

*Ceratophyllum demersum L. and Typha domingensis Pers as bioindicator of some PAHs compounds In Euphrates river at AL-Hindiya city*

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**Abstract:**

The present study was conducted on the Euphrates river in Al-Hindiya city river during Jul.2005 to Jun.2006. The study was included measure the concentration and distribution of some Polycyclic Aromatic Hydrocarbons (PAHs) and predict their sources in the samples of two species of two species of aquatic plant (*Ceratophyllum demersum L.* and *Typha domingensis Pers*). the lowest value among PAHs was 0.33ng/gDW recorded for Naphthalene in *C. demersum* and the highest value of 225.5ng/g DW was for Dibenzo(a,h)Anthracene in *T. domingensis*.The results of statistical analysis rerffered to non significant differences in the concentration of PAHs in both species of aquatic plants. In plant *C. demersum*, the concentration of 0.33ng/g Dw was obtained for 2 ring-PAHs, while the value of (388.93ng/gDW) was recorded for 5 ring-PAHs in *T. domingensis*. The concentrations of PAHs in both species of clams and plants were significant among seasons with exception of Naphthalene. According to the values of the ratios (Phen./Ant, Flur/Py, BaP/BghiP and LMW-PAHs/HMW-PAHs), the distribution of PAHs in aquatic plants may have the origin from different sources (Pyrogenic, Petrogenic and urban air).

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**1-Introduction :**

The term "polycyclic aromatic hydrocarbons" (PAHs) commonly referred to a large class of organic compounds found present in the environment(EHC, 1998(1); Sanders et al., 2002(2); Booker, 2004(3) ). Because of their mutagenic, carcinogenic and teratogenic properties and potential hazard to human health and natural life, they have attracted agood deal of attention (Zaid and Imam, 1999(4); Basheer et al., 2004(5), ATSDR(6)). PAHs concentration in the environment are often closely related to local and regional sources, although remote areas can be sites of PAH deposition through

atmospheric processes (Verrhiest et al., 2001(7); Rhea et al., 2005(8)).The bioconcentration of PAHs by aquatic plants involve several processes which include the partitioning of PAHs between the sediment and root system of plant and the movement of PAHs between the root and shoot (McGiynn and Livingston,1997(9)).Recently due to its low cost and relatively high effeciency ,treatment using aquatic plants combined with microbial degradation became popular, however in a study two aquatic plants *Ceratophyllum demersum* and *Naja gramunea* were used to study their effeciency to sorb PAHs compounds as phenanthrene and

pyrene. The results indicated that the two aquatic plants demonstrated great potential in application used for treating waste water containing PAHs due to low energy and the cost of devices (Hsu, 2004(10) ). The principal aims of the present work is to assess the qualitative and quantitative determination of PAHs in two species of aquatic plants (Ceratophyllum demersum and Typha domingensis ) collected from Euphrates river and determine the possible sources of these compounds in aquatic plants.

## Material and method

### Study area

Figure (1) showed the selected site along the Euphrates river under the study. Two species of aquatic plants which are C. demorsum and T. domingensis, were collected in AL-Hindiya city directly adjacent to populated area .Domestic and industrial effluents are directly discharged in to the river .Due to sedimentation ,the water level is very low with the presence of aquatic plants as C. demorsum, T. domingensis, On return back to the laboratory,the aquatic plants washed with distilled water, dried on papers, wrapped in aluminum papers, labeled and freezed at -20 C° till analysis(Kayal and Gnnell, 1995(11)).

### Extraction of PAHs from aquatic plant :

- At least about 50 gm of each plant was dried in fan-incubator at 15 C° and sieved through 63 mesh metal sieve(Ibrahim, 1999(12)).
- 10 gm of dried sample with three replicates was put in pre-extracted cellulose thimble and soxholet extraction with 250ml Hexan for 12 hr.
- The extract was carefully reduced to about 10 ml.To this 20 ml of triply distilled water was added and mixture washed with 30 ml of(0.1M) Na<sub>2</sub>CO<sub>3</sub> (Ogunfowokan et al., 2003(13)).This step is necessary to remove saponified lipids .

- Non-saponified lipids and other complex components removed by chromatographic column. using silica gel(70 -150 mesh) neutral aluminum oxide and 1-2 cm anhydrous sodium sulphate. Activated copper was added to the top to remove reduced sulphur (Sanders et al., 2002(2)).

