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Distribution of total petroleum hydrocarbons in water, sediment and its impact on six commercially important fishes of Kundalika estuary, west coast of India

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Kundalika estuary, which opens into the Arabian Sea along the west coast of India, receives effluents from industries situated on its bank. The Total Petroleum Hydrocarbons (TPHs) were studied using fluorescence spectrophotometer in water, sediments and fishes along the Kundalika estuary. The values of TPHs varied in water (2.0-19.1µgL^{-1}) and surface sediments (2.1-16.8µgg^{-1}, dry wt) with maximum concentrations recorded in the upstream region. The concentration of TPHs (dry wt) in the sediment cores collected at mouth, middle and upper parts of the estuary ranged 1.4–4.4 µgg^{-1}, 4.5–27.5 µgg^{-1} and 6.9–29.1µgg^{-1}, respectively. Sediments with higher mud (silt+clay) content showed higher TPHs enrichment due to the larger specific surface area and showed significant positive correlation between mud and TPHs concentrations. The accumulated TPHs values of fin fishes (0.5-2.2µgg^{-1}; wet wt) were lower than hazardous levels, although there was no statistical significance between TPHs and total length and weight of fish.

[Keywords: Total petroleum hydrocarbons (TPHs); Kundalika estuary; Sediments; Accumulation; Fish]

Introduction

The Indian coast is most vulnerable to contamination of petroleum hydrocarbons (TPHs) due to oil spill which affects the marine ecosystem[1-2]. Estuarine regions are very prone to contamination by TPHs originated from domestic and industrial effluents, boating and shipping activities, and marine operations including tanker traffic and oil production[3]. Although a significant portion of petroleum hydrocarbons entering the marine environment is removed by evaporation, a portion of it gets disseminated in water, accumulates in sediment and is transferred to biota[4]. TPHs have received special

particulate matter and settle to the bottom sediment[5], which ultimately acts as a reservoir for hydrophobic contaminations. Predominance of hydrocarbons, such as normal alkanes (saturated, n-alkanes), unsaturated hydrocarbons, non-symmetric cyclic hydrocarbons (terpanes) and polycyclic aromatic hydrocarbons (PAHs) in the environment indicate petroleum pollution[6-7]. Once the petroleum compounds are released in water through wastewater, spills or leakage, certain hydrocarbons evaporate and others float on surface making a thin film. These hydrocarbon components belong to the family of carcinogens and neurotoxic organic pollutants[8], which affect a variety of biological processes and entities[9]. Oil comes in direct contact with fish which contaminates their skin or gills, whereas toxic and volatile components of oil present in the water column are absorbed by their eggs and larvae. Besides, fishes accumulate toxins in their tissues[10] through food ingestion, which is magnified in the food chain[11]. Human population, which largely depends on fishes for food, may get affected due to presence of carcinogenic organic matter[12]. Therefore, fish has been used widely as a bio-indicator to assess the contamination levels in the environment[13].

Kundalika estuary, which opens into the Arabian Sea near Revdanda along the west coast of India, receives effluents from many large and medium scale industries situated on its bank[14]. The present study aims to understand the current environmental status of water, sediments and fishes of Kundalika estuary with respect to TPHs contamination. Though there is no direct source of oil pollution which can enhance TPHs concentration in the estuary, industries of multiple products including petrochemicals situated in Dhatav industrial region discharge their effluents in the estuary. The study of sediment cores is an excellent tool for establishing the effects of anthropogenic and

natural processes on depositional environments[15]. Earlier studies have reported deteriorated condition of Kundalika estuary[14, 16], but there is no information, which can explain the status of TPHs in the estuary. Hence, this study would provide useful evidence about anthropogenic impact in water, sediments and biota of Kundalika estuary.

The prime objectives of this study are: (i) To determine the spatial distribution of TPHs in water, sediments along the Kundalika estuary. (ii) To evaluate the vertical distribution of texture and TPHs contamination in the sediments of Kundalika estuary, and (iii) To estimate the level of TPHs in six commercially important fish species along the Kundalika estuary.

