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# STATUS OF POLYCYCLIC AROMATIC HYDROCARBON CONTAMINATION IN PALLIKARANAI WETLAND: FISH AS AN INDICATOR

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## ABSTRACT

Pallikaranai Wetland, within Tamil Nadu, India, receives runoff from the surrounding urban area, including polycyclic aromatic hydrocarbons (PAHs). PAH have been listed as Priority Pollutants by US EPA (2014) due to their carcinogenic, genotoxic and teratogenic potential in humans and form DNA adducts. In addition to the run-off that the area receives, dumping and burning of waste occurs here and hence, this study was designed to evaluate PAH contamination in three fish species commonly found in the wetland considering the ongoing indiscriminate dumping of assorted waste here. Between January and April 2018, fifty fish comprising of seven subjects of *Oreochromis aureus,* thirty four subjects of *Oreochromis mossambicus* and nine subjects of *Oreochromis niloticus* were collected from Pallikaranai wetland. The fish were then necropsied and were analysed for fifteen PAH. Total PAH load was greatest in Oreochromis mossambicus. Naphthalene and phenanthrene were detected in all samples. While levels of sum of four carcinogenic (PAH4: Sum of Benzo (a) pyrene, Chrysene, Benzo (a) anthracene and Benzo (b) fluoranthene) in one subject of *Oreochromismossambicus* manifolds higher than the toxic levels as per the European Union guidelines, and was closely trailing the limits in one individual of *Oreochromisaureus*, the levels of the other 48 fish were below the limit of concern. *Oreochrmoismossambicus* is near threatened in its home range (Mozzambique) according to the IUCN and PAH may be contributing to the threats that the species faces. While the Oreochromis mossambicus is not threatened in India, it is imperative to assess the accumulation patterns of PAH in the species elsewhere to contribute data to the conservation in the home range.

**KEYWORDS:** ***Polycyclic Aromatic Hydrocarbons, Pallikaranai, Tilapia, fish, POP Contamination, PAH4.***

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# INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAH) are omnipresent in the environment due to the diverse nature of their sources. Among different habitats, wetlands suffer the maximum exposure due to the fact that petroleum spills, industrial effluents, municipal waste water, and surface run-off ultimately find their way into aquatic bodies. While individual PAH can be toxic to aquatic organisms including fish, there are also PAHs that are carcinogenic to humans [1]. Fish are good bio indicators because they occupy a range of positions in different trophic niches. In India, fish protein contribute 14.9% to total animal protein consumption[2] Tilapia are a group of omnivorous subtropical to tropical freshwater fish in the Cichlidae family. They are good indicator organisms for monitoring PAH concentrations in tropical waters[3,4,5]. While O.niloticus and O. aureus are species of least concern[6,], O.mossambicus is Near Threatened[7]according to the IUCN red list. While habitat alteration, agro-chemicals, cross-hybridization and increases in fishing pressure for food present the greatest threats to fish populations[8], PAH exposure may be adding to the list of threats in certain locations. This may have consequences when a species is on the brink of being threatened. PAH can have toxic effects as the metabolized, parent molecules; however, fish can metabolize many PAHs, which can cause adverse effects as reactive metabolites. Reactive metabolites of some PAHs can bind to DNA and cellular/sub-cellular proteins as adducts[9]. Moreover, the metabolism of PAHs can generate reactive oxygen species (ROS) which can cause oxidative stress, including lipid peroxidation, oxidation of proteins, and oxidation of nucleic acids The biochemical disruptions and cell damage as a result of the binding, may lead to mutations, developmental malformations, tumours and cancer [9]. Although studies on the ill effects of PAH on fishes in China[10,11,12], Europe[13,14] and USA[15, 16, 17] are aplenty, in India, very few studies have been carried out on PAH exposures in fresh water ecosystems[18,19,20]. Pallikaranai is an important wetland within the city limits of Chennai. According to the solid waste department of the Chennai Corporation, the city generates about 3,200 tonnes of garbage on a daily basis. This garbage is collected from trash bins in 15 zones, 8 of which transport to Pallikaranai. 2200 tonnes is dumped in Pallikaranai alone[21].Around 32 million litres of untreated sewage was being released every day into Pallikaranai by Metro water, Chennai which has contaminated the already leached out water. Close to 4000 lorry loads of trash are dumped here so documenting the magnitude of contamination[22], particularly PAH, assumes significance. The present study was conducted to assess PAH contamination in 3 species of fishes belonging to genus Oreochromis (invasive) in Pallikaranai wetland, Chennai.

