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Influence of Salinity on PAH Uptake from Water Soluble Fraction of Crude Oil in *Tilapia mossambica*

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Abstract Accidents during marine transport and offshore production facilities often are responsible for oil spills in the open sea. In few cases, these oil slicks drift towards the shore and further into the estuaries, which serve as an important spawning and nursing grounds for many fish species. This study examined the role of salinity in the uptake and accumulation of toxic PAH from crude oil in select somatic and reproductive organs of *Tilapia mossambica*. Our results showed significantly (ANOVA, $p < 0.01$) lower PAH solubility in higher salinity waters and its uptake by fish. The differences were largest with the low molecular weight (LMW) two (naphthalenes) and three (phenanthrene) ring compounds as compared with higher molecular weight (HMW) compounds such as pyrene (four ringed).

Keywords Oil spills · Toxic PAH · Salinity · *Tilapia mossambica*

Oil spills are associated with marine transport and offshore production facilities; they often take place in the open sea. In some cases, the oil slicks drift towards the shore and into estuaries. These near-shore habitats serve as an important spawning and nursing grounds for many fish species. Since the physico-chemical characteristics of these coastal areas are different from those that persist in open sea, environmental factors such as temperature and salinity could significantly influence the natural fate and distribution of the oil. Salinity of water may influence the solubility of toxic hydrocarbons that are present in the crude oil and the accumulation of these hydrocarbons by aquatic organisms that dwell in different salinity mediums.

Crude oil is a mixture of several fractions of hydrocarbons, with varying solubilities, which depend on their octanol–water partition coefficients (Kow). Amongst them, polycyclic aromatic hydrocarbons (PAHs) are ranked as relatively soluble; more soluble than alkanes that comprise an equal number of carbon atoms (McAuliffe 1987). Since many PAHs rank among the most toxic components of crude oil (Ramchandran et al. 2006), PAH solubility is an important feature of oil spills. McAuliffe (1987) found solubilities of toluene in 3.5% and 20% NaCl to be 70% and 16% of that in distilled water. Sutton and Calder (1975); reviewed in McAuliffe (1987) found that the mean reduction in solubility for 12 aromatic hydrocarbons was 68 ± 4.4% less at 25°C in seawater relative to fresh water. Therefore, the concentrations of aromatic hydrocarbons after an oil spill would increase in the water column of low salinity coastal water or estuaries and would have a greater adverse impact on aquatic organisms than that can be expected in the case for spills in open ocean waters. Hence, the results of bioassays conducted with marine test organisms in salt water would not be useful to predict effects on fresh or brackish water organisms.

Fish can accumulate soluble petroleum hydrocarbons very rapidly (Collier et al. 1995) and the fish that are placed in water contaminated with crude oil will take up dissolved hydrocarbons until a steady state is established between the fish and water.

The lighter PAHs volatilize and solubilize easily, the heavier and more toxic fractions are less soluble. The hydrophobic nature of the more toxic fractions enables them to partition directly from crude oil to lipid rich tissues

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Table 1 PAH composition of WSF of crude oil

PAH	Water soluble fraction (WSF) concentration in ppm			Method detection limits (μg/L)
	WSF (0‰)	WSF (15‰)	WSF (30‰)	
Naphthalene	19.9	14.9	11.2	1.6
Phenanthrene	0.8	0.6	0.5	5.4
Fluorene	1.4	1.26	1.18	1.9
Pyrene	0.5	0.42	0.36	1.9
Chrysene	0.46	0.40	0.0	2.5

coming into contact with oil droplets. Uptake of soluble PAH may also be influenced by changes in osmoregulation, if PAHs are taken up across the gills by transport with water.

