Bio-concentration of Polycyclic Aromatic Hydrocarbons in the grey Mangrove (Avicennia marina) along eastern coast of the Red Sea

1 Introduction

Mangroves are intertidal wetland plants usually found in tropical and subtropical coastal areas. They are considered as one of the most important ecological habitats due to their unique features such as high productivity, global extent, also because they represent the connection between the terrestrial and marine environment [1,2]. They play an important role in the export of carbon and nutrients to the coastal zone and oceans [3]. Mangroves provide foods for marine animals as the primary producers do, serve as nursery habitats for marine biota, and enhancing the diversity of the terrestrial and aquatic organisms [4]. In the last few decades, mangrove ecosystems were exposed to high risk of extinction through anthropogenic activities near to mangrove swamps [5]. Organic carbon richness, availability of detritus, and high productivity are the most important characteristics which make the mangrove ecosystem liable to the accumulation of a wide range of organic pollutants like polycyclic aromatic hydrocarbons (PAHs) [6,7].

Polycyclic aromatic hydrocarbons are ubiquitous anthropogenic semi-volatile pollutants consisting of two or more fused benzene rings. Numerous inputs of PAHs to various environmental compartments represented in industrial emission, oil spills, ship traffic, urban runoff, wastewater and atmospheric deposition [8]. Sources of PAHs in the environment can naturally occur (biogenic) or anthropogenic due to human activities. Anthropogenic sources could be either petrogenic that directly associated with petroleum hydrocarbons and/or pyrogenic as a result of incomplete combustion of recent and fossil organic matter. Polycyclic aromatic hydrocarbons are categorized as one of the most dangerous and harmful substances to the environment and human health due to their toxicity, mutagenicity, and carcinogenicity [1]. That is why, 16 PAH congeners were classified as priority pollutants by United States Environmental Protection Agency (USEPA) [9]. In mangrove swamps, PAHs are transferred from sediments through roots and translocated into the upper part of the...
plants and cause serious lesions like irregularity in growth rate [10], damage the cell membrane of the plant shoots [7], depletion in the exchange with the atmospheric gas [10], and increase the lethality and mutation percentage. The degree of contamination of PAHs in mangrove swamps varies significantly and hot spots could be found even in relatively clean swamps. Based on the proximity of a pollution source, very high levels of PAHs (11098 ng/g) were detected in mangrove sediments in Hong Kong [11], and mangrove plant (68518 ng/g) in India [12], while low levels of PAHs were detected in mangrove plants in China (24 ng/g) [13].

Saudi Arabia is one of the biggest countries in production, oil exploration and exploitation all over the world. Production and transportation of oil usually leads to considerable amounts of PAHs as well as other organic pollutants being introduced into the environment [14]. Despite the acuteness of the environmental activities in the Red Sea Saudi coast, only heavy metals levels were studied in the mangrove ecosystems [15,16]. To our knowledge, there has been no specific survey for PAHs levels in the mangrove swamps which are widespread along the Red Sea coast. The objectives of the present study are to (1) provide baseline data for PAHs levels in nine mangrove swamps (surface sediments, roots and leaf) along the eastern side of Red Sea (2) compare total and individual bio-concentrations and translocation of PAHs for different studied swamps with other swamps all over the world and (3) evaluate the probable sources of PAHs for the studied swamps.

2 Study Area

The coastal area of Saudi Arabia covers around 79% of the eastern part of the Red Sea coast, extending over a distance of 1840 km between Yemen in the South and Jordan in the North. Two mangrove species (Avicenna marina and Rhizophora mucronata) are spread over this area. Avicenna marina is the most dominant species at the coastal line area [17]. The present study covers mangrove swamps in the coastal area extended from 16°43'50.49"N to 20°45'5.48"N and from 38°55'13.38"E to 42°42'22.91"E. All stations were located in remote areas far from direct anthropogenic effects, except station 1 (Jazan) which is located near to a fish farm and station 8 (Al-Kharrar Rabigh) which is located in the way of torrent stream. The mangrove samples had a mean height of 2.5-3.0 m and its age was estimated to be 25-30 years old, except station 8 (Al-Kharrar Rabigh) where the mangrove plants were characterized by longer and intensive leaf. The growth conditions of all swamps were comparable, therefore significant differences in PAHs contamination due to this factor is excluded. Figure 1 shows the map locations for the mangrove samples along the eastern Red Sea coast.

