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Original Article

Application of some aquatic plants as bio-indicators for petroleum hydrocarbon compounds in Um Alnaaj Marshes, Iraq

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Abstract: Petroleum hydrocarbon compounds are one pollutant that contributes to water pollution in Um Alnaaj Marshes in Iraq. The present study aimed to estimate the role of some aquatic plant species in the accumulation of petroleum hydrocarbon compounds. Four plant species viz. *Typha domingensis, Phragmites australis, Azolla filiculoides,* and *Hydrilla verticillatafrom)* were collected from Um Alnaaj marshes in southern Iraq and used as a bioindicator for petroleum hydrocarbon compounds. The results showed that Total Petroleum Hydrocarbons (TPHs) concentrations ranged from 21.3 to 53.9 µg/g, dw in *A. filiculoides* and *T. domingensis*, respectively. In addition, the levels of total PAHs were 2376.501, 1127.139, 541.17, and 19.062 ng/g, dw in *T. domingensis, H. verticillatafrom, A. filiculoides,* and *P. australis,* respectively. Higher molecular weight PAHs were more than lower molecular weight PAHs in all plants studied. The results also revealed that the origin of PAHs in the studied plants according to LPAHs/ HPAHs ratio and Fluo/ Pyr ratio was a mix of pyrogenic and petrogenic. Furthermore, the results showed that the sources of n-alkanes, according to Carbon Preference Index (CPI) ratios, were biogenic in all plants except *P. australis*, which was anthropogenic. However, the Pristine /Phytane ratio was less than one in all studied plants. A high accumulation of hydrocarbon compounds was observed in studied plants, which indicated the possibility of using aquatic plants as a bio-indicator for petroleum hydrocarbon pollutants in the marshes of Iraq.

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Introduction

As organic chemical pollutants, petroleum hydrocarbon compounds pose a significant threat to soil and aquatic ecosystems, endangering all living organisms (Shahriari et al., 2006; Abha and Singh, 2012; Aziz et al., 2024). This complex mixture of high and low-molecular-weight compounds, including asphaltenes, resins, N-alkanes, and polycyclic aromatic hydrocarbons (PAHs), is used for various purposes, such as machines and domestic fueling. However, their presence in ecosystems is a clear indicator of pollution and has a detrimental effect on water quality and aquatic biota. The impact of these compounds on the aquatic environment is influenced by factors such as the amount of oil spills, the type of ecosystem, and environmental conditions (Jazza and Khwadem, 2021; Saleh et al., 2021). These compounds accumulate in plant cells and inhibit processes of photosynthesis by reducing the electron transport capacity, reducing primary photochemical production, and oxygen release (Aksmann and Tukaj, 2008).

PAHs prevent the absorption of nutrients and CO_2, leading to reduced primary productivity, destroying the cell membrane of plants, affecting the synthesis of protein and nucleic acid, and subsequently leading to mutations of plant cells (Bopp and Lettieri, 2007). Singh and Gaur (1990) stated that PAHs damage DNA in plant cells and prevent DNA replication, and subsequently, cells miss their ability to undergo the division process, leading to an increase in cell size. Also, hydrocarbon compounds can enter the seeds and kill the embryo or destroy metabolic reactions, inhibiting plant growth (Gallegos et al., 2000). Also,

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the effect of mixtures of polycyclic aromatic hydrocarbons on fish causes cardiac dysfunction, edema, spinal curvature, and reduction in the size of the jaw and other craniofacial structures (Incardona et al., 2004; Saad et al., 2023).). Jazza et al. (2016) pointed out that aquatic plants of *Ceratophyllum demersum* and *Paspalum pespaioides* in the Al-Kahlaa River could be used as bio-indicators of hydrocarbon compounds because they can accumulate these compounds. Hassan et al. (2020) showed a high accumulation of PAHs in *C. demersum,* indicating their potential use as a bioindicator for petroleum compounds in aquatic ecosystems. Therefore, the present study aims to use four aquatic plant species as bio-indicators for petroleum hydrocarbon compounds in the Um Alnaaj marshes in southern Iraq.

Materials and Methods

Samples collection and extraction: Plant samples of *Typha domingensis, Phragmites australis, Azolla filiculoides*, and *Hydrilla verticillatafrom* were collected from Um Alnaaj marshes, southern Iraq. The plants were rinsed thoroughly with tap water and then distilled water in the lab. Plant samples were cut into small parts and left to dry at 15℃. Next, the samples were grounded and sieved through a 63 µm metal sieve and kept in a clean glass vial labeled and stored for further analysis.

Based on Grimalt and Oliver (1993), hydrocarbon compounds were extracted from plant samples. Briefly, 10 g of dried plants were placed in a thimble and the soxhlet for extraction with 200 ml benzene: methanol (1:1 ratio) for 36 hours. After that, the mixture was saponified for 2 hours with a solution of (4N) KOH and left until forming two layers. The unsaponified layer passes through the chromatographic column provided with glass wool, silica gel (100-200 mesh), alumina (100-200 mesh), and a layer from anhydrous Na_2SO_4. Then, 50 ml of n-hexane was added to isolate the aliphatic fraction, and 30 ml of benzene was added to isolate the aromatic fraction. These samples were reduced to a suitable volume before analysis. A spectrofluorometer was used to measure the total petroleum hydrocarbons (TPHs), and Gas chromatography was used to determine PAHs and N-alkanes in plant samples.