This study examined the role of salinity in the uptake of PAH from crude oil by *Tilapia mossambica*. *Tilapia mossambica* was chosen to enable comparisons with freshwater data from previous experiments across salinities within their zone of tolerance (0–30‰). Teleosts, the bony fish inhabit both fresh water and seawater environments. Regardless of adaptation, the gills of these animals possess a highly specialized cell type called the chloride cell (Karnaky 1986). Tilapia, a euryhaline species, enabled testing at higher salinities up to 30‰ without confounding results by osmotic stress. They are highly amenable to laboratory studies, which is also a common and abundant species, found along the west and east coast of India and would almost certainly be affected in case of coastal oil spills.

Materials and Methods

The crude oil used to make up water soluble fraction (WSF) was obtained from a production platform in the Bombay high oil field, West Coast Offshore Region, India. The oil had a viscosity of 5–10 CSt (Centistokes) at 38°C. The crude oil was weathered by sparging with air for 130 h to simulate the loss of volatile components at sea shortly after a spill (about 14% by weight of whole oil).

Tilapia mossambica were chosen for exposure bioassays at salinities of 0‰, 15‰ and 30‰ because of their ability to tolerate wide fluctuations in salinity. Tilapias were collected from a hatchery' present near the seashore (near Mumbai). They were placed in a 1,200 L holding tank with a recirculating water system at 30°C. Salinity was maintained at 15‰ by adding 10 g/L of sea salt to dechlorinated municipal water. To acclimate Tilapia to 30‰, salinity was increased gradually over a week (about a 3‰ increase per day).

In order to estimate small scale variability, water soluble fractions of a crude oil sample were prepared in duplicates following conditions described by Ramachandran et al. (2004) using water, adjusted to 15‰ and 30‰. A 1:9 mixture of oil and water was mixed for 18 h at 18°C, settled for 1 h and the WSF layer was separated from surface oil to use as exposure solution.

To determine the PAH composition, the WSFs were subjected to liquid–liquid extraction by means of separatory funnel, using methylene chloride (HPLC grade, E. Merck Germany) as the solvent (Standard Methods, APHA, AWWA, WEF, 2005, Method 6410 B). The extracts were cleaned up in a silica gel column and then concentrated to 1 ml over a Kuderna Danish apparatus, on a boiling (100°C) water bath. The polycyclic aromatic hydrocarbons (PAHs) analyses were performed using gas chromatography with mass spectrometer (GC/MS) (Standard Methods, APHA, AWWA, WEF, 2005, Method 6410B). Five parent PAHs (naphthalene, fluorene, phenanthrene, pyrene and chrysene) were quantified and the standards for these PAHs were obtained from M/s Acros Organics Ltd. Belgium. Table 1 shows the detection limits for selected PAHs along with mean concentration of individual PAH in both the WSFs, which were considered as the representative PAH concentration.

The acute toxicity of the WSF to Tilapia was determined at different WSF:water ratios. The ratio that resulted in the lowest LT_{50} (time to 50% mortality in fixed concentration) was used to expose the fish at varying salinities (0‰, 15‰ and 30‰). Five fish of similar size (ranged about 15 ± 2 cm) were placed in each of the exposure tanks with salinities of 0‰, 15‰ and 30‰, respectively. Triplicates were run at each of the salinities along with a control.

After 96 h of exposure two fish from each replicate were anaesthetized and killed by severing the spinal cord. Their livers, muscles, gills and gonads were removed, weighed and subjected to 6 M methanol KOH saponification (Tremblay et al. 1992). The digests were diluted with water and purified using a solid phase extraction column packed with silica gel (Activated at 200°C for 17 h and deactivated 5% w/v with HPLC grade water). The column was washed with hexane (HPLC, Lichrosolv, Germany) and the sample was applied as a 1 mL extract and PAHs were eluted with 10 mL hexane:dichloromethane (4:1 v/v). Extracts of biological tissues were analyzed using high-resolution gas chromatography (Agilent 5973 N, USA) coupled to a mass selective detector. The following GC (Ultra 2, 0.17 μm