- The aquatic plants samples were re-extracted and treated as blank. The standard mixture used for calibration contain the following compounds: Naphthalene, Acenaphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a) anthracene, Chrycene, Benzo (b) Fluoranthene, Benzo(k) Fuoranthene, Benzo(a) pyrene, Dibenzo (a,h) anthracene, Benzo (ghi) Perylene and Indeno (1,2,3-cd) pyrene.

### PAHs analysis

The PAHs extracts of plants were analyzed by high performance liquid chromatography (KNAUE) series system at the college of Pharmacy/Medicine Hawler University.

Retention time	water%	Acetonitrile%
0-2	65	35
2-16	10	90
16-22	5	95

The mobile phase was HPLC-grade acetonitrile and Distilled water in a linear gradient program as follows:

Ten µl of sample was injected into a stationary phase capillary column with a dimension of(15cm×4.6mm ID) and determined with UV detector at 254 nm . The flow rate of mobile phase was kept at 1.5 ml/min.

### Results

Seasonal changes in the concentration of PAHs in two species of plant (Ceratophyllum demersum and Typha domingensis) were studied as demonstrated in table(1) and

figure(2) ,(3) AND (4).. The mean concentration of 2 ring-PAHs(Naphthalene) in plant *C. demersum* and *T. domingensis* were(0.33 ng/g DW and 8.75 ng/gDw) respectively. As related to 3ring-PAHs(Acenaphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene) in *C. demersum*, the range between(10.6-107.2 ng/g Dw ) were recorded during summer and autumn respectively with the mean concentration of(39.55ng/g Dw) and mean percentage of(6.12%) of the total PAH. In plant *T. domingensis*, the concentrations ranged(35.0-100.3 ng/gDW) during spring and autumn respectively with mean concentration of(54.15 ng/g Dw) that represent about( 7.88%) of the total PAHs. The concentrations of 4 ring-PAHs (Fluoranthene, Pyrene, Benzo(a) anthracene, Chrycene,) in plant *C. demersum* ranged between(10.6 and 220.4 ng/gDW) during summer and spring respectively, with mean concentration of(141.3ng/g dw) and mean percentage of(21.94%) of the total PAHs. In plant *T. domingensis*, the concentrations of 4 ring-PAHs ranged between (83.8-238.7 ng/gDW) during summer and autumn respectively, with the mean concentration of (207.1 ng/g Dw) and represents (30.14%) of the total PAHs. As related with the concentration of 5 ring-PAHs (Benzo(b) Fluoranthene, Benzo (k) Fuoranthene, Benzo (a) pyrene) in plant *C. demersum*, the concentrations ranged between(212.5ng/g dw-611.5 ng/g Dw) during summer and autumn respectively with the mean concentration of(388.93 ng/g DW) and mean percentage of (60.83%). In *T. domingensis*, the 5 ring-PAHs concentration ranged between(34.6ng/g Dw and 558.6 ng/g DW) during summer and winter respectively with mean concentration of(348.8 ng/gDW) and represents (50.76%) of

the toatal PAHs. The concentration of 6 ring-PAHs( Dibenzo (a,h)anthracene, Benzo (ghi) Perylene and Indeno(1,2,3-cd) pyrene) in *C. demersum* ranged between(4.4 ng/g DW and121.7 ng/g DW) during summer and autumn respectively, with mean concentration of(69.23 ng/g DW) and mean percentage of(10.83%) of total PAHs. The results of the study for 6 ring-PAHs in plant *T. domingensis* referred to high concentration of(86.5 ng/g DW) during the autumn, while the low concentration (36.1 ng/g DW) was recorded during summer with mean concentration of(68.28 ng/g DW) and mean percentage of(9.94%) of the total PAH. In general, the mean concentration of total PAHs in *C. demersum* was (639.34ng/g Dw) and HMW-PAHs represents (93.76%, 599.64 ng/gDW), while LMW-PAHs recorded only (6.24% and 39.88ng/gDW). As related to plant *T. domingensis*, the mean concentration of PAHs was (687.08 ng/g DW) and mean percentage of(90.84 % and 9.15%) and mean concentratiuon of (62.9 ng/gDW and 624ng/gDW) was recorded for HMW-PAHs and LMW-PAHs respectively(Figure 5).. As related to concentration of PAHs in both plant species with those in water and sediment, Positive correlation was recorded for PAHs in plants with those in water and sediment (Figure 6).

