Polycyclic Aromatic Hydrocarbons (PAHs) in Plants of Shadegan Wetland: *Halocnemum strobilaceum* and *Suaeda maritima*

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**Abstract**

Plants are known to bioaccumulate many contaminants. Petroleum-derived compounds, such as saturated hydrocarbons and PAHs are widespread persistent environmental contaminants. Deposition of soot and precipitation of black rain have occurred in the Shadegan Wetland. In this study, PAHs in plant parts of two prominent species of halophytes, *Halocnemum strobilaceum* and *Suaeda maritima* were determined. PAHs were found in plant parts of *H. strobilaceum* and *S. maritima*. PAHs quantities differed significantly between shoots and roots of plants and between *H. strobilaceum* and *S. maritima*. Concentration of these compounds was greater in plants growing in the southern part of the wetland (south of Abadan-Mahshar road) than those growing at the northern part. This finding corroborates findings of investigation on prevalence of heavy metals associated with crude oil; for example, Ni and V in sediments of Shadegan wetland. Detecting PAHs in plant organs indicates the potential for their bioaccumulation and transfer in trophic levels in the food chain. Although, determining threshold levels for ill-effects of PAHs, such as reduction in vegetation coverage area, plant growth and development, physiological and metabolic changes as well as changes in biodiversity indices, require further investigation, the bioaccumulation by plants and subsequent removal of such plants from contaminated areas, the wetland ecosystem could recover itself in few years.

**Keywords**: Plants, Polycyclic Aromatic Hydrocarbons, Shadegom wetland

1. **Introduction**

The release of massive amounts of oil combustion related pollutants are common in oilfields and around refineries in Iran. However, precipitation of black rain and deposition of soot resulted from burning Kuwaiti oilwells caused widespread contamination of many parts of Iran including the Shadegan Wetland in 1991. Aminipouri, et. al., (1997, 1998, 1999 a and b) reported on the introduction of soot and pollutants from burning oilwells of Kuwait into Iranian territory and subsequent reduction in vegetation coverage area. Zare-maivan, et. al. (1999 a) and Zare-maivan (2004) reported damage to wetland ecosystems of Southern Iran as a consequence of pollutants from Iraq-Kuwait war. Korury, et. al. (1999) reported increased stress enzyme levels on the vegetation of soot-affected areas of southern and southwestern Iran.

There are many persistent and less biodegradable compounds in petroleum that could easily enter the food chain. Petroleum-derived compounds, such as saturated hydrocarbons and polycyclic aromatic
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Hydrocarbons (PAHs) are widespread environmental contaminants and many of them are known as potent carcinogens. Zare-maivan, et al., (1999b) demonstrated chromosome aberration in lima beans exposed to petroleum in vitro. Saeed, et al. (1998) demonstrated presence of PAHs as a result of Iraq-Kuwait war in the atomosphere of Kuwaiti towns with potential health hazards. Since, PAHs are persistent in the environment and plants, as primary producers of the ecosystem can absorb PAHs, plant parts of two prominent species of halophytes, Halocnemum strobilaceum and Suaeda maritima were examined for their PAHs contents in order to determine the potential for PAHs transfer in the foodchain and also, advocate possibility of phytoremediation in hot spots.

2. Material and Methods

2.1. Sample Collection and Preparation

Samples were collected from branches and leaves of Halocnemum strobilaceum and Suaeda maritima plants in March 2003 from Shadegan Wetland (Table 1). Density of each species was also recorded for each sampling station. Samples were kept cold on ice, transferred to the laboratory, freeze dried immediately and were kept at -20°C until further analysis. Plant species were identified using Zohari (1973) and Rechinger (1963-1997) keys.

Table 1- Station Coordinates of Sampled Plant Species of Shadegan wetland

<table>
<thead>
<tr>
<th>Station No.</th>
<th>N Latitude</th>
<th>E Longitude</th>
<th>Dominant Plant Species</th>
<th>Density/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30°34'176</td>
<td>48°53'216</td>
<td>H. strobilaceum</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>30°33'521</td>
<td>48°45'673</td>
<td>S. maritima</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>30°32'541</td>
<td>48°42'084</td>
<td>S. maritima</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>30°33'338</td>
<td>48°44'227</td>
<td>S. maritima</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>30°26'563</td>
<td>48°7'130</td>
<td>H. strobilaceum</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>30°25'178</td>
<td>48°23'513</td>
<td>H. strobilaceum</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>30°25'259</td>
<td>48°23'047</td>
<td>H. strobilaceum</td>
<td>6</td>
</tr>
</tbody>
</table>

2.2. Extraction

Shoot and root samples were crushed in a china mortar and were placed in solvent under ventilated hood. Samples were then concentrated through evaporation of the extracted elute in a gently shaken water bath and under mild nitrogen flow. Then, the residue was dissolved with 100 μL of dichloromethane and analyzed by gas chromatograph (GC).