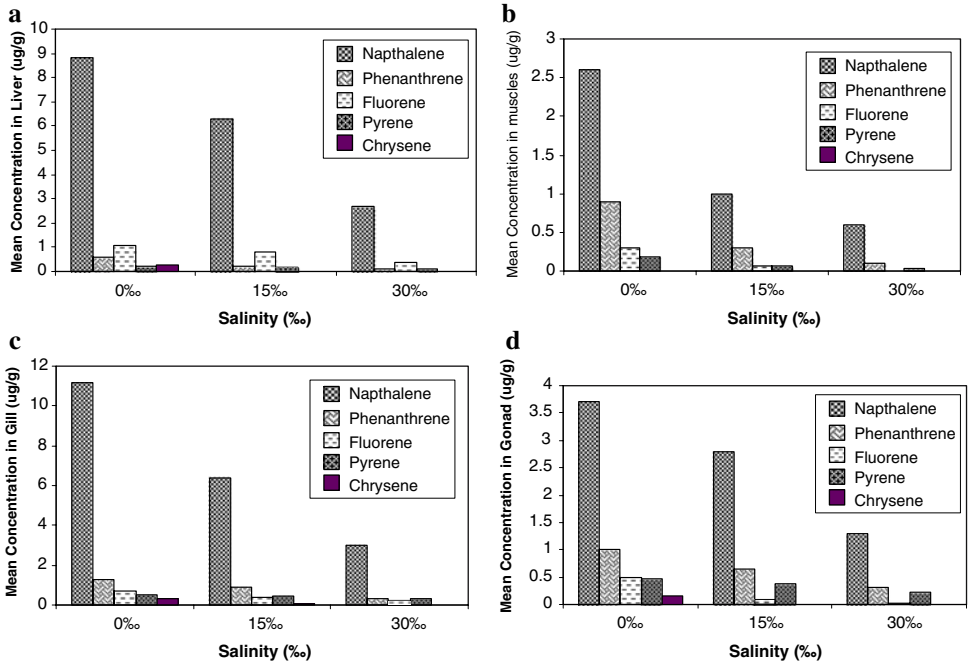

Fig. 1 Mean concentrations of individual PAHs in somatic and reproductive organs of Tilapia, **a** liver; **b** muscle; **c** gills; and **d** gonad expressed on a wet weight basis (μg/g)

capillary column Supelco, USA) conditions were used for the analysis; splitless injection 225°C, temperature programs 60°C hold 2 min. ramp at 6°C min. to 300°C hold 13 min. A duplicate certified reference material and operational blank was routinely performed with each batch of ten samples. Following the above mentioned procedure percent recovery was estimated for somatic and reproductive organs spiked with 0.1 μg/g PAH. Two, three, four and five ringed aromatics were calculated as naphthalene, phenanthrene, fluorene, pyrene and chrysene equivalents.

The data generated during the study was processed with the aid of Statistica (5.1) software. The data characteristics such as mean, standard deviation, range, etc. were determined and the One-Way Analysis of Variance test was carried out to check the difference between the PAH accumulation in fish (fish organs such as liver, muscle, gill, and gonad) exposed to different salinities.

Results and Discussion

The mean concentrations of different classes of PAHs found in somatic and reproductive tissues of Tilapia exposed to varying salinities (0‰, 15‰ and 30‰) are shown in Fig. 1.

A consistent PAH recovery of more than 90% was obtained from fortified fish tissues (Table 2). Table 3 shows the accumulation of individual PAHs in select somatic and reproductive organs (mean ± standard deviation) of Tilapia. Relatively low concentrations of PAHs were accumulated in somatic and reproductive tissues. Gills were the primary site for bioaccumulation of

Table 2 Percentage recovery of PAH from fortified fish tissues

PAH	Liver (%)	Muscle (%)	Gills (%)	Gonads (%)
Naphthalene	92.83	90.53	92.93	91.97
Phenanthrene	90.29	93.9	90.47	93.40
Fluorene	90.17	90.50	90.28	92.93
Pyrene	92.47	92.2	93.63	90.24
Chrysene	91.68	90.87	92.78	91.62

hydrocarbons closely followed by the liver. Muscle showed least accumulation. The highest level of accumulation was noted for naphthalenes and the lowest for chrysene.

