90.0	0.8-1.4	1.1
<i>Pellona ditchela</i>	4	14.0-16.0	25.4-34.8	1.0-1.8	1.4
<i>Pampus argenteus</i>	5	14.5-18.0	50.5-85.4	1.7-2.9	2.1
<i>Boleophthalmus</i>	7	13.2-16.7	18.0-27.0	0.5-1.2	0.8
<i>Johnius glaucus</i>	10	17.0-19.0	28.3-70.6	0.8-1.6	1.1
<i>Trichiurus savala</i>	7	55.0-56.8	110-118.6	2.2-3.7	3.1
<i>Harpadon nehereus</i>	23	9.7-16.9	10.6-21.0	0.7-1.4	1.0

Table 3 Range and mean values of TPHs ($\mu\text{g/g}$; wet wt) in different fish species of Amba Estuary collected during December, 2014 (average atmospheric temperature 26.6°C)

Fish species	n	Length (cm)	Weight (g)	TPHs range	TPHs mean
<i>Coilia dussumieri</i>	17	10.8-17.5	9.2-25.4	1.4-2.5	1.8
<i>Arius maculatus</i>	10	20.5-30.8	42.6-78.4	1.2-1.9	1.6
<i>Kathala axillaris</i>	3	15.6-18.4	23.7-30.4	2.5-2.9	2.6
<i>Otolithoides biauritus</i>	5	22.4-27.6	40.8-96.8	1.0-1.8	1.3
<i>Pellona ditchela</i>	3	15.7-16.2	28.7-40.2	1.2-1.9	1.6
<i>Pampus argenteus</i>	4	14.4-20.8	48.5-100.5	1.7-2.5	2.2
<i>Boleophthalmus</i>	6	13.0- 17.2	15.6-25.4	0.7-1.5	1.0
<i>Johnius glaucus</i>	5	9.0-16.5	16.3-65.4	1.2-1.8	1.4
<i>Trichiurus savala</i>	9	47.6-54.2	90.8-108.4	2.6-3.9	3.2
<i>Harpadon nehereus</i>	20	11.0-16.0	14.0-19.4	0.8-1.8	1.3

Table 4 Comparison of TPHs concentration ($\mu\text{g/l}$) in water samples of Amba Estuary with those from selected marine areas.

Study area	TPHs ($\mu\text{g/l}$)	Source
Amba Estuary, India	7.2-39.7	Present study
Orissa coast, India	2.4-3.8	Ansari et al. (2012)
Tamilnadu coast, India	2.28-14.02	Veerasingam et al. (2011)
Andhra Pradesh coast, India	0.81-45.72	Natarajan et al. (2006)
Tamilnadu coast, India	0.2-5.8	Natarajan et al. (2006)
Amba Estuary, India	1.1-6.0	Chouksey et al. (2004)
Bassein–Mumbai, India	2.9-39.2	Chouksey et al. (2004)
Thane Creek, India	7.5-39.2	Chouksey et al. (2004)
Ulhas Estuary, India	3.3-21.3	Chouksey et al. (2004)
Chennai coast, India	11-139	Selvaraj et al. (1999)
Arabian sea, India	1.6-11.1	Sen Gupta et al. (1993)
Southern Bay of Bengal, India	34-165	Topgi et al. (1982)
Northern Indian Ocean, India	0.6-26.5	Sen Gupta et al. (1980)
East coast of Peninsula, Malaysia	1.4-21.8	Tahir et al. (1997)
Winyah Bay, USA	0.23-25	Bidleman et al. (1990)
Gulf of Lyons	18-23	Marchand et al. (1988)
Qatar	1.2-428	El-Samra et al. (1986)
Saudi Arabia	4.3-546	El-Samra et al. (1986)
Kuwait	2.1-3.6	El-Samra et al. (1986)
English channel and North sea	2.1-43.7	Marchand and Caprais (1985)
Shatt Al-Arab and NW Arabian Gulf	2.7-86.7	DouAbul (1984)
Southern Baltic Sea	2.0-130	Law and Andrulawic (1983)
Tamar Estuary, England	1-50	Readman et al. (1982)
U.K Marine waters, U.K	1.1-74	Law (1981)

Table 5 Correlation coefficients (r) between TPHs, total length and total weight of the ten fish species (p<0.05).

Fish species	TPHs &Total length			TPHs &Total weight	
	n	r	p	r	p
<i>Coilia dussumieri</i>	51	0.06	0.68	0.23	0.10
<i>Arius maculatus</i>	22	0.26	0.25	0.24	0.28
<i>Kathala axillaris</i>	10	0.44	0.20	0.26	0.47
<i>Otolithoides biauritus</i>	19	0.48	0.23	0.14	0.73
<i>Pellona ditchela</i>	10	0.30	0.56	0.35	0.50
<i>Pampus argenteus</i>	12	0.16	0.76	0.11	0.84
<i>Boleophthalmus</i>	18	0.11	0.84	0.13	0.81
<i>Johnius glaucus</i>	23	0.13	0.81	0.04	0.94
<i>Trichiurus savala</i>	21	0.20	0.70	0.20	0.70
<i>Harpadon nehereus</i>	60	0.19	0.82	0.33	0.53