3 Material and Methods

3.1 Samples collection and pre-treatment:

Approximately the top 0-5 cm of sediments were collected from each studied swamp and placed in aluminum bags, refrigerated, and transported to the laboratory within 8 hours of collection and kept frozen at -20°C. Plant samples were washed by tap water followed by deionized water before being freeze-dried. The dried plant samples were ground into powder using a blender. The sediment
samples were freeze-dried for 24 hours, ground into a fine powder using a pestle and mortar, and filtered through an 80-mesh screen-sieve. All samples (sediments and plants) were transferred to glass bottles and kept at 4°C in a refrigerator for further analysis.

3.2 Organic carbon and grain size analysis

Total organic carbon in the sediments was analyzed by a wet dichromate-sulfuric acid oxidation method [18]. A standard dry sieving technique was used for sediment grain size fractions and were classified as fine (mud; < 0.063 mm), sand (0.063 – 2 mm) and gravel (> 2 mm) [19].

3.3 Extraction and purification of PAHs:

Ten grams of each sediment sample (5 g leaf, 5 g root) was subjected to a soxhlet extraction using 300 ml dichloromethane (DCM) for 24 hours. All samples were spiked with 250μL deuterated surrogate standard mixture (naphthalene-d8, phenanthrene-d10 and chrysene-d12) before extraction [20]. The crude extract was concentrated on a rotavapor at 35°C. Particulate impurities for plant samples were filtered. The extracts were concentrated to approximately 1 ml and transferred to a silica column for cleanup and fractionation. The column was packed from top and bottom with pre-combusted anhydrous Na₂SO₄ at 450°C for 6 hours. Silica gel (230-400 mesh) was activated at 230°C for 12 hours then partially deactivated with 5% deionized water. The elution was done using n-hexane for the aliphatic fraction followed by n-hexane/DCM (70:30 v/v) for the PAH fraction [21]. PAH fractions were concentrated using a gentle stream of pure N₂ to nearly 1 ml DCM. Deuterated internal standard mixture (acenaphthene-d10, fluorene-d10 and perylene-d12) (100 µl, 5 ppm) was added just before injection to the GC-MS.

3.4 Identification and Quantification of PAHs:

A GC-MS (Schimatzu 2010) with DB-5MS column (30 m*0.25 μm, RTX) was used for analysis of the samples. The programmed temperature starts at 100°C with 1-minute hold, and then ramped at 6°C/minute to 300°C then hold for 3 minutes. The electron energy of the mass spectrometer was 70 eV. Individual PAHs were identified on both retention time and the mass spectrum of selected ions with the external calibrated standards for the priority 16 PAH parent targets in addition to 1 and 2- methylated naphthalene.

3.5 Calculation of Bio-concentration and Translocation Factors:

The bio-concentration factor (BCF) for roots and leaf was calculated by the formula: \[ \text{BCF}_{\text{root}} = \frac{C_{\text{root}}}{C_{\text{S}}} \] and \[ \text{BCF}_{\text{leaf}} = \frac{C_{\text{leaf}}}{C_{\text{S}}} \], where \( C_{\text{root}} \) and \( C_{\text{leaf}} \) are the concentration of total PAHs in mangrove roots and leaf respectively, and \( C_{\text{S}} \) = concentration of PAHs in sediment [22]. The translocation factor (TF) was calculated from the formula \[ \text{TF} = \frac{C_{\text{leaf}}}{C_{\text{root}}} \] to estimate the transfer of PAHs from root to leaf in mangrove plant [23].

3.6 Quality control and quality assurance

Duplicate samples (10% of the analyzed samples) and procedural blanks (1 blank for each 5 samples) were performed at the same time as the analysis. At the beginning of each working day, calibration standards were run before each sample batch to establish the calibration curves for PAHs. Before extraction, all samples were spiked with surrogate deuterated mixture for recovery calculations. Before GC-MS injection, deuterated internal standard mixture was added for all samples. The recoveries of sediment samples ranged between 66-104% with RSD% < 18%. The concentrations in the procedural blanks were no more than three times the method detection limit. Detection limits (DL) were calculated through a five-point calibration curve and extrapolated for determining the y axis intercept [24]. All results were expressed as dry weight basis, and those samples with concentrations less than the DL were reported as not detected (ND).

Ethical approval: The conducted research is not related to either human or animals use.