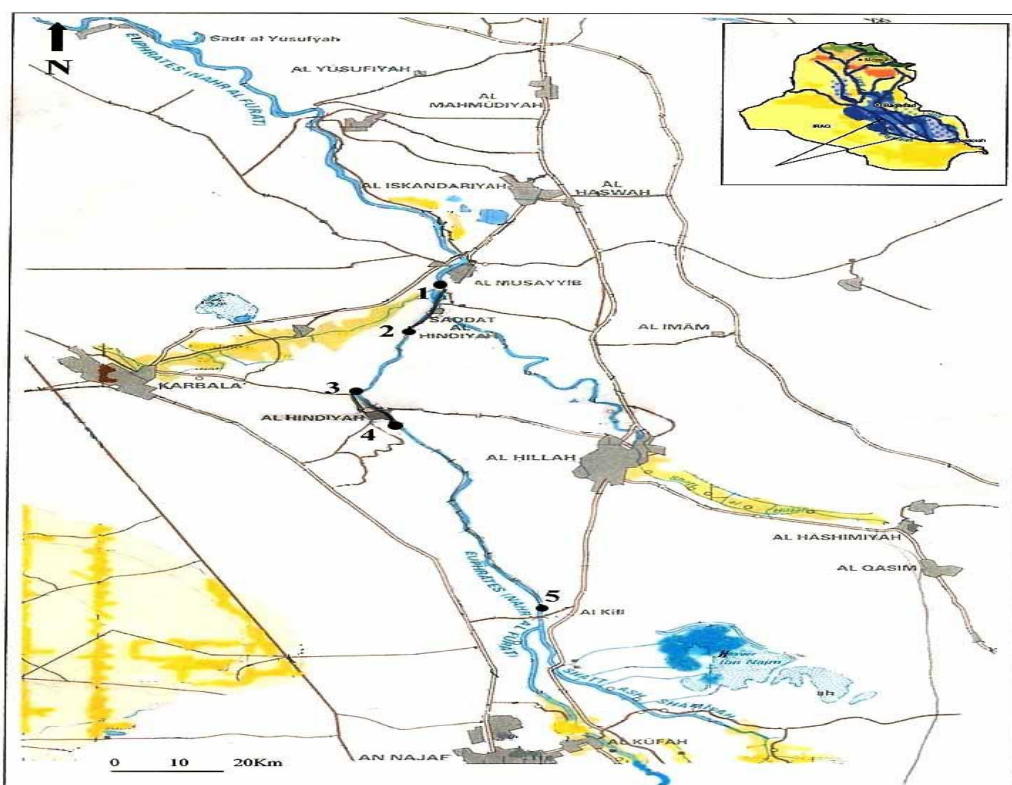
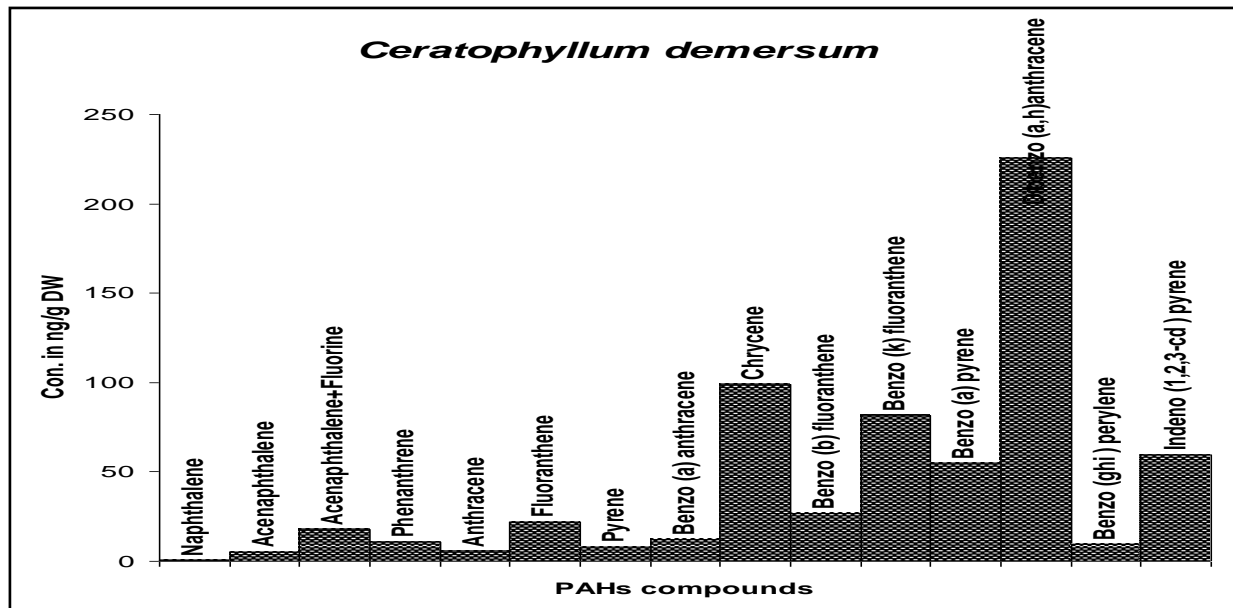