Materials and Methods

Study area description

The Kundalika river which is the mainline of drainage for central Raigad district (Fig. 1) rises in the Sahyadris near the Garbolot Pass where it meets the Arabian Sea at Revdanda after a westerly course of about 65 km. After the construction of Dam on Kundalika river, the water is used on a large scale for paddy fields and growing vegetables. A small bund of 1.5 m high built upstream of Roha meets the freshwater requirements of the town and the Industrial estate. Several small islets covered with thick mangroves exist in the mid-estuarine zone. The Revdanda Port is an important fish landing centre near the mouth of estuary and provides shelter for many fishing boats. The wastewater from Dhatav and Roha industrial area is treated and released in the mid estuarine zone. The shallow estuary experiences considerable tides with the spring range of 3.8 m in the mouth decreasing to 1.3 m at 35 km inland and from 0.7 to 0.5 m during neap[14]. The spring tidal range decreases significantly from lower to middle estuary[17].

Sampling and chemical analysis

Water and surface sediment samples were collected from 10 locations during March 2014 along the Kundalika estuary (Fig. l). The water sample 1 L was collected from 1 m depth at each station using an amber coloured glass bottle[18]. The bottle was pre-cleaned with n-hexane, rinsed with the ambient seawater before sampling. Surface sediment samples at each station were collected using a van-Veen grab (0.04 m^2). Three sediment cores were collected during the same period from the intertidal mudflat area using a hand-operated sediment corer of 1.5 m length (ID 5.5 and OD 6.5 cm) each from the mouth (K1), middle (K2) and upper (K3) regions of the estuary (Fig. 1). Collected core samples were carefully sectioned at 2 cm interval from top to bottom. The surface sediments as well as the core sections were transferred in the aluminum foil and were kept frozen at –20 ˚C until analysis. Six commercially important fishes were collected along the Kundalika estuary during March 2014. After collection, fishes were

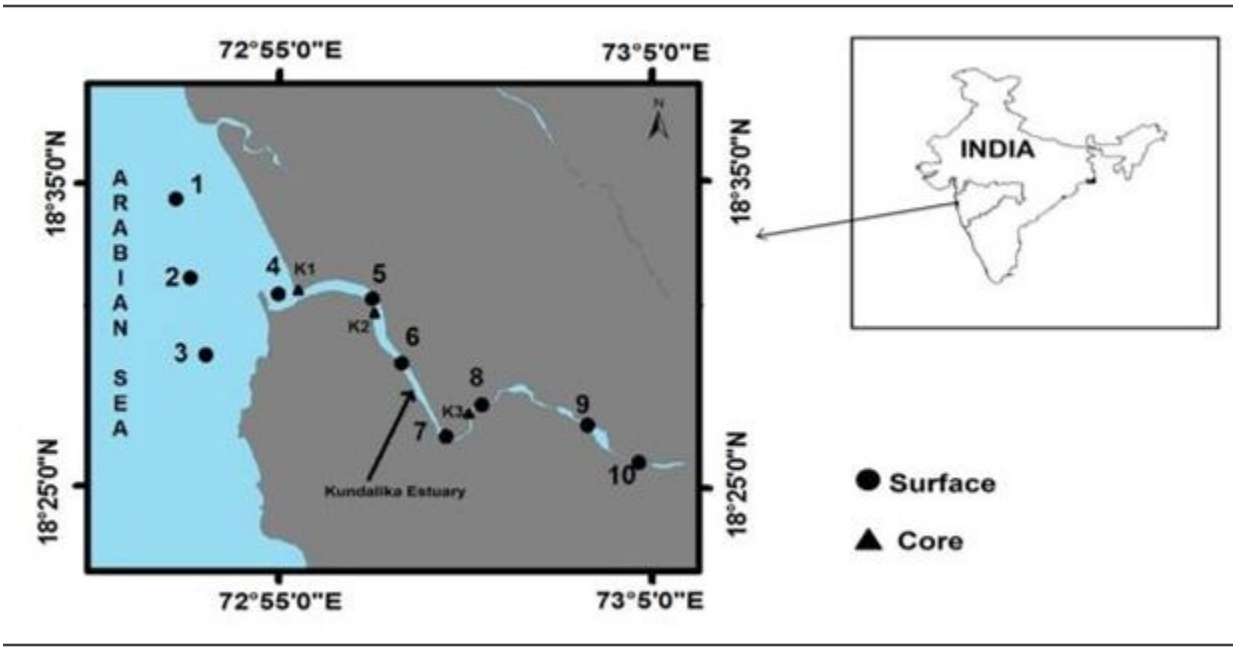

Fig. 1 — Map showing study area and sampling locations.