The same superscript letters indicate significant differences ($p < 0.05$, verified by repeated measure one-way ANOVA) between the recorded bioaccumulation concentrations at different salinity concentrations.

A hypothesis to explain salinity effects on PAH uptake by fish states that salinity controls PAH solubility and bioavailability in an aquatic system. The solubility of hydrophobic organic contaminants is low at higher salinities (Schlautman et al. 2004). This holds true for PAH, as corroborated by the PAH data from this experiment (Fig. 1) and by the results of other research (Whitehouse 1984; Ramchandran et al. 2006). At 0‰ there were higher concentrations of individual PAHs in solution than at 15‰ and still lower at 30‰, with the differences being statistically significant (ANOVA, $p < 0.01$). The differences were largest with the low molecular weight (LMW) two (naphthalenes) and three (phenanthrene) ring compounds as compared with higher molecular weight (HMW) compounds such as pyrene (four ringed). As stated by

Table 3 Recorded accumulation of individual PAHs in select somatic and reproductive organs of *Tilapia mossambica* (N = 6)

Treatment	Fish organs	PAH	Salinity		
			0 ‰ Mean ± SD	15 ‰ Mean ± SD	30 ‰ Mean ± SD
Control	Liver	Naphthalene	2.60 ± 0.15	2.40 ± 0.02	2.60 ± 0.02
		Phenanthrene	0.40 ± 0.02	0.40 ± 0.02	0.30 ± 0.03
		Fluorene	0.40 ± 0.02	0.40 ± 0.02	0.40 ± 0.03
		Pyrene	0.26 ± 0.02	0.25 ± 0.03	0.22 ± 0.03
		Chrysene	ND	ND	ND
	Muscle	Naphthalene	0.70 ± 0.03	0.60 ± 0.02	0.70 ± 0.03
		Phenanthrene	0.09 ± 0.003	0.10 ± 0.03	0.06 ± 0.003
		Fluorene	ND	0.02 ± 0.003	0.01 ± 0.003
		Pyrene	0.10 ± 0.03	0.09 ± 0.003	0.10 ± 0.03
		Chrysene	ND	ND	ND
	Gills	Naphthalene	1.30 ± 0.02	1.20 ± 0.02	1.10 ± 0.02
		Phenanthrene	0.40 ± 0.02	0.39 ± 0.03	0.30 ± 0.02
		Fluorene	0.80 ± 0.03	0.85 ± 0.03	0.78 ± 0.02
		Pyrene	0.20 ± 0.02	0.20 ± 0.03	0.17 ± 0.04
		Chrysene	ND	ND	ND
	Gonad	Naphthalene	1.00 ± 0.02	0.90 ± 0.03	1.20 ± 0.02
		Phenanthrene	0.40 ± 0.03	0.36 ± 0.03	0.41 ± 0.02
		Fluorene	0.40 ± 0.03	0.38 ± 0.03	0.39 ± 0.03
		Pyrene	ND	ND	ND
		Chrysene	ND	ND	ND
Exposed	Liver	Naphthalene	8.80 ± 0.11^{a}	6.30 ± 0.12^{ab}	2.70 ± 0.02^{ab}
		Phenanthrene	0.60 ± 0.02^{a}	0.20 ± 0.02^{ab}	0.09 ± 0.003^{ab}
		Fluorene	1.10 ± 0.02^{a}	0.80 ± 0.02^{ab}	0.39 ± 0.02^{ab}
		Pyrene	0.22 ± 0.03^{a}	0.18 ± 0.02^{ab}	0.11 ± 0.02^{ab}
		Chrysene	0.28 ± 0.03	ND	ND
	Muscle	Naphthalene	2.60 ± 0.02^{a}	1.00 ± 0.02^{ab}	0.60 ± 0.02^{ab}
		Phenanthrene	0.30 ± 0.03^{a}	0.07 ± 0.003^{a}	ND
		Fluorene	0.90 ± 0.02^{a}	0.30 ± 0.02^{ab}	0.10 ± 0.03^{ab}
		Pyrene	0.18 ± 0.02^{a}	0.07 ± 0.003^{ab}	0.03 ± 0.002^{ab}
		Chrysene	ND	ND	ND
	Gills	Naphthalene	11.2 ± 0.02^{a}	6.40 ± 0.02^{ab}	3.00 ± 0.11^{ab}
		Phenanthrene	0.70 ± 0.02^{a}	0.40 ± 0.02^{ab}	0.17 ± 0.04^{ab}
		Fluorene	1.30 ± 0.02^{a}	0.90 ± 0.02^{ab}	0.30 ± 0.03^{ab}
		Pyrene	0.53 ± 0.02^{a}	0.46 ± 0.03^{ab}	0.31 ± 0.03^{ab}
		Chrysene	0.30 ± 0.02^{a}	0.05 ± 0.002^{a}	ND
	Gonad	Naphthalene	3.70 ± 0.02^{a}	2.80 ± 0.03^{ab}	1.30 ± 0.02^{ab}
		Phenanthrene	0.50 ± 0.03^{a}	0.10 ± 0.02^{ab}	0.03 ± 0.004^{ab}
		Fluorene	1.00 ± 0.02^{a}	0.65 ± 0.03^{ab}	0.32 ± 0.03^{ab}
		Pyrene	0.46 ± 0.03^{a}	0.38 ± 0.03^{ab}	0.22 ± 0.03^{ab}
		Chrysene	0.15 ± 0.03	ND	ND