4 Results and Discussions

4.1 Levels of PAHs in Mangrove Swamps

From the 18 inspected PAH congeners (priority 16 PAHs in addition to 1 and 2- methylnapthalenes), only 7 were detected either in sediments or in mangrove organs which are: naphthalene, 1-methylnaphthalene, phenanthrene, fluorene, anthracene, benzo[a]anthracene and pyrene, while the rest of congeners remained below...
the detection limit. Mangrove sediments present in variable compositions of silt, clay and sand contents [25]. Although several biogeochemical processes are available in mangrove swamps to host organic pollutants [26], sediment particle size is an important factor affecting the concentration of PAHs [27]. Table 1 represents proportional ratios for sand, mud, CaCO₃, in addition to total organic carbon in the mangrove sediments for the nine studied stations. The particle size nature of sediments in the present study were found mainly sandy (range: 91.60-99.8%) with large particles and smaller surface area that diminishes adsorption of the PAHs [28]. The low values of total organic carbon detected in the mangrove sediments in the study area (range: 1.09-4.46%) was consistent with the sandy nature.

Figure 2 represents the total PAHs concentrations (∑PAHs) in the nine studied mangrove stands (sediments, roots and leaf) along the eastern Red Sea coast of Saudi Arabia. The total PAHs concentration in sediment samples ranged from 1.06 to 6.97 ng/g with an average value of 2.98 ng/g. These values seems to be very low relative to the extremely high PAHs levels detected in polluted mangrove sediments of different swamps in Hong Kong (11098 ng/g; 3785 ng/g) [11, 29]; and in Shenzhen, China (4480 ng/g) [6]. The detected levels from this study are also lower than the values detected in moderate polluted areas such as in Shantou mangrove wetlands in China (57 to 238 ng/g) [30], and in the Sunderban wetlands (Bay of Bengal, India, 20 to 839 ng/g) [31]. The low levels of PAHs in the sediments in the present study are attributed in principle to the fact that almost all studied swamps were in remote areas and far from direct anthropogenic effects, moreover, geochemical factors such as particle size and total organic carbon are consistent with the interpretation of these low levels. Aerosol deposition of PAHs could be the unique anthropogenic source in the studied mangrove swamps, especially for the low molecular weight PAH congeners [32,33]. Three member rings phenanthrene and anthracene were found as the most dominant congeners and represent 79.4%, 84.8% and 90.07% from total PAHs in mangrove sediments, roots and leaf respectively. Meanwhile, anthracene showed the highest detected concentrations in the sediment (2.39 ng/g in Al-Lith), roots (26.47 ng/g in Wadi Omq) and leaf (83.15 ng/g in Jazan). According to sediment quality guidelines (SQG), individual and total PAHs were found below the effect range low (ERL) [34].

Mangrove plant samples showed significant increase in the total PAHs levels, where roots values ranged from 1.11 to 30.96 ng/g with an average of 8.57 ng/g, and further increase in leaf (3.24 to 89.75 ng/g with an average of 23.43 ng/g). The trend of total PAHs concentrations in all sites showed the descending order: leaf > roots > sediments. The observed accumulation trend of PAHs in this study is compatible with the mangrove swamps in Shenzhen (Chania) [6]. On the other hand, the highest levels of PAHs were detected in the mangrove roots followed by leaf in Mumbai sediments (India) [12] explained by the fine particle size nature in this area.

### 4.2 Bio-concentration and translocation of PAHs in mangrove plant

The accumulation of PAHs in a mangrove plant takes place through two pathways; the substantial mechanism is the sediment/root uptake [7,35,36] and the other one is leaf/atmospheric uptake [33]. In the first mechanism, the PAH uptake prevails in the fine particle sediment around roots [33], and the low water solubility and lipophilic nature of PAHs smoothing the plant uptake that is easily accumulated in the lipid storing cells [37], then translocate
in the upper shoots (leaf) [38]. In the leaf/atmospheric mechanism the uptake of the PAHs, either in gas phase and/or particulate phase, is through the stomata of the large surface areas of mangrove leaf that usually covered with thick waxy layers, which can accumulate the lipophilic PAHs from the atmosphere. The opportunity for PAHs uptake depends also on the volatility of PAH congeners [33]. It should be noted that both mechanisms are possible and that may lead to unsystematic correlations between PAHs in sediments and mangrove leaf and roots occasionally [39], as in stations 1 (Jazan) and 9 (Om Al-Goar Rabigh) in the present study.