placed in ice box and brought to the laboratory where they were kept at -20 ºC in deep freezer for further analysis of TPHs. The muscle tissue of each species was dissected, kept in aluminum foil and was frozen until analysis. All dissection apparatus were pre-cleaned and precaution was taken to minimize contamination while handling. 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[18-19]. The texture (sand, silt and clay) of collected sediment core samples was estimated using wet-sieving method[20]. For estimation of TPHs, the IOC-UNESCO[21] method was used for the sediment and fish samples. Before analysis, the sediment samples were freeze-dried and fish tissue was thawed. Then, the sediment and homogenized fish samples were saponified using KOH (3 g)–methanol (100 ml) mixture and were filtered through Whatman filter paper number 1. The filtrate was extracted with n-hexane and the organic layer was dried over sodium sulfate and evaporated to a small volume. 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) at excitation wavelength 310 nm and emission wavelength of 360 nm[21]. Each sample was tested for quenching by dilution as recommended[18]. 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 TPHs in water, sediment and fish were calibrated using Saudi Arabian Mixed (SAM) crude oil[4,19] and appropriate blanks were analyzed with each set of samples. Standard reference material (Chrysene) was used for quality assurance/quality control (QA/QC). To authenticate the result, recovery for spiked samples was processed, which ranged from 95% to 97%, whereas precision agreed within ±5%. The detection limit (3σ) of the method was 0.2 μgL^{-1} and 0.1 μgg^{-1}. All experiments were conducted in triplicate and averages of the values were reported. The chemicals used for all analyses were of analytical grade (AR) 99% purity of KOH and Na_2SO_4 (Merck, Germany) and 99% purity of n-hexane, dichloromethane and methanol (HPLC grade, Spectrochem, India).

Statistical analysis

Pearson's correlation analyses were performed between TPHs in water, surface sediments and distance of the study area, and between TPHs in sediment cores, sand and mud (silt+clay) content. ANOVA test was applied from which the correlation coefficients between TPHs content in fish, total length and weight of the fish were obtained. All statistical calculations were performed using STATISTICA 7.0 (Statsoft, US) software.

Results and Discussion

Spatial distribution of TPHs in water and surface sediments of the study area

Concentration of TPHs in water samples along the estuary varied over a wide range (2.0 to 19.1 μgL^{-1}) (Fig. 2a) with the highest concentration (19.1 μgL^{-1}) of TPHs at station 9 (upper zone). A systematic decrease was observed in the concentration of TPHs from upper estuarine zone to offshore area which showed a negative correlation ($r= -0.93$; $p< 0.001$) with distance (Fig. 2b). According to FAO[22], seawater containing hydrocarbon levels of less than 2.5 µgL-1 are considered as the natural back ground levels in a region. The TPHs concentrations in the Kundalika estuarine waters were considerably higher (station 4 to station 10) than the background levels recorded from station 1 to 3. Comparatively, these values were lower than Amba estuary, India[19] (39.7μgL^{-1}); Ulhas estuary, India[4] (3.3–21.3 μgL^{-1}); and East coast of Peninsula, Malaysia[23] (1.4–21.8 μgL^{-1}). Concentration of TPHs in surface sediments varied in the range from 2.1 to 16.8 μgg^{-1} (Fig. 2a) and the highest concentration of 16.8 μgg^{-1} was obtained in the upper zone (station 9) with negative correlation ($r= -0.91$; $p< 0.001$) of TPHs vs distance (Fig. 2b). This indicated a hydrocarbon source in upstream zone, which might be mainly due to sewage, industrial effluents and other land, based activities. Whereas, the lowest concentration of TPHs was recorded at station 1, which is about 33 km away from station 9 indicating that the TPHs released to the aquatic environment, is adsorbed on particulate matter and settles in the sediment near the source[24]. The present

values are compared with those reported for selected coastal areas including the water and sediment of the Indian coast (Table. 1).

