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Original Article

Using *Ceratophyllum demersum* as a bioindicator for PAHs pollution in Hor Al-Azim, Southern Iraq

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Abstract: This study was carried out in Hor Al-Azim, to determine the concentrations of total petroleum hydrocarbons in water, sediments, and plant and to use *Ceratophyllum demersum* as a bioindicator to determine the concentrations of polycyclic aromatic hydrocarbons. The study recorded the highest seasonal and local changes in the total hydrocarbons in water, sediment, and aquatic plant, results were as (3.88, 10.99, and 6.74, respectively. The results also showed significant differences in the concentrations of TPHs in *C. demersum* between the study stations and seasons. The results recorded an increase in the PAHs in *C. demersum* during the autumn and winter, while the lowest rates in spring and summer. The range was from 5.605 ng/g dry weight in the first station in the summer season and 91.866 ng/g of dry weight at 3rd station in autumn. The ratio of BaA/(BaA+Chr) was 0.000-0.859 during the study seasons, and InP/ (InP + Bghip) between 0.000-0.887 indicating that most of the PAHs sources in this plant are pyrogenic, and few of them petrogenic. The ratio of Flouranthene to most Pyrene compounds is greater than 1, and few are less than 1 indicating that some PAHs sources are petrogenic, and most of them pyrogenic.

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Introduction

In recent years, the aquatic environment has suffered from increased pollutants (Shariati et al., 2019). Oil pollution is one of the most severe problems in water bodies due to its direct impact on living organisms (Bhalerao, 2013). Crude oil is a complex and solid organic compound mixture (Akpor et al., 2007). The hydrocarbons make up 50-98% of the crude oil (Zhu et al., 2001), remaining in the aquatic environment for a long time (Oh et al., 2014; Shariati et al., 2019). They significantly affect living and non-living components in the water ecosystem (Seiyaboh and Jackson, 2017). The hydrocarbon compounds in aquatic environments originate from many sources, such as natural ones (Hakeem et al., 2020), defusing from the bottom of the seas and oceans (Aigebura et al., 2017), and compounds resulting from anthropogenic activities (Khadim et al., 2019). The increase of hydrocarbons has recently become a source of concern because of their significant impact on water quality and aquatic organisms threatening human health (Doong and Lin, 2004; Karbassi et al., 2015). Also, the sediments have a significant role in the biodegradation of PAHs, having a long-term effect on the aquatic environment (Spasojevic et al., 2017).

Oil fields, refineries, electric-powered energy manufacturing stations, and oil pipelines close to agricultural lands, residential areas, and water bodies are the main origin of hydrocarbon pollution. The researchers have worked on aquatic plants as bioindicators of petroleum pollutants; because the phytoremediation techniques are environment friendly to remedy and low cost compared to other methods (Martins et al., 2014). Many studies have indicated the widespread use of plants as indicators for many pollutants, including petroleum pollutants (Markert et al., 2003).

Hor Al-Azim is an aquatic system of ecological and economic importance. It is characterized by its high productivity and biodiversity, including aquatic

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plants. The purpose of this study was to determine the concentrations of total petroleum hydrocarbons in water, sediments, and plant and to use *Ceratophyllum demersum* as a bioindicator to determine the concentrations of Polycyclic aromatic hydrocarbons (PAHs), which is one of the plants that has been proven its efficiency in removing and accumulating petroleum hydrocarbons. It is considered one of the submerged plants widely spread in the Hor Al-Azim.

Materials and Methods

Hor Al-Azim is a vital river located in southern Iraq's Al-Musharrah district in the Maysan Governorate. It is a suitable environment for many living organisms. It is also one of the wetlands extending on both sides of the Ahwaz border, fed by two branches of the Tigris and Euphrates rivers in Iraq and the Karkheh River from Iran. For this study, samples were collected from four stations (Table 1, Fig. 1).

The samples were collected during the four seasons of 2020. The water was collected using dark brown colored glass bottles with 5-liter capacity. Sediments were collected using Van Veen Grab Sampler from the middle of the marsh, preserved in plastic storage bags, and placed in the refrigerator. The plants were collected manually and washed well with marsh water to remove the mud and other residues. The sediment and *C. demersum* were dried at 50 g by a Freeze Dryer, then finely ground with a small electric mill and sieved with a metal sieve with a diameter of 63 mesh metal sieve, and placed in small glass bottles and marked for the extraction process.