# MATERIALS AND METHODS

***Study area***

Pallikaranai wetland (12° 56′ 15.72″ N, 80° 12′ 55.08″ E) is a fresh water marsh running parallel to the Bay of Bengal situated in Chennai city with a geographical area of 80 km2 (Figure1). The Tamil Nadu state Government declared 317 hectares (780 acres) of the Pallikaranai wetland as a reserve forest. Pallikaranai has a rich biodiversity and is a bird watchers' paradise. It provides suitable habitat for many organisms particularly birds, fishes, amphibians. With rapid urbanization, indiscriminate dumping of waste, uncontrolled vehicle emissions and rampant oil spills, PAH have become a predominant group of contaminants in Pallikaranai. Part of Pallikaranai is a reserve forest and contamination may have both ecological and social consequences on the wetland[23].

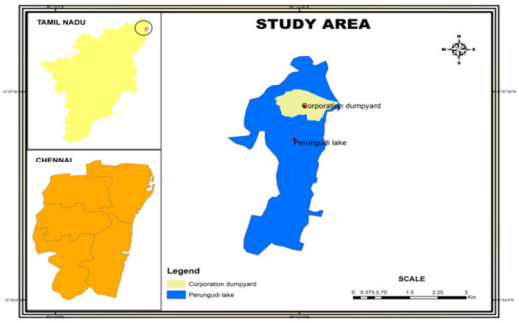


**Figure 1**

***Perungudi Lake located in Pallikaranai wetland, Tamil Nadu***

***Sample collection and processing***

Samples of fishes were collected after obtaining all the necessary permissions to collect fish from a protected area from Tamil Nadu Forest Department. Care was taken to make sure that the fish were not under stressed conditions and that no physiological damage occurred till sacrifice. They were maintained, handled, and tested under conditi ons that did not create such responses. Compliance of Animals (Scientific Procedures) Act 1986[24] was strictly adhered to. Sample collection was between January and April 2018 on different days from Perungudi, the lowest point of the Pallikaranai Marsh, where corporations routinely dump waste. In total, , 50 individual fish were collected from the waterbody with the help of local fishermen[25]. Gill net, scoop net and line net methods were used to collect the fish. Samples were packaged, labelled and transported over ice to the laboratory at Salim Ali Centre for Ornithology and Natural History, Coimbatore. Out of the 50 fishes, 34 were *Oreochromis mossambicus*, 7 were Oreochromis aureus and 9 were *Oreochromis niloticus*. All the fish were necropsied and muscle tissue dissected, wrapped in aluminium foil and stored at -20ºC until analyses. Muscle was the tissue of choice because it is usually consumed[26,27].

***Chemicals and reagents***

PAH calibration standards (certified reference material) were obtained from Sigma-Aldrich, GmbH. Chromatography grade acetonitrile and sodium chloride (Merck, India) and anhydrous magnesium sulphate (Himedia, India) were used. Clean up reagents, namely Primary Secondary Amines (PSA), C18 bulk sorbent and graphitized carbon block (GCB) were supplied by Agilent Technologies, USA. Ultrapure water was produced in a Milli-Q-DQ3 (Millipore) in the laboratory.

***Sample processing***

QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe)[28] multiresidue extraction method was adopted for processing of samples and analyses of PAH residues. Two to ten grams of homogenized tissues were accurately weighed and placed in a 50 mL centrifuge tube. 5 mL of water and 10 mL of acetonitrile were added to it and shaken vigorously for a minute in a shaker. To the centrifuge tube, 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride were added and shaken vigorously in a shaker for one minute and centrifuged at 5000 RPM for 5 minutes. After centrifugation, 4 mL of the organic layer of the centrifugate was transferred into a 15 mL centrifuge tube, which contained 100 mg of Primary Secondary Amines (PSA), 100 mg of C18 bulk sorbent and 500 mg of anhydrous magnesium sulphate and shaken well. This was then centrifuged at 3000 RPM for 5 minutes and 2 mL of the centrifugate was transferred into vials for chromatographic analyses.