2.3. Determination

One μL of concentrated sample was injected to GC with split/splitless mode. A Varian CP-3800 gas chromatograph (GC) with a flame ionization detector (FID) was used for the analysis. Operating conditions were as follows: 25 m×0.32 mm i.d. fused silica capillary column with film thickness of 1.2 μm (CP-Sil 8-CB), split ratio = 5, nitrogen carrier gas (1.7 ml/min), injector and detector temperature 250 °C and 330 °C, respectively, and temperature program: 60 °C (2 min), 60-320 °C (20 °C/min), 320 °C (2 min), RSD% = 8 (EPA, 1979).

2.4. Alumina Column

The column was slurry packed using 6 mL of alumina. Elution was performed using 5 mL of dichloromethane. Ten grams of each sample were extracted with a Soxhlet extractor with 200 ml of methanol. After the extraction was completed, approximately 3 hours, 20ml of 0.7 M KOH and 30 ml of water were added to the flask and the extraction is continued for 2 more hours. The content of the extraction flask was transferred into a separatory funnel and extracted with 90 ml of hexane and re-extracted again twice with 60 ml of dichloromethane.

2.5. Clean-up

Silicagel and alumina were cleaned with dichloromethane and hexane. A chromatography column was prepared using a 50 ml burette in which a piece of glass wool was added near the stopcock to
maintain the packing. Then the column was filled with n-Hexane and 10 g of silica was transferred into the column gradually while the column was knocked in order to compact the particles. After silica gel, 10 g of alumina and on top 2 g anhydrous sodium sulfate granular was added in order to avoid the disturbance of the layer when solvents were poured into the chromatographic column. The extracts were concentrated down to exactly 5 ml using rotary evaporator and then, the final solution was evaporated to 100 μL by nitrogen blow down.

2.6. QA/AC

This research was conducted in Plant Ecology Laboratory of Tarbiat Modarress University. The laboratory passed SOP and GLP standards of US-EPA. Some samples were duplicated. The deviation for duplicate analyses for 16 PAH’s was an average of 12%. All solvents and reagents were supplied from Merck-Germany. Instrument Blank and Extraction Blank were used for monitoring for QA/AC. Spike samples were used. Recovery percent was average 88% for 16 PAH’s compounds. Standard curves of each PAH was prepared and used to calculate area under curve.

3. Results and Discussion

Results of PAHs in plant roots and shoots are summarized in Table 2. All PAHs were detected in both roots and shoots of *H. strobilaceum* and *S. maritima*. Concentration of PAHs was significantly different (P≤ 0.05) between *H. strobilaceum* and *S. maritima* and in their shoots and roots. *S. maritima* had accumulated 1.6 folds total PAHs than that of *H. strobilaceum* (3198 to 1195 ppb, respectively). Ratio of bioaccumulation in roots and shoots of *S. maritima* to *H. strobilaceum* differed (0.70 and 0.42, respectively); Bioaccumulation ratio in shoots of *S. maritima* and *H. strobilaceum* was greater in the northern section of the wetland than that of the southern section (0.56 and 0.42, respectively). Results showed that *H. strobilaceum* bioaccumulated total PAHs almost equally in northern and southern sections of the wetland, but *S. maritima* had greater bioaccumulation in the southern section.

Floranthene (Fl) was detected in the highest quantities in both sections of the wetland in shoots and roots of both plants. However, Naphthalene (N) was the least bioaccumulated PAH in both sections of the wetland. Naphthalene was also detected in the least quantities in roots of both plants and in shoots of *S. maritima* in the southern section. *H. strobilaceum* had bioaccumulated the Acenaphtalene (Ac) the least in the southern section.