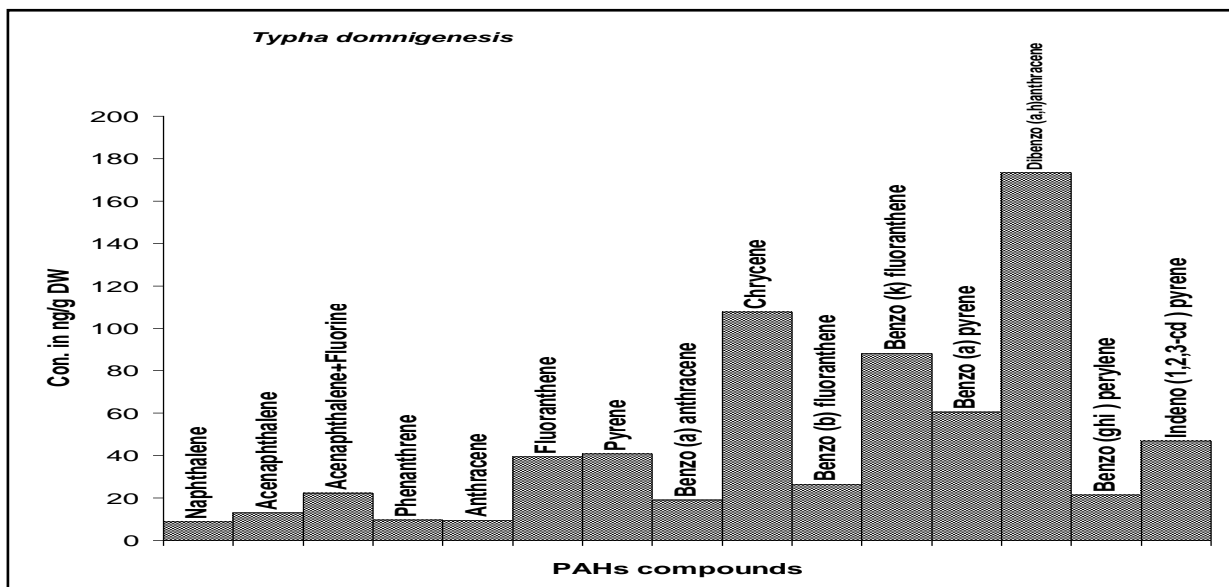
Figure (1) : Map represents study sites on the Euphrates river ● Study sites

Table(1):Seasonal changes in the concentration of some PAHs Compounds in Two species of plants (*Ceratophyllum demersum* and *Typha domingensis*) .