***PAH quantification***

Samples were analysed for presence and quantification of 15 PAH, namely Naphthalene, Acenaphthene, Anthracene, Flourene, Pyrene, Fluoranthene, Chrysene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(e)acenaphthene, Benzo(k)fluoranthene, Dibenzo(a,h)anthracene, Indeno(1,2,3-cd)pyrene and Benzo(g,h,i)pyrelene listed by United States Environmental Protection Agency as priority pollutants . PAH was quantified with High Performance Liquid Chromatography (Agilent 1100 Series) equipped with fluorescence detector, fitted with Eclipse-PAH (4.6 mm X 150 mm, 5µm) column.

***Quality control***

The instrument was calibrated with 1, 2, 5, 10, 20, 50 and 100 ng/mL of PAH standard mixture and linear calibration curves with R2 values>0.99 were obtained for all individual PAH. PAH residues were identified by comparison of their retention time to the peaks from the calibration standards and quantified using calibration curve. Limit of Detection (LoD) and Limit of Quantification (LoQ) were evaluated by the concentration of analyte required to produce signal to noise ratio of 3x and 10x, respectively. LoQ of compounds ranged from 0.2 to 3 ng/g. Samples were analysed in a batch of 10 samples plus 3 quality controls, namely blank, method blank and mid-range standard. Estimated values, greater than the LoQ were expressed in ng/g or ppb (wet weight$\pm$ SD). The average recoveries of all the compounds from fortified samples were above 94.50% at three concentrations (10ng/g, 20ng/g and 30ng/g, n=6 for each concentration. Results were not corrected for percent recovery.

***Analyses***

Analyses for PAH residues were performed on a Agilent 1100 High Performance Liquid Chromatograph (HPLC) equipped with programmable Florescence Detector (FLD) and controlled by Chemstation software. Fluorescence detection is employed generally for trace analysis of PAHs for better selectivity and sensitivity. Hence, fluorescence detector was used to quantify the PAHs. The optimum excitation and emission

wavelengths varied with individual PAHs, and hence FLD was programmed accordingly to obtain high sensitivity and low limit of detection for individual PAHs. The chromatographic separation was achieved on Agilent Zorbax Eclipse PAHs (4.6*150mm, 3.5µm) column by gradient elution with a binary system of water and acetonitrile with 1.5ml per minute flow rate. Chromatographic conditions were as follow;

**Column** : Agilent Zorbax Eclipse PAHs (4.6*150mm, 3.5µm)
**Mobile phase** : (A) Water
(B) Acetonitrile

## DATA ANALYSIS

The PAH concentration data did not meet the assumption of normal distribution (Shapiro-Wilk test, $p<0.05$), so non-parametric test (Kruskal-Wallis test) was employed. The test groups comprised of the three species versus the individual PAH. The values have been expressed as median and range although for discussion and comparison with other studies, mean with standard deviation has been considered. Values below detection limit were not considered to calculate mean. Standard deviation was not calculated where the number of samples with detectable levels was less than 3. Variation in percentage of detection among the PAH was tested using Chi-Square test. P values less than 0.05 were considered statistically significant. All statistical tests were performed using statistical software SPSS student version 17.