Vertical distribution of texture and TPHs in sediment cores of the study area

The distribution of sand and mud studied in three sediment cores from the Kundalika estuary is depicted in Figure 3. The core K1, collected from the lower estuary was divided into three sections, upper (0–20 cm), middle (22–40 cm), and bottom (42–58 cm). In the upper section, sand and mud were nearly constant. While, in the middle section, mud percentage decreased and sand percentage increased with some variations, and it was vice versa in the bottom section. The core K2, collected from the middle estuary was divided into three sections, namely, upper (0–24 cm), middle (26–38 cm) and bottom (40–56 cm). In the upper section, mud percentage decreased and sand percentage increased. In the middle section, mud percentage inclined while sand percentage declined. In the bottom section, mud percentage decreased while sand percentage showed elevation with some variations. Similarly, core K3, collected from upper estuary, and was divided into three sections, namely, upper (0–30 cm), middle (32–44 cm) and bottom (44–60 cm). In the upper section, the percentage of mud and sand was inversely proportional to each other. However, in bottom and middle section, mud and sand remained constant with some variations. From the observed percentage of sand and mud in three sediment cores, it was observed that the percentage of sand was minimum at the upper estuary (K3) and maximum at the mouth of the estuary (K1), which indicates increasing energy conditions from upper to mouth of the estuary[31]. The relatively high tidal energy in lower estuary results in higher hydrodynamics near the mouth which facilitates the

Table 1 — Comparison of TPHs in water and sediment of the Kundalika estuary with those of selected marine areas.

Study area	TPHs(μgL^{-1})	TPHs (μgg^{-1})	Source
Kundalika Estuary, India	2.0-19.1	2.1-16.8	Present study
Amba Estuary, India	7.2-39.7	-	Rao et al. 2016
Mandovi estuary, India	-	5.4–12.34	Veerasingam et al. 2015
Todos os Santos Bay, Brazil	-	0.22 – 40101	Silva et al. 2014
Visakhapatnam coast, India	-	0.34 – 19.70	Venkatachalapathy et al. 2013
Orissa coast, India	2.4-3.8	-	Ansari et al. 2012
Tamilnadu coast, India	2.28-14.02	-	Veerasingam et al. 2011
Guanabara Bay, Brazil	-	77 – 7751	Da Silva et al. 2007
Gulf of Fos, France	-	7.8 – 180	Mille et al. 2007
Mumbai coast, India	2.9-39.2	2.0-42.8	Chouksey et al. 2004
East coast of peninsula, Malaysia	1.4-21.8	-	Tahir et al. 1997