compound	<i>Ceratophyllum demersum</i>				<i>Typha domingensis</i>			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
Naphthalene	ND	0.6	ND	0.7	19.0	16.0	ND	0.6
Acenaphthalene	6.2	5.0	ND	9.8	21.4	19.1	ND	10.0
Acenaphthalene & Fluorene	ND	62.3	3.7	4.9	5.5	58.1	17.6	7.4
Phenanthrene	2.9	31.3	7.8	1.4	6.3	11.9	18.0	3.3
Anthracene	1.5	8.6	3.6	9.2	3.1	11.0	9.1	14.3
Fluoranthene	6.6	10	34.6	37.1	39.4	28.3	34.1	56.0
Pyrene	1.5	ND	2.6	28.2	20.1	98.0	6.4	39
Benzo (a) anthracene	1.7	19.5	ND	27.1	5.1	30.3	ND	40.6
Chrycene	0.8	105.7	161.8	128.0	19.2	82.1	148.5	181.1
Benzo (b) fluoranthene	ND	28.5	18.9	59.3	ND	ND	34.8	71.1
Benzo (k) fluoranthene	2.8	199.2	29.3	96.0	ND	210.0	30.0	112.0
Benzo (a) pyrene	11.8	ND	79.0	128.6	34.6	ND	64.9	142.2
Dibenzo (a,h)anthracene	197.9	383.8	320.6	ND	ND	348.6	345.2	ND
Benzo (ghi) perylene	ND	37.7	ND	ND	7.1	78.4	ND	ND
Indeno (1,2,3-cd) pyrene	4.4	90.0	85.7	59.1	29.0	8.1	67.5	83
Total PAHs	238.1	982.2	747.6	589.4	210.1	1000.1	776.1	760.6



Figure(2):Mean concentration of PAHs in plant *Ceratophyllum demersum* and *Typha domingensis* collected from Euphrates river site during the study period (Jul.2005-Jun.2006)



Figure(3):Mean concentration of PAHs in plant *Ceratophyllum demersum* and *Typha domingensis* collected from Euphrates river site during the study period (Jul.2005-Jun.2006)