## RESULTS AND DISCUSSION

The concentration of individual PAH varied among the 3 species of Oreochromis (Table 1). The percentage of individual PAH detected also varied significantly on subjecting to Kruskal-Wallis test ($n = 48$ , $\chi2 = 179.2$, $p < 0.05$) among the three species. While the total subjects of fish were 50, 48 of them did not carry detectable levels of high molecular weight PAH. The 48 subjects comprised of 6 oreochromis aureus, 33 Oreochromis mossambicus and 9 Oreochromis niloticus. Naphthalene and phenanthrene were detected in the muscle tissue of all individuals of all the 3 species. Naphthalene was the most prevalent parent PAH compound. This may be because of its better solubility in water when compared to other PAH congeners with greater molecular weight[29]. The greatest concentration was detected in O. niloticus (39.39 ng/g + 15.64) and the least was in O.aureus (29.23 ng/g +5.61). This is in good agreement with the findings of DouAbul et al., (1997)[30] who found naphthalene to be the most abundant PAH compound in Solea solea (Dover Sole) collected from Yemen. Naphthalene contributed the maximum to the total PAH load at 67% in O.aureus, 62% in O.mossambicus and 74% in O. niloticus. In the present study, the second most abundant compound was Phenanthrene which is a principal PAH compound of crude oil along with Pyrene. This was in good agreement with the findings of Ohiozebau et al.,(2017)[31] in Athabasca and Slave River, Canada. Phenanthrene was detected the maximum in O. aureus (9.09 ng/g +2.3) and least in O. niloticus (7.37 ng/g +2.52). Phenanthrene contributed 19%, 17% and 14% to the total PAH load in O. aureus, O. mossambicus and O. niloticus respectively. High molecular weight PAH were detected only in 2 individuals so these two individuals were not considered for statistical analyses, one O. aureus (8. 67 ng/g) and one O. mossambicus and the levels in O.mossambicus(41.42 ng/g) were several fold greater than the safe concentrations (12 ng/g) for humans by the European Union[32], while the concentrations in O.aureus were closely trailing the limit. The rather low detection of certain PAH, namely (Benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(e)acenapthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd) pyrene and benzo(g,h,i)pyrelene) in the fishes may be attributed to their rapid rate of depuration or biotransformation[33]. While Low Molecular Weight (LMW) PAH (PAH with 2-3 aromatic rings) are linked with acute toxicity, High Molecular Weight (HMW) PAH (PAH with 4 and above rings) are associated with carcinogenicity[34]. Bioaccumulation of PAH in fish is influenced by multiple factors particularly the route of exposure (gills, skin and ingestion), duration of exposure and age of the fish, fat content available to accumulate lipophilic contaminants, temperature, salinity of the habitat and other environmental factors, elimination and biotransformation rates for particular species, sex and the effects caused by other toxicants (potentiation, additive or synergism[35, 36]. The rate at which bioaccumulation occurs is dependent on many factors, in particular, their trophic position, feeding preference[37]. Fishes metabolize PAH in the liver before they get concentrated and excreted[35, 38.39]. This may be the reason why the concentrations of many PAH were Below Detection Limits (BDL) (Table 1). Deb et al. (2000)[33] reported greater concentrations of low-molecular weight PAHs in the fish, similar to the findings reported here.

**Table 1**

***Concentration means, standard deviation, median, and ranges of individual PAH among 3 species of fishes collected from Pallikaranai***