UNEP IETC (1999) method was used to extract the total hydrocarbons TPHs dissolved in water by adding 10 milliliters of CCl_4 per liter of filtered water, and the sample was mixed well using a mixer device for 30 minutes. The contents were transferred to the separating funnel and left. To stabilize for some time and separate the organic layer because it is heavier than water, the process was repeated a second time by adding 15 ml of carbon tetrachloride (CCl_4) and agitating well with the mixer to extract the remaining total petroleum hydrocarbons with water.

IOC/WMO (1982) was used to extract PAHs in sediments by taking 20 g of the dried sediment and placing it in a thimble for extraction. The method of intermittent extraction was applied using Soxhlet intermittent extraction using a mixture of methanol: gasoline at a ratio of 1:1 for 24-36 hours and at a temperature not more than 40 °C.

Extraction of PAHs from Plants was done based on Grimalt and Oliver (1993) by taking 5 g of dried plants, placing it in a thimble, and adding to it about 100ml of methanol: benzene mixture at a ratio of 1:1 for each flask in the extraction device, where the Soxhlet Intermittent extraction process was conducted for a period 24 hours. A temperature of 40 °C was used to evaporate the extract, as the fats were isolated from the solvents. Then, saponification of the extract was done by adding 20 ml of KOH solution for 2 hours at 40 °C. Then the extract was transferred to a separating funnel, and 50ml of n-hexane was added to it. The formed hexane layer was taken using a rotary evaporator with a rotation speed of 220 rpm and a temperature of 40°C for 60 min. A concentration of 10ml was obtained, then it was used for the chromatography column. The types and concentrations of aromatic compounds in *C. demersum* were measured using gas chromatography (GC) device equipped with a flame ionization detector (FID) in the Basrah oil company.

Diagnostic ratios *(*INP/(INP + BghiP) and BaP/BghiP*),* semi-quantitative methods, were used to distinguish between pyrogenic and petrogenic sources of PAHs, but their application can be limited in further distinguishing between specific pyrogenic or petrogenic sources because of the potential for overlap among ratios (Galarneau, 2008).

Statistical Analysis: The Statistical Package Social Sciences (SPSS) program was used to analyze the results and test the least significant difference between the average values at a significant level of 0.05.

Results and Discussion

Total Petroleum Hydrocarbons (TPHs): Figure 2 shows the highest TPHs in water during the cold seasons. This is due to transferring oil residues with rains into the marshes or fuel and oil combustion by

Table 1. The coordinates of the study stations.

Stations	x	y	
S_1	47°69'15.59"	31°69'44.72"	Northwest al Azim marsh, the limits of Al Swda marsh
S_2	47°67'05.68"	31°61'63.67"	Southwest al Azim marsh, near Umm Al Niāj marsh
S_3	47°79'35.0"	31°70'21.86"	Northeast al Azim marsh near the station
S_4	47°76'86.2"	31°60'92.77"	Southeast al Azim marsh, near alsada