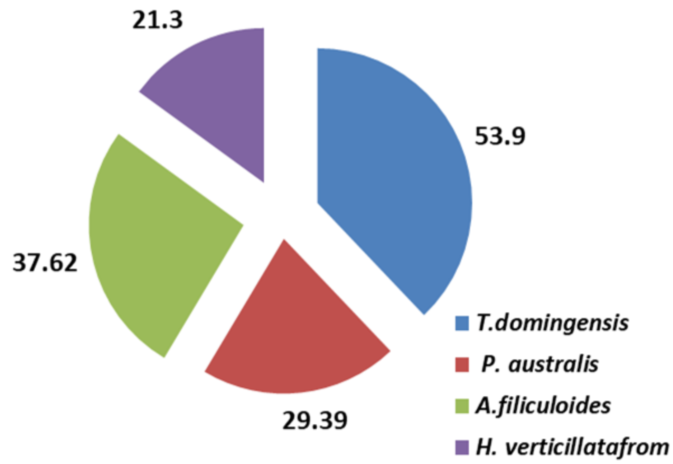

Figure 1. Concentrations of TPHs (µg/gr Dry Weight) in the studied aquatic plant species.

Results and Discussions

TPHs in aquatic plants: Plants play an essential role in the ecosystem because they are the primary energy source for living organisms in the terrestrial and aquatic environments. Aquatic plants can accumulate chemical pollutants like hydrocarbons. Thus, they can be used as bioindicators to assess levels of pollution (USEPA, 2011). Based on the results, concentrations of TPHs in aquatic plants were 53.9, 37.62, 29.39, and 21.3 µg/g, dw in *T. domingensis, A. filiculoides, P. australis*, and *H. verticillatafrom*, respectively (Fig. 1). The results revealed that TPHs were present in different levels in four aquatic plants and this may be attributed to the variation abilities of species to eliminate petroleum hydrocarbon compounds to the ambient or accumulate of in their tissues (Al-Saad, 1994; Rushdi et al., 2006). The accumulation processes of TPHs depend on lipid content in their tissues and some environmental factors such as total organic carbons, pH, salinity, temperature, amount of nutrients, and dissolved oxygen (Jazza et al., 2016; Hassan et al., 2020; Kadhim et al., 2022).

PAHs in aquatic plants: The results showed that the levels of total PAHs were 2376.501, 1127.139, 541.17, and 19.062 ng/g, dw in *T. domingensis, H. verticillatafrom, A. filiculoides*, and *P. australis,* respectively (Table 1). There are variations in levels

Table 1. Levels of PAHs (ng/g, dw) in the studied aquatic plant species.

PAHs individuals	*T. domingensis*	*P. australis*	*A. filiculoides*	*H. verticillatafrom*
Ace	51.455	7.5204	31.941	3.5624
Phen	78.481	69.385	58.432	65.802
fluo	18.905	2.931	50.239	11.506
pyr	1821.92	13.51	273.667	46.532
chr	103.245	42.287	32.503	ND
BaA	73.961	ND	50.592	ND
BbF	20.009	4.694	ND	592.56
BkF	104.843	4.752	9.607	ND
BaP	10.909	4.281	3.072	371.772
InP+ DahA	34.732	ND	ND	21.733
BghiP	58.041	19.062	31.117	13.672
Total PAHs	**2376.501**	**168.422**	**541.17**	**1127.139**
LPAHs	129.936	76.9054	90.373	69.3644
HPAHs	2246.565	91.517	450.797	1057.775
LPAHs /HPAHs	0.057	0.840	0.200	0.065
Fluo/ Pyr	0.010	0.216	0.183	0.247

* ND = not detected

of PAHs between the studied species attributed to the fatty content of each plant, the extent of tolerance of each plant to the environmental conditions, and differences in seasons, which subsequently can have effects on temperature, long periods of solar radiation, abundance of nutrients, and the ability of plants to absorption of PAHs from waters and sediments. Therefore, it becomes contaminated with these compounds, and their accumulation in aquatic plants is associated with the amounts of chemical pollutants that enter the marshes from the deposition of atmospheric, domestic, and industrial wastes (Jazza et al., 2016; Sheikh-Abdullah et al., 2020). Moreover, each plant's surface area will affect the accumulation rate (Hassan et al., 2016).

The results revealed that higher molecular weight PAHs were more than lower molecular weight PAHs in all studied plants (Table 1). This may be due to high concentrations of HPAHs in the water and sediments of Um Alnaaj marshes that are absorbed by roots, leaves, and stems and accumulate in their tissues. However, LPAHs are soluble in water, easily volatilize, and are more degradable; they cannot stay for long periods in water or sediments (Jazza, 2015; Sushkova et al., 2020; Kadhim et al., 2022).