| Individual PAH | Mean ±SD | | | Median | | | Range | | |
|---|---|---|---|---|---|---|---|---|---|
| | OA (n=7) | OM (n=34) | ON (n=9) | OA (n=7) | OM (n=34) | ON (n=9) | OA (n=7) | OM (n=34) | ON (n=9) |
| Naphthalene* | 29.23±5.61 | 32.21±13.64 | 39.39±15.64 | 25.14 | 26.74 | 39.49 | 24-39 | 16-68 | 18-68 |
| Acenaphthene* | 2.12 | 2.11 | 1.68 | --- | --- | --- | --- | --- | --- |
| Flourene* | 1.54 | 1.19±0.36 | 1.64 | --- | 1.00 | --- | --- | BDL-3 | --- |
| Anthracene* | 4.09 | 5.02+9.56 | 1.18 | | 1.09 | --- | --- | BDL-38 | |
| Phenanthrene* | 9.09±3.24 | 8.73±2.3 | 7.37±2.52 | 9.36 | 8.68 | 8.41 | 2-11 | 2-18 | 2-12 |
| Flouranthene# | 2.43 | 1.9±0.35 | BDL | --- | --- | --- | --- | --- | --- |
| Pyrene# | 6.22 | 7.68±5.57 | 8.99±4.65 | --- | 5.11 | 10.82 | --- | BDL-25 | BDL-15 |
| Benz (a) anthracene# | BDL | 1.93 | BDL | --- | 1.93 | --- | --- | BDL-2 | --- |
| Chrysene# | BDL | 9.54 | BDL | --- | 9.54 | --- | --- | BDL-10 | --- |
| Benzo (b) fluoranthene# | 8.67 | 23.33 | BDL | 8.67 | 23.33 | --- | BDL-9 | BDL-24 | --- |
| Benzo (k) fluoranthene# | BDL | 7.62 | BDL | --- | 7.62 | --- | --- | BDL-8 | |
| Benzo (a) pyrene# | BDL | 6.61 | BDL | --- | 6.61 | --- | --- | BDL-7 | --- |
| Benzo (g,h,i) pyrelene# | BDL | BDL | BDL | --- | --- | --- | --- | --- | --- |
| Indeno(1,2,3-cd)pyrene# | BDL | 64.24 | BDL | --- | 64.24 | --- | --- | BDL-65 | --- |
| Dibenzo(a,h)anthracene# | BDL | 17.44 | BDL | --- | 17.44 | --- | --- | BDL-18 | --- |

***(ng/g wet weight± SD ) --- Not applicable***

****Low Molecular Weight PAH # High Molecular Weight PAH***

***OA- Oreochromis aureus, OM- Oreochromis mossambicus, ON- Oreochromis niloticus***

***Values below detection limit were not considered to calculate mean and SD since the number of samples with detectable levels was less than 3.***

Total PAH concentrations (Figure 2) among the three species of fishes did not vary significantly (Kruskal-Wallis test, n = 50, H=1.00, P>0.05). Total PAH concentrations ranged between 26 and 57 ng/g in O.aureus, 23 and 106 ng/g in O. mossambicus and 29 and 87 in O.niloticus which is much greater than PAH concentrations reported by Cheung et al., 2007[40] in fresh water fish collected in Hong Kong The average levels recorded in the present study are comparable with the levels reported by Kong et al., (2005)[41] in tilapia collected from fishponds and local fish markets in Hong Kong and are not greater than the guidelines prescribed by US EPA (2000)[42], with the exception of 2 individuals which had extremely high levels of PAH4.

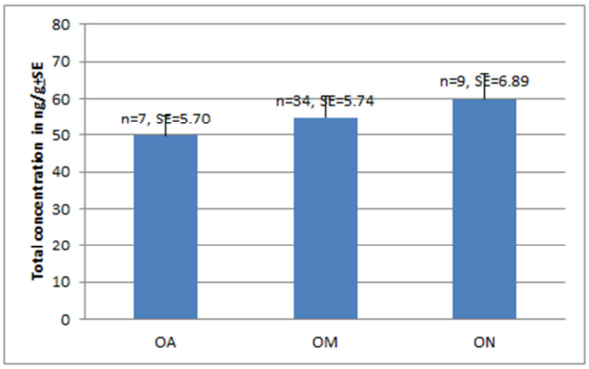


**Figure 2**

***Mean total PAH concentration and standard error (ng/g wet weight+SE) in the 3 species of fishes***

***Legend: OA- Oreochromis aureus, OM- Oreochromis mossambicus, ON- Oreochromis niloticus collected in Pallikaranai wetland.***