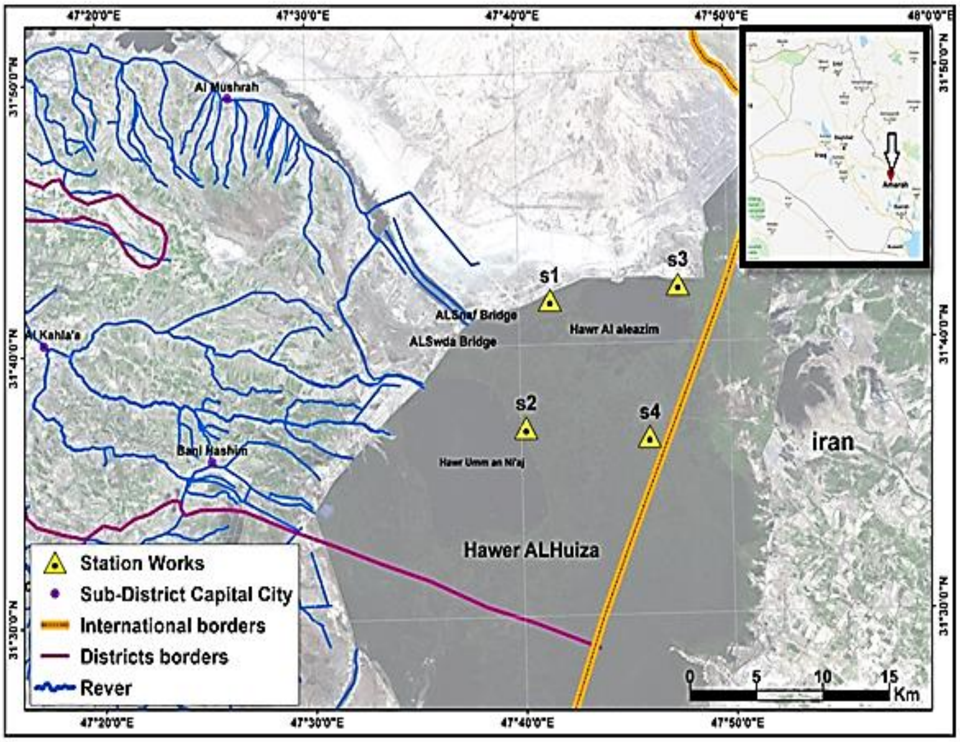

Figure 1. Map showing study stations in Hor Al Azim-Maysan Southern Iraq.

the people during the cold seasons (Abdel-Shafy and Mansour, 2016). The lowest TPHs in water was 1.39µg/l in the first station in the summer. This may be due to a rise in temperature, which plays an essential role in evaporating petroleum hydrocarbons compounds on the surface layer of water, exposed to direct sunlight. The photo-oxidation happens depending on the day's lighting period, which coincides with high temperatures in the summer (Vergeynst et al., 2019). These results agree with the findings of Nasir (2007), Farid et al. (2014) and Lazim (2019).

Marsh sediments are places to accumulate various pollutants because the sedimentation factor works to transfer contaminants from the surfaces of aquatic environments to the sediments. They are deposited directly due to their weight and the inability of water currents to carry them and thus deposit in turn to the bottom. The increase in hydrocarbons (sediments) is due to an increase in nearby industrial activities and air pollutants (Fragkou et al., 2021).

The summer and autumn had the lowest TPHs in the sediments; in contrast, the winter and spring recorded the highest TPHs (Fig. 2). This may be due to the low temperatures that lead to a decrease in evaporation and biodegradation processes by bacteria and fungi. Also, the reduction in temperature during the hot months affects organic matter's sedimentation rate, and the solubility of hydrocarbon compounds in water increases sedimentation (Hassanshahian and Cappello, 2013).

The increase in the death rates of aquatic plants and phytoplankton during the winter leads to a rise in the organic matter in the sediments and thus an increase in the percentage of hydrocarbon compounds adsorbed on the surface of the sediments. Therefore,

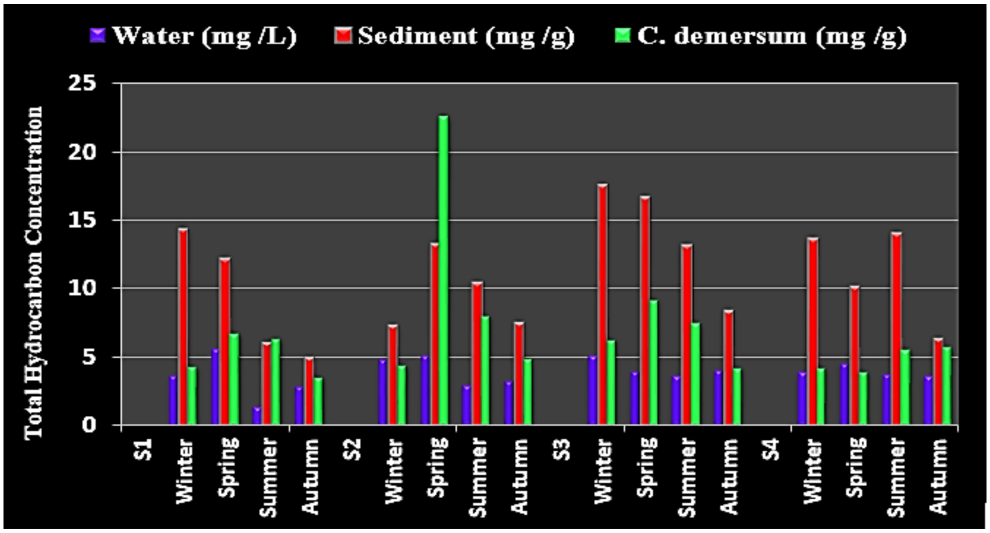

Figure 2. TPHs concentration in water, sediment, and *Ceratophyllum demersum* in the study stations.