Ramachandran et al. (2006), we also found the LMW PAHs to be more soluble in water than HMW compounds, with salinity affecting the solubility of the LMW compounds to a greater extent than HMW compounds. Interactions between PAH and particulates might also be affected by salinity, but their interactions were probably unimportant in these assays. Fish were not fed for 48 h prior to testing to avoid fecal material and exposures were carried out in filtered water. Hence, there would be few particulates available for binding with PAH.

Although osmoregulation was not examined in our test animals, it may have been an essential factor since the fish regulate osmotic balance as salinity changes (Hoar 1966). In hypo-osmotic environments, fish are subjected to diffusion of water from the surrounding medium into the gills, as is the case with freshwater fish. As the salinity of the surrounding medium is increased, this process slows until iso-osmotic conditions prevail (about 15‰) (Ramchandran et al. 2006). Increased salinity markedly reduced (ANOVA, $p < 0.01$) PAH uptake from water-soluble fraction by *Tilapia mossambica*. Tilapias are euryhaline and are quite adapted to changing salinities and have chloride cells to excrete salt in saline conditions (Karnaky 1986). If water uptake or loss via the gills acts as a transfer medium for PAH across the gills, in addition to passive diffusion across lipid membranes, the reduction in PAH uptake at higher salinities might be due to water and PAH efflux in response to osmotic gradients. However, the efflux of water with increased salinity is counteracted by the requirement that fish drink water at high salinities, which might provide a dietary loading of PAH equivalent to the efflux of PAH with water via the gills. These possibilities cannot be resolved without physiological experiments to partition uptake and excretion rates among the gills, intestine and possibly the kidney to develop a mass balance of water flows and PAH loading. Considering that hydrophobicity drives partitioning of PAH from water into lipid membranes, the trans-membrane transport of PAH with water should decrease in importance with an increasing molecular size and octanol water partition coefficient. Overall, the reduction in PAH uptake with increasing salinity in fish exposed to WSF of crude oil likely reflects the combined effect of lower PAH solubility and osmoregulation. Therefore, the potential risks to aquatic life of PAH toxicity following oil spills are enhanced in lower salinity waters such as estuaries and near coastal zones.

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