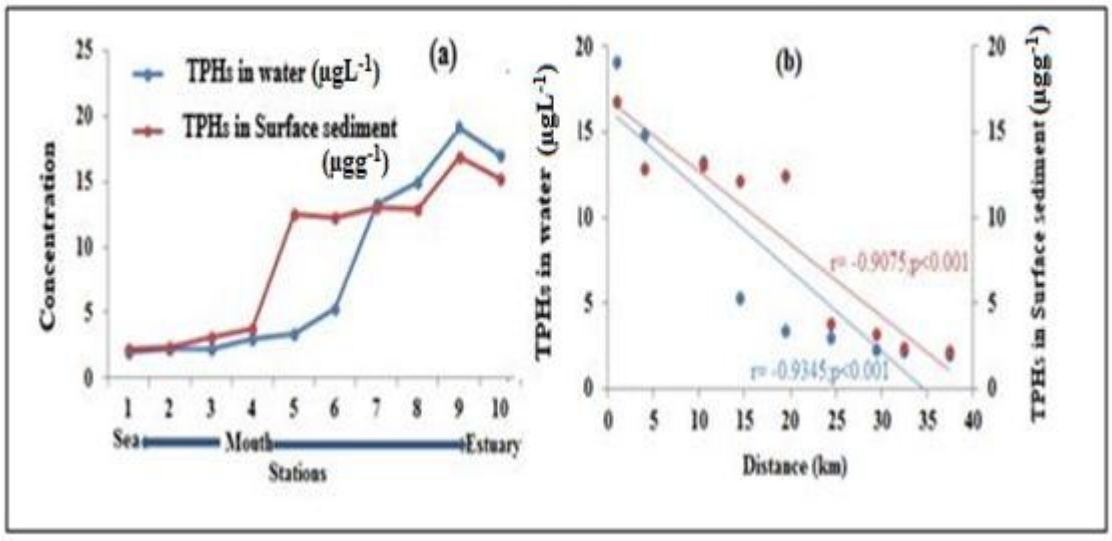

Fig. 2 — (a) Spatial distribution of TPHs in water samples (µg/l) and surface sediments (µg/g; dry wt) along the Kundalika estuary. (b) Correlation between TPHs content in water Vs. distance and surface sediments Vs. distance along the Kundalika estuary.

deposition of coarser sediments, as finer sediments are more mobile and are carried to middle and upper part of the estuary where tidal currents are weaker resulting in the deposition of mud[32,16]. Sudden decrease in mud percentage and an increase in sand percentage in middle section of core K1 indicate a period of relatively higher energy conditions predominant of the past in the study area, or sand is transported as bed load during strong flood and ebb currents during spring tides and due to high river discharge[33]. One or more of these factors may be responsible for higher sand content in the middle section. Moreover, factors such as land based activities like sand mining, agricultural, anthropogenic activities, might be contributing to increased sand content[34]. Upper and middle section of core K2 showed increase in mud percentage and decrease in sand percentage. Sand content decreased whereas mud content increased in the upper section of the core K3. This indicates that other factors such as changes in sediment source, biological factors and physiographical parameters in addition to hydrodynamics may also affect the grain size distribution of tidal wetlands[35-36].

TPHs concentration in sediment cores K1, K2 and K3 varied in the range of 1.4–4.5 μgg^{-1}, 4.5–27.5 μgg^{-1}and 6.9–29.1 μgg^{-1} respectively, showing an increasing trend towards surface (Fig. 3). Concentration of TPHs (1.9-2.1 μgg^{-1}) in the bottom section (52-58 cm) of core K1 which is about 20.5 km away from the industrial discharge point was in the range recorded from the surface sediment of open shore region and may be considered as background concentration. However, concentration of TPHs was 4.5 μgg^{-1} in the bottom (56 cm) section of core K2 and 6.9 μgg-1 in the bottom (60 cm) section of core K3. These concentrations increased towards surface with intermediate maxima, indicating continuous source of TPHs in the upper estuary. However, a definite decrease of TPHs in 10 cm surficial sections of core K3 may be attributed to the enhanced enforcement to reduce discharge of pollutants in the aquatic environment in recent past. The above results reveal that most of the TPHs released through anthropogenic wastes get accumulated near the source and redistributed with resuspension of the sediment due to local currents. TPHs showed significant positive correlation with mud and negative correlation