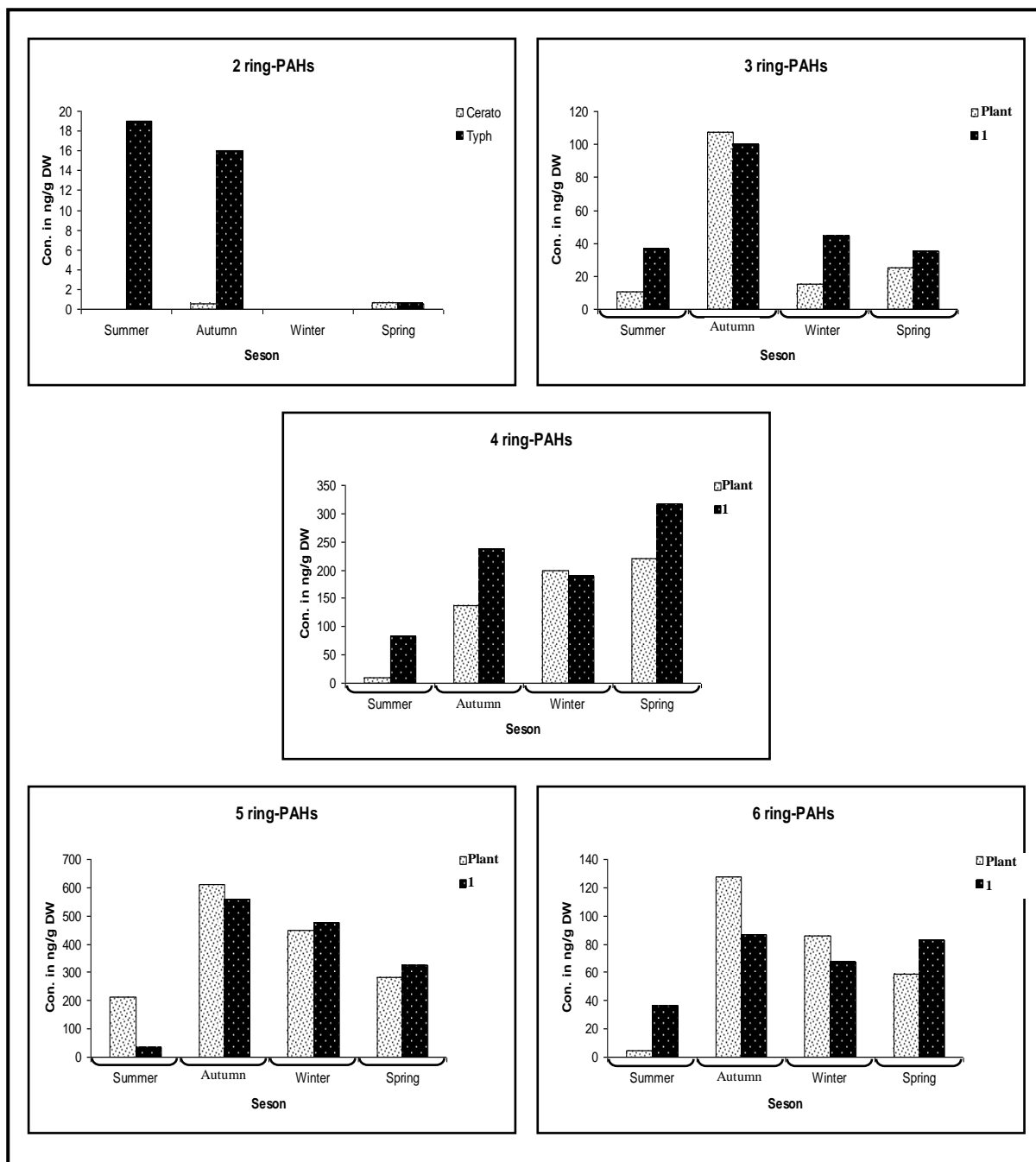
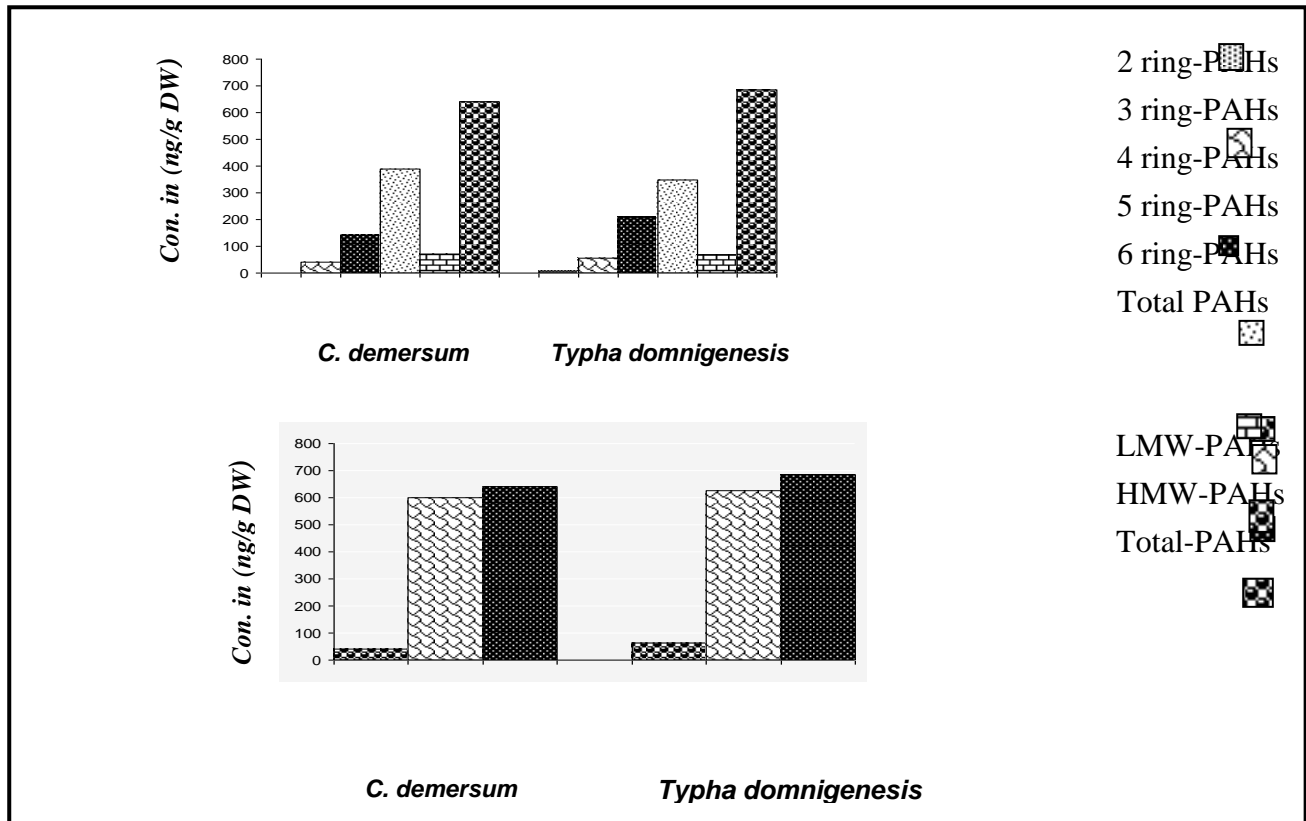