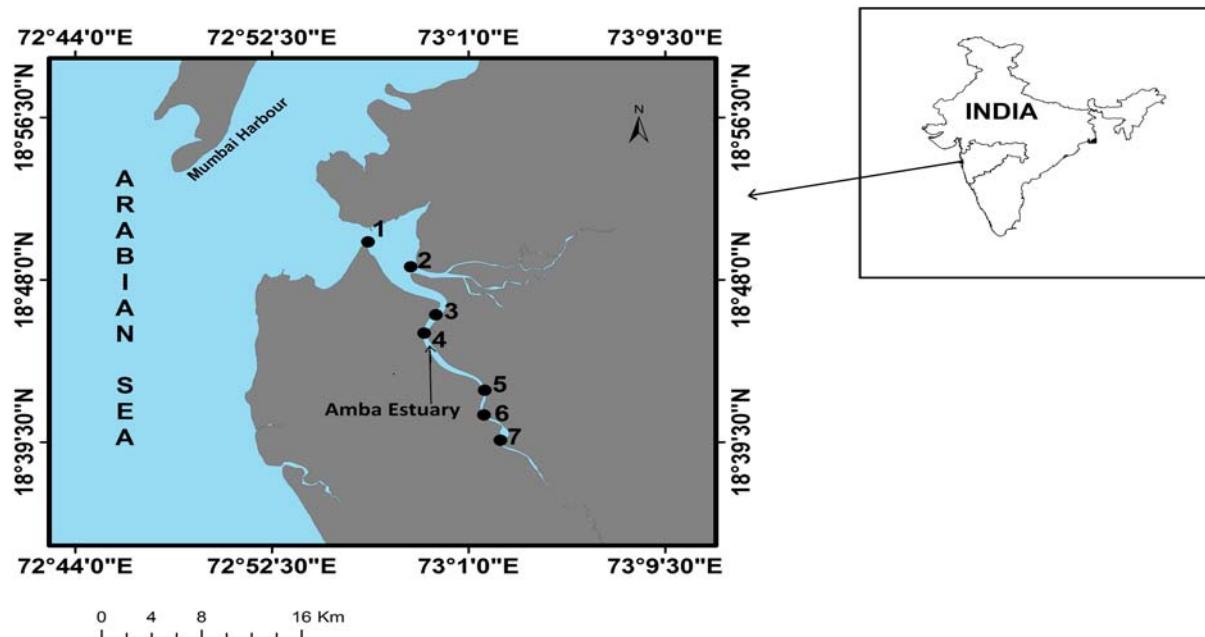


Fig. 1 Map showing the sampling locations in Amba Estuary.

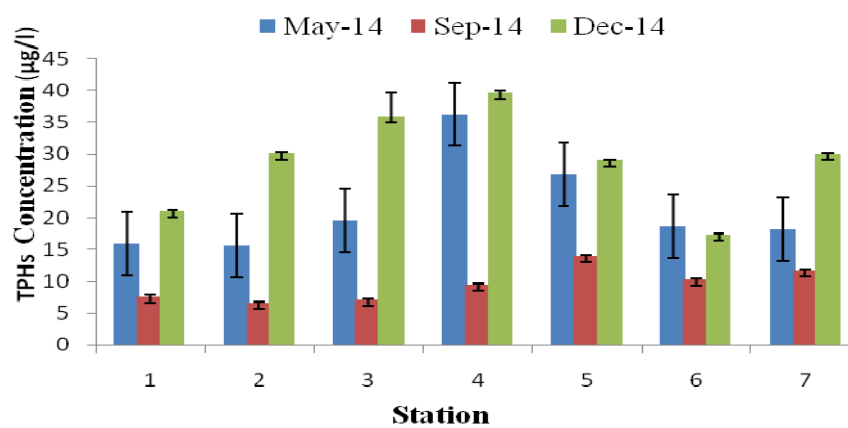


Fig. 2 Monthly variations of TPHs in water (µg/l) in the Amba Estuary (mean ±sd).

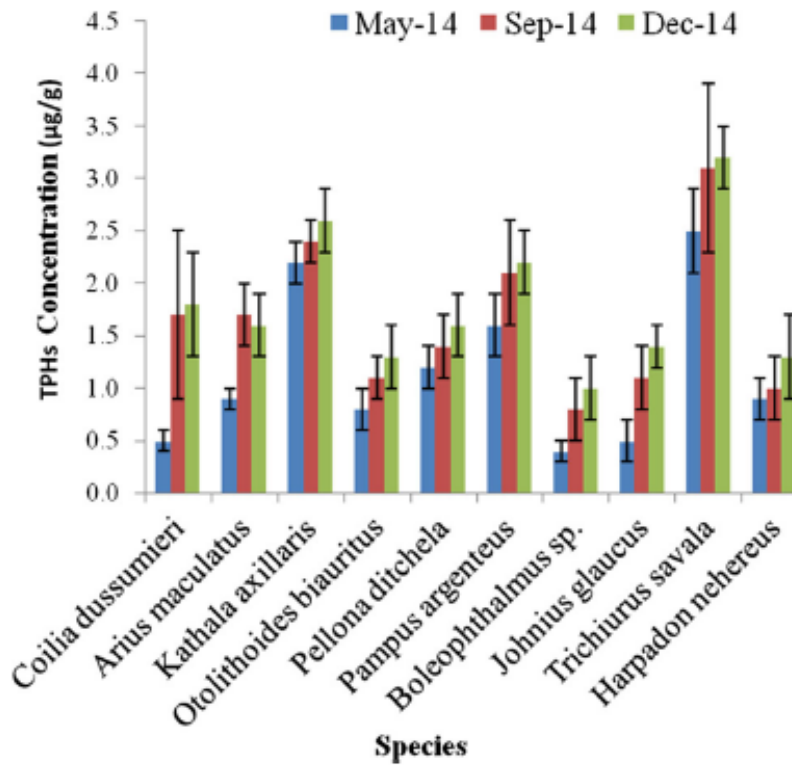


Fig. 3 Monthly variations of TPHs in fish species ($\mu\text{g/g}$; wet wt) in the Amba Estuary (mean $\pm\text{sd}$)