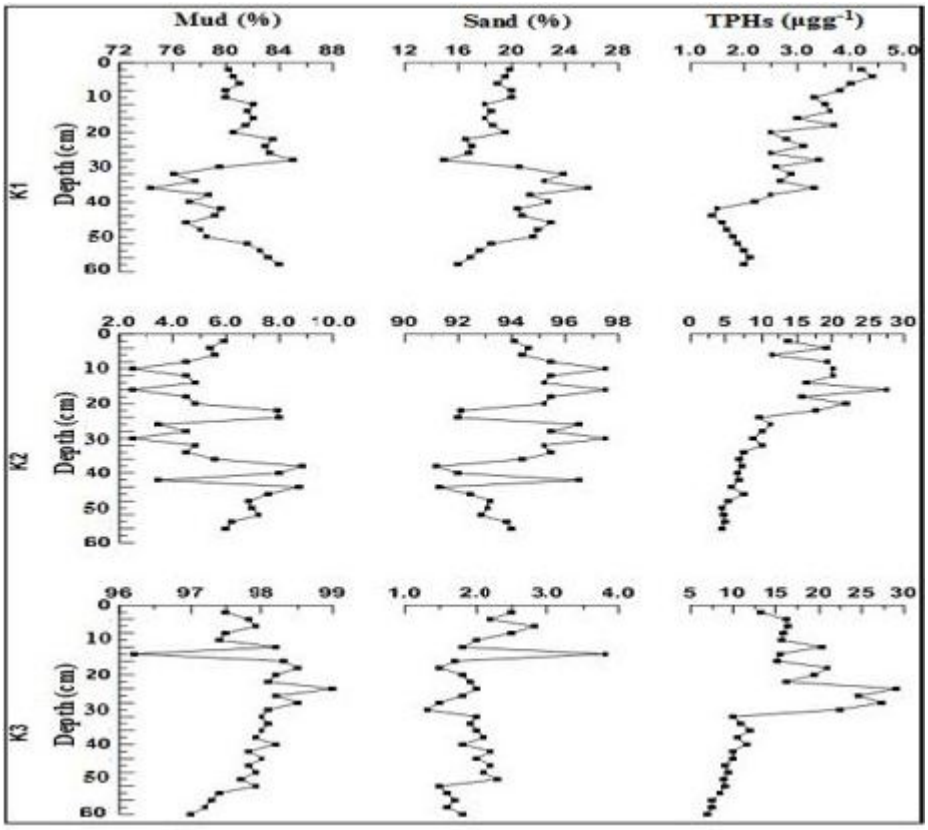

Fig. 3 — Depth-wise distribution of TPHs (μg/g; dry wt), sand (%) and mud (%) content in sediment cores of the Kundalika estuary.

with sand in K2 and K3 cores, while no significant correlation was observed in K1 core between mud and sand (Fig. 4) which inferred that sediments with more mud (silt + clay) contents had higher TPHs concentrations than sandy sediments. The reason may be attributed to the capacity of mud to adsorb and trap more TPHs due to the large specific surface area as compared to poor ability of sand[5]. Also the sediments having higher percentage of clay and silt have higher binding sites available for the adsorption of the pollutants due to the anthropogenic addition and alteration as a part of diagenesis, organic carbon may not be used as normalizing element[37]. The concentration of TPHs in the upper section 0-24 cm and 0-30 cm of sediment cores (K2 and K3) was significantly higher than the background values (TPHs values at the bottom of sediment core K1). The overall TPHs in the Kundalika estuarine sediments, although higher than the recently reported values (5.4–12.34 μgg^{-1}) from Mandovi estuary[5] do not indicate much contamination when compared with the values reported from the Qua Iboe estuary, Nigeria[38]. Thane creek, west coast of India[4].

TPHs in fish species along the study area

The TPHs analyzed in six commercially important fish species along Kundalika Estuary varied between 0.5 and 2.2 μgg^{-1} wet wt (Table 1). The average TPHs values were the lowest in *Thryssa malabarica* (0.5 μgg^{-1}) and the highest in *Coilia dussumeiri* (2.2 μgg^{-1}). In recent published information, TPHs values were reported in Arabian Gulf [39] (2.1-7.4 μgg^{-1}), Bay of Bengal[27] (1.45–17.56 μgg^{-1}; Avg. 13.70 μgg^{-1}), Amba Estuary[19] (0.4-3.2 μgg^{-1}) and North-West Arabian Gulf [40] (2.45-7.65 μgg^{-1}). It is noted in the present study that the TPHs concentrations recorded in fish tissues were below the higher values reported in other regions. TPHs, total length and total weight of the studied fish species were checked for their correlation coefficient through ANOVA. No significant correlation (Table 2)

Table 2 — Range, average values (±sd) of TPHs (μgg^{-1}; wet wt.), the correlation coefficients (r) between TPHs, total length and total weight of the six fish species ($p<0.05$).