Among different congeners of PAH, two-ring PAH Naphthalene with Molecular Weight 128, three-ring PAH Phenanthrene with Molecular Weight 178 and Flourene with Molecular Weight 166 have dominated the distribution in fish muscles. Such patterns are the properties of PAH mixtures that get into the environment from petrogenic sources[33]. The petroleum-derived residues contain greater concentrations of two- and three-ringed PAH[43]. The ratio of low molecular weight PAH (LMW-PAH) to high molecular weight PAH (HMW-PAH) have been used to determine the origin of PAH in the environment[44]. LMW PAH (2–3 rings) have higher water solubility, greater bioavailability and higher rates of uptake compared to HMW PAH (4–7 ring) which have higher rates of depuration[45]. Flouranthene was not detected in any of the individuals of O.niloticus. Anthracene was detected in one individual of this species at low concentrations when compared to the other two species. Mechanism of PAH toxicity vary according to the number of rings, as well as other factors. They may cause developmental toxicity, photo-induced toxicity, oxidative stress and cancer[46]. A more reliable indicator of PAH exposure for human health is PAH4 ( sum of levels of Benzo (a) pyrene, Chrysene, Benzo (a) anthracene and Benzo (b) fluoranthene) (Joint FAO/WHO Expert Committee on Food Additives[32] and the CONTAM Panel[1]. This is considered more reliable than BaP by itself. Concentration of PAH4 in 2 subjects out of the 50 which had detectable levels of PAH4 (Table 2).

**Table 2**
***Fish samples with measurable concentrations of Carcinogenic PAH (PAH4) Collected from Pallikaranai***

| SPECIES | No. of individuals | Level of PAH4 (Benzo (a) pyrene, Chrysene, Benzo (a) anthracene and Benzo (b) fluoranthene) |
|---|---|---|
| OA | 1 | 8.67 |
| OM | 1 | 41.42 |

The European Union Commission's regulation (EC) No 1881/2006[47] states that the maximum limit for human consumption is 2 µg/kg benzo (a) pyrene and the maximum permitted level of the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b) fluoranthene and chrysene which is 12 µg/kg. The global per head consumption is estimated at 22.3 kg/year[48]Fish consumption pattern of 47g/ day in a city in Tamil Nadu, India. Based on the per capita consumption and information available on consumption of fish, the levels of only two of the 50 seem to be a cause for concern[49]. Organisms, such as fish and crustaceans possess an MFO system which is highly developed and capable of metabolizing PAH and only tend to accumulate PAH in highly polluted localities. Two individuals of the analysed samples had PAH4 greater than the limits set by European Union. The levels in these two fish were several folds greater than the permissible limits set for human consumption. One must also keep in mind that waste is being dumped indiscriminately in Pallikaranai. It should also be noted that PAH are more prevalent in urban areas and usually co-exist with other contaminants like dioxin, dioxin-like PCBs, PCBs, pesticides and metals. The concentration- to- effect relationships can be better understood using controlled exposure experiments. But, this still cannot fully give an insight on the effects that other contaminants have in combination to PAH[50]. The combination effects (potentiation, additive, synergistic or antagonistic effects) are not fully understood and hence, cannot be discounted.

## CONCLUSION

Although the levels of individual PAH in the fishes of Pallikaranai marsh are lower than the maximum limits set for human consumption, the results reiterate the need for systematic monitoring of environmental contaminants in fish while also assessing the guidelines published for human health and safety. Due to the increasing concern about Persistent Organic Pollutants (POPs) and their hazardous impact on the environment and human health, especially on the possible linkage between fish consumption and their effects on humans, it is necessary to improve and update data on different contaminants in fish to establish a proper framework for their management and control.

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## AUTHORS CONTRIBUTION STATEMENT

Ms Mythreyi Devarajan conceptualized, gathered data, analysed and designed the manuscript. Dr S Muralidharan analysed the results and critically revised the manuscript to shape it. Dr Nambirajan standardized the methodology followed for processing the samples. Mr Karthikeyan was involved in the processing and analysing the samples.

## ABBREVIATIONS

ON- Oreochromis niloticus, OM- Oreochromis mossambicus, OA- Oreochromis aureus, PAH- Polycyclic Aromatic Hydrocarbon, DNA- Deoxyribo Nucleic Acid, UV- Ultra Violet, LMW- Low Molecular Weight PAH, HMW-High Molecular Weight PAH, PAH4- Sum of 4 carcinogenic PAH namely Benzo (a) pyrene, Chrysene, Benzo (a) anthracene and Benzo (b) fluoranthene, BDL- Below Detection Limit, POPs- Persistent Organic Pollutant

## CONFLICT OF INTEREST

Conflict of interest declared none.

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