Regarding the sources of PAHs according to the LPAHs/ HPAHs ratio, the results revealed that the ratio was less than one in all plants, indicating that the origin of PAHs in these plant species was pyrogenic. Fluo/ Pyr ratio was less than one, which suggests that the sources of PAHs in all species were petrogenic. Based on the results, the origin of PAHs in the studied plants was mixed pyrogenic and petrogenic, and this agreed with the findings of previous studies (Vrana et al., 2001; Sander et al., 2002; Jazza, 2015; Kadhim et al., 2022).

N-alkanes in aquatic plants: Concentrations of total N-alkanes were 10129.64, 6735.03, 6563.313, and 2961.03 µg/g, dw in *A. filiculoides, T. domingensis, H. verticillatafrom,* and *P. australis*, respectively (Table 2). This difference can be due to the different abilities of plant species to accumulate N-alkanes compounds, which are affected by some environmental factors such as pH, temperature, long periods of sunlight, abundance of nutrients, salinity, dissolved oxygen, and the type of plant and lipid content (Al-Saad, 1994; Rushdi et al., 2006).

The results of the present study showed that the levels of odd-carbon N-alkanes were higher than even-carbon N-alkanes in all studied plants except *P. australis* (Table 2). Abed Ali (2013) and Jazza (2015) observed that odd-carbon N-alkanes are derived from bacteria, phytoplankton, zooplankton, and fungi (biogenic origin).

Based on our findings, carbon Preference Index (CPI) values ranged from 0.09 and 6.75 in *P. australis* and *T. domingensis*, respectively, which were more than one in all plants except *P. australis*. This

Table 2. N-alkanes (μg/g, dw) levels in the studied aquatic plant species.

Carbon no	*T. domingensis*	*P. australis*	*A. filiculoides*	*H. verticillatafrom*
C7	ND	ND	ND	ND
C13	ND	2.654685	10.80602	ND
C14	ND	ND	ND	ND
C15	9.537668	7.079187	13.47408	3.800028
C16	2.650224	4.036564	6.056323	3.124508
C17	3.172635	4.889521	15.11627	8.285778
Pri	8.528052	4.59092	10.62373	3.343698
C18	58.49667	13.48244	60.13184	18.07027
Phy	12.9158	6.698825	24.90672	4.929868
C19	10.26048	22.2713	16.82255	7.821086
C20	73.42545	1911.744	82.1763	25.06404
C21	3862.034	44.593	2371.243	793.1878
C22	123.1424	47.96127	254.5592	83.41756
C23	29.6675	29.5086	98.03492	87.38126
C24	26.07423	24.03298	369.6777	123.7354
C25	273.2411	11.35104	105.7434	44.00839
C26	51.65285	40.13996	40.87096	9.995566
C27	139.902	9.53791	37.98058	14.76704
C28	33.06573	34.5244	11.0702	5.524763
C29	124.0809	11.85077	22.52801	9.473023
C30	37.09071	56.55372	13.18862	11.54076
C31	53.84242	84.56405	118.3262	68.40584
C32	86.06526	525.3298	117.0181	47.10323
C33	680.8279	31.56273	383.8717	417.5576
C34	291.8714	19.76678	33.00031	11.59522
C35	171.6072	12.34605	113.8077	61.40066
C36	81.90235	2.614527	22.29751	38.3638
C37	489.9743	ND	5294.755	3012.353
C38	ND	ND	492.3599	1649.063
Total n-alkanes	**6735.03**	**2961.03**	**10129.64**	6563.313
Odd	5848.148	249.8871	8602.509	4528.442
Even	865.4381	2699.853	1491.600	2026.597
CPI	6.75	0.09	5.76	2.23
Pri/Phy	0.66	0.68	0.42	0.67

* ND = not detected

indicates that the origin of N-alkanes compounds in *H. verticillatafrom,* *A. filiculoides,* and *T. domingensis* were biogenic, whereas *P. australis* was anthropogenic. Pristine /Phytane values were less than one in all plants, which ranged from 0.42 and 0.68 in *A. filiculoides* and *P. australis*, respectively. This shows that the sources of N-alkanes were biogenic (Table 2). The results of the present work agree with those of other local studies in the marshes of Iraq (Al-Khatib, 2008; Talal, 2008).

Conclusions

The current study revealed that the selected aquatic plant has a high ability to accumulate petroleum hydrocarbon compounds (TPHs, PAHs, and N-alkanes) in their tissues, which can be used as a bioindicator for petroleum hydrocarbon pollutants in marshes of Iraq. The sources of PAHs in the studied plants according to the ratio of LPAHs/ HPAHs and Fluo/ Pyr were mixed pyrogenic and petrogenic, whereas the sources of N-alkanes according to the ratio of CPI and Pri/Phy were biogenic, and few anthropogenic.

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