Fish species	n	Length (cm)	Weight (g)	TPHs range (Av)	Correlation of TPHs & total length		Correlation of TPHs & total weight	
					r	p	r	p
Coilia dussumieri	10	12.5-18.6	4.2-14.5	2.2±0.4	0.0444	0.903	-0.3682	0.295
Johnius aneus	5	13.5-15.2	5.0-10.3	0.9±0.3	0.5255	0.226	0.3695	0.415
Harpadon nehereus	7	17.0-23.5	9.4-54.6	0.7±0.2	0.4894	0.218	-0.0937	0.825
Opisthopterus tardoore	6	11.3-15.5	3.8-23.0	0.8±0.3	0.5929	0.215	0.6165	0.192
Liza parsia	5	12.0-13.3	9.0-16.8	0.6±0.2	0.2290	0.719	0.2137	0.730
Thryssa malabarica	4	13.2-16 .7	8.1-13.4	0.5±0.2	-0.120	0.985	0.0231	0.971

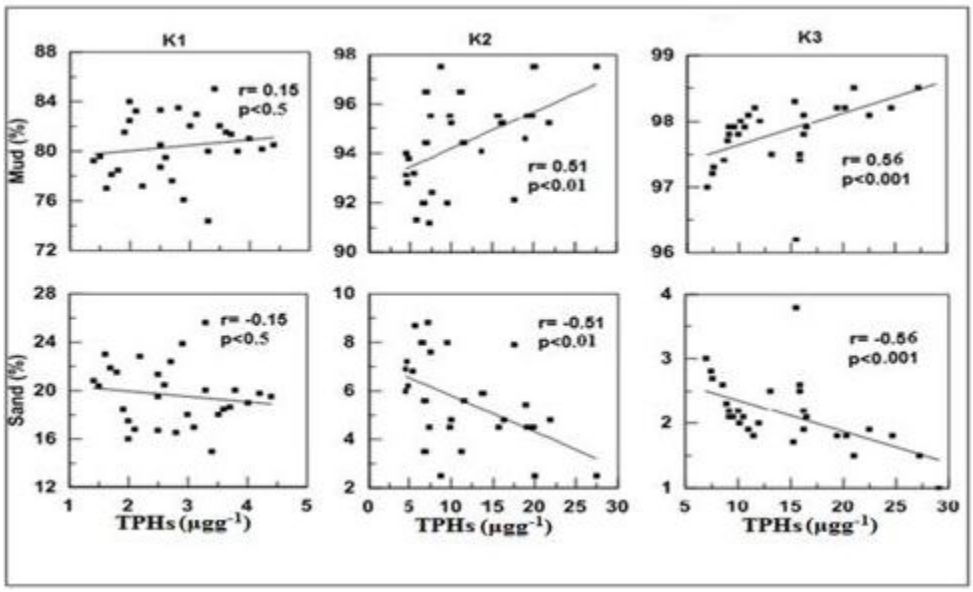

Fig. 4 — Correlations between TPHs, sand and mud content in sediment cores of the Kundalika estuary.

was found between TPHs and length and weight of the fish which indicated that these fishes might not be able to accumulate hydrocarbons in their tissues. The similar phenomena were observed in marine fish from Bay of Bengal[27] and Amba estuary[19]. In the present study, maximum concentration of TPHs (2.2 μgg^{-1}) was recorded in *Coilia dussumeiri*. According to the results, this species can be used as a good biological indicator for TPHs pollution in water[19]. Marine fishes are found to accumulate TPHs in the range of 0.1–10 μgg^{-1} (wet wt) [4], whereas fish from areas contaminated by TPHs can have concentrations of 10–1000 times higher[40]. Though the concentration of TPHs in water was noticeably higher than that of the background level in upper estuarine zone, there was no substantiation for its elevated concentration in fish of Kundalika estuary. Moreover, TPHs residue levels in all fish samples analyzed in this study were considerably lower than hazardous levels[41-42].

Conclusion

A clear picture of TPHs content along the studied Kundalika estuary was obtained where concentration in water and surface sediments of upper estuary was higher than the middle and lower zones due to sewage, industrial effluent and land-based wastewater discharges. Similarly, the TPHs values were higher than the background level (bottom section of sediment core K1) in upper sections of sediment cores (K2 and K3); which indicates anthropogenic discharge of TPHs and its build up in the sediment over the years. The highest concentration of TPHs was observed in fish *Coilia dussumeiri* compared to other fish species. Overall, TPHs levels in fish species were within the permissible limit, although it might accumulate within the higher organisms through biomagnification. This study may be useful tool for long-term monitoring in future to curtail the pollution load and restore ecological conditions.

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