Translocations (TF) and bio-concentration factors for roots (BCF$_{\text{root}}$) and leaf (BCF$_{\text{leaf}}$) were calculated only for phenanthrene and anthracene congeners that were detected in most mangrove sediments, roots and leaf. A bio-concentration factor (BCF)>1 means that an organism absorbs PAH congener at a rate faster than that at which the substance is lost by catabolism and excretion. The results of the regression analysis for roots / sediments and leaf / roots indicated p-values < 0.05 that confirm a significant correlation for both congeners, harmonize with sediment/ root mechanism. BCF$_{\text{root}}$ for both congeners was >1 in all stations except the Al-Birk station for anthracene (<1), and the highest value (4.13) was recorded at Wadi Baysh for phenanthrene; the mean value for BCF$_{\text{root}}$ for anthracene (1.72) was lower than BCF$_{\text{root}}$ for phenanthrene (2.68). On the other hand, the bio-concentration factors for leaf (BCF$_{\text{leaf}}$) elucidate relatively high accumulation, where BCF$_{\text{leaf}}$ recorded 5.41 and 5.57 for phenanthrene and anthracene, respectively. BCF$_{\text{leaf}}$ was < 1 for phenanthrene at the Al-Kharrar-Rabigh and Om Al-Goar-Rabigh stations. The highest value for BCF$_{\text{leaf}}$ (18.37) was recorded at Al-Kharrar-Rabigh for anthracene. The high BCF$_{\text{leaf}}$ values may support the presence of both mechanisms for PAH accumulation, either from sediment/root way or leaf atmospheric deposition way [36,40]. TF consist with the bio-concentration factors that recorded 6.45 as the highest value at Al-Kharrar-Rabigh for anthracene (Figure 3).

It is mentioning that the presence of flourene and phenanthrene in mangrove leaf at Al-Lith and Al-Modather stations, respectively, which were not detected in the roots and sediments preclude the sediment/ root mechanism and outweigh the leaf/atmospheric deposition uptake mechanism especially for such light congener [41], that could represent the main source for three ring congeners to mangrove in these specific sites [42].

4.3 The probable origin of PAH in the study area

The common sources of PAHs in the marine environment originate from anthropogenic activities related to either pyrogenic and/or petrogenic processes [43]. It is very difficult to discriminate between different origins of PAH mixtures due to the complexity governing the distribution of PAHs in marine sediments and mangrove. However, isomeric ratios Phenanthrene/Anthathene and Anthrathene/(Phenanthrene+Anthrathene) were selected to differentiate between pyrogenic and petrogenic origin of PAHs due to the dominance of these congeners in most studied sites. Values higher than 10 for the Phenanthrene/Anthathene ratio indicate petrogenic origin, while the ratio of Anthrathene/(Phenanthrene + Anthrathene) indicate pyrogenic origin if higher than 0.1 [34]. In the present study, only roots at station 4 (Wadi Omq) and leaf at station 3 (Wadi Baysh) showed petrogenic origin, while the rest of PAHs in roots and leaf in all other sites including sediments showed pyrogenic origin (Figure 4). It is clear that the probable origin of PAHs is site specific depending on the anthropogenic sources; where mangrove swamps in Shenzhen (China) showed mainly pyrogenic origin as in the present case [6], while it showed mainly petrogenic origin in Mumbai (India) [12].

Figure 3: Bio-concentration factors (roots and leafs) and translocation factors for phenanthrene and anthracene in mangrove plants in the study area.
5 Conclusion

The ranges of total PAHs in the studied mangrove swamps along the eastern coast of the Red Sea showed a gradual increase from sediment to root and the highest values were recorded in leaf. This trend supports PAH uptake through the sediment/root mechanism. The atmospheric deposition/leaf uptake mechanism found in a few stations where phenanthrene and anthracene were detected in mangrove leaf and not the sediments and roots. The relatively low levels of PAHs either in sediments or mangrove organs attribute to sandy nature and low organic carbon content of sediments in the studied swamps, moreover the presence of mangrove swamps apart from direct anthropogenic effects decrease the contamination probabilities. Despite the low recorded PAH values, bio-concentration factors in most stations showed values higher than one and reaching 18.37 for anthracene in the mangrove leaf as the maximum value for accumulation. PAH origin of most studied mangrove swamps was found mainly pyrogenic.

Acknowledgment: Mr. El-Amin Bashir and Mr. Rasiq K.T are grateful to deanship of graduate studies, King Abdulaziz University for providing the PhD fellowship.

Conflict of interest: Authors state no conflict of interest.

References