Table 2. Rate of seasonal and stations changes in the concentration of some TPHs Compounds in aquatic plant *Ceratophyllum demersum* (μg/g) dry weight.

Season / Station	S1	S2	S3	S4
Winter	4.36	4.47	6.26	4.27
Spring	6.8	22.69	9.23	3.93
Summer	6.36	7.99	7.54	5.59
Autumn	3.55	4.88	4.27	5.78
L.s.d	season		2.1642	
	station		0.959	

the rise in TPHs means increased deposited organic matter (Kayukova et al., 2020).

The second station recorded high TPHs in *C. demersum* in the spring, and the lowest in the first station in the fall (Table 2). The highest TPHs recorded in the spring due to the higher growth of the plant i.e. the seasonal variation in TPHs in *C. demersum* has shown in accordance with the natural growing season of the plant (Hassan et al., 2010). The results also showed significant differences in TPHs in *C. demersum* between the stations and seasons. This is due to the presence of a close source of oil pollution, revealing that aquatic plants remove petroleum hydrocarbons from the aquatic environment.

Plants differ in their ability to accumulate petroleum hydrocarbons inside their bodies (Basumatary et al., 2017). The pollution of the aquatic environment with PAHs is mainly related to anthropogenic activities (Yan et al., 2012). Because of the great diversity of aquatic plants in the marshes region of Iraq, different species have been used as bioindicators of water pollution with various pollutants, particularly PAHs. The high PAHs were recorded in *C. demersum* in autumn and winter (Table 3), whereas the lowest was in the spring and summer (Table 4).

This is due to the decreased activity of microorganisms in cold seasons. In warm seasons, the increase in degradation may affect the degradation of PAHs by microorganisms, or it may be due to high photo-oxidation for the length of the day (Koudryashova et al., 2019).

The polycyclic aromatic compounds in *C. demersum* ranged from 5.605 ng/g dry weight in the first station during the summer to 91,866 ng/g dry weight in the third station during the autumn (Tables 5 and 6). This may be due to the presence of residential communities near this station e.g. fuel for fishing boats. Regarding water, the rivers of Tigris and

Table 3. Station changes rates for PAHs in aquatic plant *Ceratophyllum demersum* during winter (ng/g) dry weight.

Compound	S1	S2	S3	S4
Anthracene	0.643	0	0.890	0
Phenanthrene	0.371	0.336	0.518	0.223
Fluoranthene	16.428	0.732	17.112	12.645
Pyrene	1.625	1.811	1.812	1.806
Chrysene	1.247	1.244	1.712	1.412
Benzo(a)anthracene	7.117	6.513	9.147	8.620
Benzo(k)fluoranthene	2.852	3.317	6.623	5.120
Benzo(b)fluoranthene	9.563	8.520	12.330	6.318
Dibenz(a) pyrene	5.711	5.711	5.710	5.090
Indeno(1,2,3-cd) pyrene + Dibenz (a,h)anthracene	12.398	8.888	14.820	10.450
Benzo(g,h,i)perylene	4.385	4.172	4.420	4.235
Total	62.34	41.244	75.094	55.919
LPAHs	17.443	1.068	18.522	12.868
HPAHs	44.903	40.179	56.577	43.053
LPAHs/HPAHs	0.388	0.026	0.327	0.298
BaA/(BaA+Chr)	0.850	0.839	0.842	0.859
InP(InP+Bghip)	0.738	0.680	0.770	0.711
Fl/py	10.105	0.404	9.439	6.999

Table 4. Station changes rates for PAHs in aquatic plant *Ceratophyllum demersum* during spring (ng/g) dry weight.

Compound	S1	S2	S3	S4
Anthracene	0.821	0.719	0.560	0.833
Phenanthrene	1.610	2.576	3.001	0.888
Fluoranthene	11.074	9.014	8.050	9.002
Pyrene	0.818	0.820	0.818	0.312
Chrysene	0.788	0.820	0.820	0.917
Benzo(a)anthracene	2.202	1.230	2.250	0.994
Benzo(k)fluoranthene	0.612	0.913	1.014	0.999
Benzo(b)fluoranthene	0.445	0.515	0.720	0.630
Dibenz(a) pyrene	0.968	0.444	0.969	0.601
Indeno(1,2,3-cd) pyrene + Dibenz (a,h)anthracene	2.593	1.305	3.010	2.59
Benzo(g,h,i)perylene	0.448	0.449	0.460	0.329
Total	22.379	18.805	21.672	18.095
LPAHs	13.507	12.310	11.611	10.724
HPAHs	8.877	6.498	10.062	7.375
LPAHs/HPAHs	1.521	1.894	1.153	1.454
BaA/(BaA+Chr)	0.736	0.600	0.732	0.520
InP(InP+Bghip)	0.852	0.744	0.867	0.887
Fl/py	10.992	9.841	9.841	28.813

Euphrates pass through many cities and therefore receive significant amounts of hydrocarbon pollutants (Al-Khatib, 2008).