Figure ( 4): Seasonal changes in the concentrations of 2, 3, 4, 5 and 6 ring-PAHs compounds in *Ceratophyllum demersum* and *Typha domingensis* (plant 1 and 2 respectively) collected from Euphrates river site



Figure(5) :Composition pattern of PAHs in plant *Ceratophyllum demersum* and *Typha domingensis* collected from Euphrates river during the study period(Jul.2005-Jun.2006)

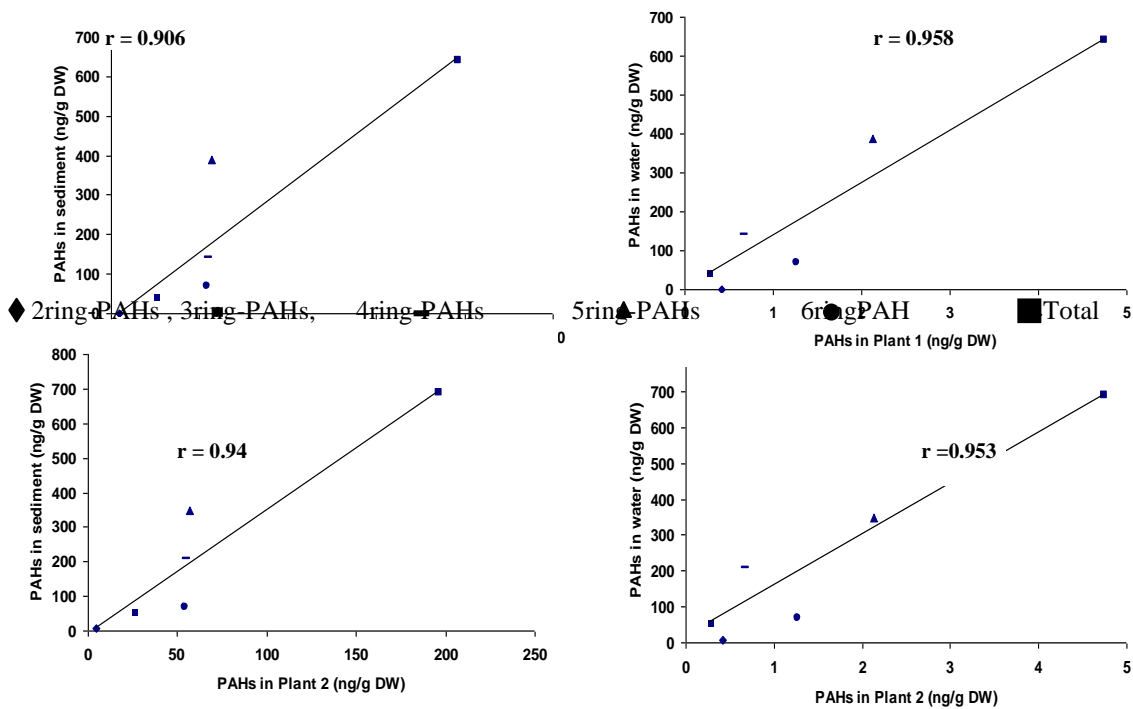


Figure (6): Relationship between the concentrations of 2ring, 3ring, 4ring, 5ring and 6ring-PAHs, total PAHs in *Ceratophyllum demersum* and *Typha domingensis* (plant 1 and plant 2 respectively) with those in water and sediment .

## Discussion

Because of their wide distribution, low cost and the efficiency to accumulate pollutants in their tissues, aquatic plants can be used to remove PAHs from contamination area (Colins and Willey, 2004(14); Hsu, 2004(10)). PAHs compounds are detected in different parts of plants including root, leaves and the concentration may be greater on plant surface than internal tissues, broad leaves than narrow leaves (Eisler, 1987(15); Irwin, 1997(16); Colins and Willey, 2004(14)). Several experimental researches and field were done to assess the accumulation potential of plants (McGlynn and Livingston, 1997(9); Hsu, 2004(10); Alfani *et al.*, 2005(17)). The present results referred to non-significant differences in the concentration of PAHs in two plants studied. This may be related to the grow of both species under the same environmental condition and exposed to the same pollution sources. The present study referred to significant differences in the concentrations of PAHs among seasons in both species of plants. This may be related with the fluctuation of different climatic conditions that affected the efficiency of plant to concentrate pollutants in their bodies. High concentrations were recorded during the cold season than the hot season with some exception. This may be attributed to the elevated PAHs photo degradation in summer caused by strong sun light and high temperature (Alfani, 2005(17)). However, some individual PAHs showed high concentrations during the summer which may be due to recent input of PAHs from atmosphere in addition to different factors