Contamination of Shadegan wetland could have originated both from oil spills and combustion of crude oil (Zare-maivan, 2004). Usually, low molecular-weight compounds (3 or less aromatic rings) and their alkylated homologues are constituents of crude oil, whereas high-temperature combustion of crude oil produces the higher molecular-weight (4 or greater aromatic rings) parental non-alkylated compounds, many of which are carcinogenic. There are 16 PAHs listed as standard of environmental concern by the EPA. Amongst these, the benzopyrene and the benzo (α) pyrene are the most harmful and carcinogenic compounds. These compounds are detected in considerable quantities in plant tissues. Worldwide measurements of PAHs have been indicated at distribution range of 0.001 to 60 ng/m2. The allowable limit for benzopyrene was set by Occupation Safety and Hazard Agency (OSHA) of the USA as 5 ug/m² and for total PAHs level was set at 200 ug/m² (Hussain, 1995). Studies have indicated the presence of N-alkanes and PAHs such as benzopyrene in the combusted particulates resulted from the burning crude oil of Kuwait in 1991 (Husain, 1995). Movements of these particulate have been indicated and their distribution to distances farther than the point source of the burning oilwells have been recorded (Husain, 1995). Considering the fact that PAHs are lighter than total suspended particulates
(TSP), their transport in the form of particulate matter to farther distance than those of TSP is noticeable. As discussed by Husain (1995), Aminipour, et. al. (1998) and Esmaili and Zare-maivan (1998) and Zare-maivan, et al. (1998 and 1999a), pollutants of burning Kuwaiti oilwells had reached the Iranian territories and had been deposited in areas closer to the Kuwait, for example in Khuzestan province and in Shadegan wetland in the form of black rain on several occasions. These compounds had also been found in the samples of black rain (Esmaili and Zare-maivan, 1998). To further this, combustion of harmful gases in refineries and petrochemical plants in the area also could contribute to further aerial transport and subsequent deposition of soot and contaminats in the region. Results of this investigation showed that PAHs were present in plant parts of *H. strobilaceum* and *S. maritima* (Table 2). Sample chromatograms of PAHs are presented in Fig. 1. This finding corroborates findings of investigation of heavy metals associated with crude oil; for example, Ni and V in sediments of Shadegan wetland (Zare-maivan, 2004). Greater bioaccumulation potential in northern section of the wetland might be because of relative calm of this section as it is less affected by diurnal tidal activities of the Persian Gulf and its discharge of fresh water to the southern section. It seems that greater density of *S. maritima* in the southern section and its greater bioaccumulation capability provides a better possibility of developing a phytoremediation protocol.

Table 2- PAH Contents (ppb) in Plant Parts of *Halocnemum strobilaceum* and *Suaeda maritima* in Shadegan Wetland

<table>
<thead>
<tr>
<th>Location</th>
<th>Plant</th>
<th>Part</th>
<th>N</th>
<th>Acl</th>
<th>Ace</th>
<th>F</th>
<th>P</th>
<th>An</th>
<th>Fl</th>
<th>PY</th>
<th>BaA</th>
<th>C</th>
<th>BbF</th>
<th>BkF</th>
<th>BaP</th>
<th>Id+DA</th>
<th>BgH</th>
<th>Total PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Part of Abadan-Mahshar Road Wetland</td>
<td><em>H. strobilaceum</em></td>
<td>Root</td>
<td>7</td>
<td>11</td>
<td>18</td>
<td>21</td>
<td>166</td>
<td>46</td>
<td>361</td>
<td>345</td>
<td>95</td>
<td>52</td>
<td>238</td>
<td>29</td>
<td>39</td>
<td>29</td>
<td>78</td>
<td>1535</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>4</td>
<td>13</td>
<td>11</td>
<td>3</td>
<td>7</td>
<td>134</td>
<td>127</td>
<td>23</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>32</td>
<td>71</td>
<td>460</td>
<td></td>
</tr>
<tr>
<td>Southern Part of Abadan-Mahshar Road Wetland</td>
<td><em>H. strobilaceum</em></td>
<td>Root</td>
<td>4</td>
<td>7</td>
<td>20</td>
<td>29</td>
<td>97</td>
<td>140</td>
<td>151</td>
<td>194</td>
<td>71</td>
<td>188</td>
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<td></td>
<td>Shoot</td>
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<td>6</td>
<td>4</td>
<td>9</td>
<td>103</td>
<td>126</td>
<td>155</td>
<td>19</td>
<td>34</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>492</td>
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<tr>
<td></td>
<td><em>S. maritima</em></td>
<td>Root</td>
<td>12</td>
<td>34</td>
<td>19</td>
<td>13</td>
<td>416</td>
<td>108</td>
<td>463</td>
<td>273</td>
<td>114</td>
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<td>2013</td>
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<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td>17</td>
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</tr>
</tbody>
</table>

Fig. 1. Sample PAHs Chromatograms in *H. strobilaceum* (top) and *S. maritima* (bottom) Growing in Shadegan Wetland

Detecting PAHs in plant organs indicates the potential for their bioaccumulation and transfer in trophic levels in the the food chain. Although, determining threshold levels for ill-effects of PAHs, such as reduction in vegetation coverage area, plant growth and development, physiological and
metabolic changes as well as changes in biodiversity indices, require further investigation, the bioaccumulation by plants and subsequent removal of such plants from contaminated areas, would enable the wetland ecosystem to recover itself in few years (Husa Lee and Banks, 1993).

References