The sources of PAHs in *C. demersum*, calculated as a ratio of LPAHs/HPAHs, was less than 1 indicating that its source in plants is pyrogenic and its toxic effect is not affecting aquatic organisms (Balmer et al., 2019). The values greater than 1 indicate the source petrogenic, i.e. from oil sources (Vrana et al., 2001).

The ratio of BaA / (BaA + Chr) ranged 0.000-0.859 during the study seasons, while the ratio of InP / (InP + Bghip) was 0.000-0.887. These indicate that most of the origin of PAHs in the plant are pyrogenic and few were petrogenic. The ratio of the compound Flouranthene to Pyrene ranged, the highest being more than 1 and a few being less than 1. This indicates that some sources of PAHs are petrogenic, and most of them are pyrogenic (Jiao et al., 2017).

Conclusions

Based on the results, the Hor Al-Azim is polluted with petroleum hydrocarbons and organic materials. The

Table 5. Station changes rates for PAHs in aquatic plant *Ceratophyllum demersum* during summer (ng/g) dry weight.

Compound	S1	S2	S3	S4
Anthracene	0.501	0.652	0.956	0.496
Phenanthrene	ND	ND	ND	ND
Fluoranthene	2.960	2.630	2.969	2.968
Pyrene	0.243	0.128	0.384	0.364
Chrysene	ND	3.09	4.103	2.413
Benzo(a)anthracene	ND	4.512	ND	ND
Benzo(k)fluoranthene	ND	ND	ND	ND
Benzo(b)fluoranthene	0.400	0.400	0.307	0.255
Dibenz(a) pyrene	0.306	0.320	0.389	0.199
Indeno(1,2,3-cd) pyrene +Dibenz (a,h)anthracene	0.685	0.682	2.042	0.630
Benzo(g,h,i)perylene	0.510	0.380	0.515	0.612
Total	5.605	12.794	11.665	7.937
LPAHs	3.462	3.282	3.926	3.465
HPAHs	2.146	9.514	0	4.475
LPAHs/HPAHs	1.613	0.345	0	0.774
BaA/(BaA+Chr)	0	0.593	0	0
InP(InP+Bghip)	0	0.593	0	0
Fl/py	12.166	20.405	7.724	8.145

Table 6. Station changes rates for PAHs in aquatic plant *Ceratophyllum demersum* during autumn (ng/g) dry weight.

Compound	S1	S2	S3	S4
Anthracene	0.879	1.310	1.314	0.899
Phenanthrene	1.011	1.115	1.134	1.105
Fluoranthene	22.952	22.841	22.960	12.000
Pyrene	0.636	ND	0.711	ND
Chrysene	1.214	50.320	51.220	3.340
Benzo(a)anthracene	1.930	5.340	3.359	1.816
Benzo(k)fluoranthene	0.775	0.881	0.939	0.742
Benzo(b)fluoranthene	0.689	0.621	0.690	0.675
Dibenz(a) pyrene	1.432	2.019	2.160	2.703
Indeno(1,2,3-cd) pyrene+ Dibenz (a,h)anthracene	6.583	2.150	5.059	2.144
Benzo(g,h,i)perylene	2.316	2.313	2.320	1.196
Total	40.417	88.91	91.866	26.62
LPAHs	24.843	25.267	25.408	14.004
HPAHs	15.579	63.647	66.461	12.619
LPAHs/HPAHs	1.594	0.396	0.382	1.109
BaA/(BaA+Chr)	0.613	0.095	0.061	0.352
InP(InP+Bghip)	0.739	0.481	0.685	0.641
Fl/py	36.051	0	32.288	0

study also confirmed that the source of petroleum hydrocarbons is biogenic and anthropogenic. The study recommends protecting the environment of the marsh from pollutants which needs more attention as one of the most important natural reserves in the world.

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