related with extraction, cleanup and analysis condition. It is also related to the properties of individual PAHs as molecular weight since it is known some high molecular weight are resistant to photo and microbial degradation so its expected to detect some HMW-PAHs during the hot season (EHC-202,(1) 1998; Eisler, 1987(15); and Irwin, 1997(16)). The concentration of PAHs in both species of plants were higher than those recorded in water and sediment. Irwin (1997(16)) cited that the concentrations of PAHs in plant can be many times higher than those in water due to bioconcentration. High concentrations of 5 ring-PAHs were recorded in comparisons with the other PAHs as in water and sediment due to their low solubility in water and tend to accumulate in the sediment and biota (Roper *et al.*, 1997(18)). Several PAHs molecular ratios were used to determine the emission sources (wood head and Matthiessen, 1999(19); yunker *et al.*, 1996(20); Walker *et al.*, 2002(21)). Compounds including phen/Ant, flur/py, Chry/BaA were also used to determine possible PAHs origin (vrana *et al.*, 2001(22)). According to the ratio of Phen/Ant., Flur/Py, BaP/BghiP and LMW-PAHs /HMW-PAHs, the sources of PAHs in plants may be pyrogenic, petrogenic and urban air. Alfani(2005(17)) suggested that the sources of emission, the distance from the source and physical-chemical properties of PAHs may be affected the concentration of PAHs in the plant. Because the plants were collected from urban site near an urban area with high traffic movement and close to the domestic sewage effluent in addition to emissions from small industries nearby, we expected the high concentration of PAHs in

plant. There are just few data present about the concentrations of PAHs in aquatic plants and most recent studies were experimental and interested in the efficiency of some orders of plants used in phytoremediation technology to remove PAHs from contaminated area (Huang *et al.*, 1997(23); Marwood *et al.*, 2001(24); Colins and Wielley, 2004(14); Hsu, 2004(10)).

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*Ceratophyllum demersum. domingensis Typha*  
في نهر الفرات عند مدينة الهندية PAHs كدلائل حيوية لبعض مركبات ال

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الخلاصة

أجريت الدراسة الحالية على نهر الفرات في مدينة الهندية للفترة من تموز 2005 ولغاية حزيران 2006 . كم ا تضمنت الدراسة قياس تركيز وتوزيع بعض المركبات الاورماتية الحلقية ومعرفة أصل هذه المركبات في نوعين من النباتات.. (*Ceratophyllum demersum L. and Typha domingensis Pers*) ( . *Pseudodontopsis*). اوضحت النتائج أن اقل قيمة (0.3 نانوغرام/ غرام وزن جاف) كان للمركب Naphthalene في نبات الشمبلان ( *Ceratophyllum demersum* ) بينما أعلى القيم (225.5 نانو غرام/غرام وزن جاف) كان للمركب Dibenzo(a,h) Anthracene في نبات البردي ( . *Typha . domingensis* ) تشير النتائج الى ان تركيز المركب ثنائي الحلقة (0.33 نانوغرام/ غرام وزن جاف) سجل في النبات ( *Ceratophyllum demersum* ) بينما (388.93 نانوغرام/غرام وزن جاف) سجل للمركب خماسي الحلقة في النبات ( *Typha domingensis* ) . أظهرت نتائج التحليل الإحصائي عدم وجود فروقات معنوية في تركيز مركبات الـ ( PAH ) بين نوعي النباتات بينما أظهرت فروقات واضحة بين الفصول باستثناء مركب الـ Naphthalene. اعتمادا على نتائج النسب ( Phen/ Ant و Flur/py و Bap/ Bghip و LMW- PAH/ HMW- PAH ) فان توزيع مركبات الـ ( PAHs ) في نوعي النباتات يأتي من أصل مصادر مختلفة ( نفطي و حراري و هواء المدن