## EFFICIENCY OF THE PHRAGMITES AUSTRALIS AND TYPHA DOMINGENSIS ROOTS IN REMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)FROM FRESHWATER SEDIMENTS S. W. Alwan

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#### ABSTRACT

This study had been conducted to quantify the concentrations of PAHs compounds in the roots of *Phragmites australis* [Cav.] Trin. ex Steudel) and *Typha domingensis* Pers. and to assess their efficiency in remediation of these pollutants from the sediment of fresh water sediment in Hilla, Daghara and Diwaniyah River. Samples (whole plants) were collected from the selective sites, seasonally during 2011-2012. Results showed the efficiency of *Phragmites australis* and *Typha domingensis* roots in the accumulation of HMW-PAHs, which were recorded (69.9 and 35.67 ng.g<sup>-1</sup> dry weight DW) during winter and spring whereas these values were (21.8 and 38.7 ng.g-1) in shoots samples during the summer and autumn for both plants respectively. In contrast, LMW-PAHs compounds were not detected in some seasons and sites whereas they were recorded (4.22 and 22.1 ng.g<sup>-1</sup>) during autumn and spring at Diwaniyah and Hilla River in root samples of both plants respectively. High value of root concentration factor (RCF) had been recorded for HMW (11.7 and 4.46) during the summer at Diwaniyah and Daghara Rivers whereas low value was detected to LMW (0.38 and 0.94) during the summer at Diwaniyah River St.4 and St.5 for both plants respectively. Values of translocation factor were recorded (0.62 and 16.7) of LMW-PAHs for both plants respectively, during spring whereas low factors were recorded (0.03 and 0.1) during summer in both plants. Current study manifested that *P. australis* and *T. domngnesis* were effective in dissipation of PAHs from sediment by the accumulation of HMW-PAHs in the roots. Accordingly, these aquatic plants might be useful in evaluating biodegradation of PAHs in freshwater sediment.

Key words: Root concentration factor, Translocation factor, Diwaniyah River, Biodegradation.

علوان

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كفاءة جذور نباتي القصب Phragmites australis والبردي Typha domingensis في ازالة المركبات المهيدروكربونية الاروماتية متعددة الحلقات من رواسب المياه العذبة

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# INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are groups of the highly persistent organic pollutants having carcinogenic, mutagenic and teratogenic properties. PAHs have reached into the environment via incomplete combustion of fossil fuels as industrial byproducts, which may be dispersed in the air and reach into the water, then accumulate in sediments and soils due to their polarity and lipophilic properties (22). High concentration of PAHs has been found in water, sediment and biota in the Euphrates River and its branches in the middle of Mesopotamia via (34, 2, 4). Many studies referred to the accumulation of the PAHs in plants and their efficiency in the dissipation of them through phytoremediation rhizosphere and microorganism biodegradation. Contribution of plants to PAH dissipation and degradation strongly dependent on the rhizosphere processes (36, 20). Remediation of PAHs in contaminated soil enhances via plants through various processes, such as stimulated on the microbial activity, community structure and functional diversity (42, 24), improvement of soil conditions (3), release of the root exudation (33, 13, 14) and enhancing gas exchange by increasing of the air flow (27,46). The effect of PAHs on wetland plant growth could be species-specific regardless of PAH types and media (52). Decreasing of the dry weight and shoot length of Phalaris arundinacea and Phragmites australis, and increasing number of young shoots are related increasing in level exposure of to Phenanthrene (23).The accumulative potential of phenanthrene in plant tissues of submersed macrophyte Vallisneria spiralis to phenanthrene in freshwater sediment was corresponded to high initial concentrations of phenanthrene in sediments and roots, which was higher than those in the shoots and concentrations of phenanthrene in stolon were lowest among these plant tissues.

*Vallisneria spiralis* plant could use adaptive mechanisms to toxic contaminants in sediment, and then change the growth patterns to decrease the toxicity on growth, (50). Toyama,*et al.* (45) showed that accelerated removal of pyrene and benzo [a] pyrene in *P. australis* rhizosphere sediments with plants by rhizosphere bacteria. Also, phenolic compounds in root exudate supported growth as a carbon source and induced benzo [a] pyrene-degrading activity of the mycobacterium. Then, biodegradation of PAHs and the concentration of phenolic compounds increase by stimulation effect in root exudates of P. australis exposed to pyrene and benzo(a)pyrene (45). Zhan et al. (51) found a relationship between root morphology and compositional factors with plant uptake of Phenanthrene in five crop plant species using a hydroponic system. In soils, the availability of PAHs have been shown to be a primary control for their uptake by plants (29, 18). PAHs tend to partition onto strongly soil solids (particularly the soil organic fractions) due to nonpolar hydrophobic molecules particularly HMW-PAHs. The removal efficiency of PAH differed significantly between LMW-PAHs (phenanthrene 3-rings) and HMW-PAHs (pyrene 4-rings), whereas Pyrene was more persistent in soils than phenanthrene. However, Zhang et al. (52) indicated that the growth of wetland plants was significantly influenced by PAHs regardless of the media (water or soil) and PAH types (naphthalene or mixture of phenanthrene and pyrene). Meng et al. (33) found that 2-4 ring PAHs were dissipating and losses to be taken up than 5-6-ring PAHs in contaminated soil, probably due to their smaller size increasing their solubility and diffusivity from the soil solution into root cell membranes. Uptake of organic molecules depending the on lipophilicity of the compounds, compositional and morphological feature of roots (37, 51). Therefore, hydrophobic chemicals (log  $K_{ow} > 3.0$ ) are not easily transported due to the high proportion of lipids located at the cell surface (41). Whereas LMW-PAHs actively transported through plant membranes because of highly water soluble and not sorbed to the roots. So, the size and molecular weight of PAHs may play a role in the ability of a plant to take up the contaminant (38). As well as, the roots and rhizomes of (P. Australis) contributes in aeration of the sediment by leakage of oxygen from the roots may create oxidized microzones. (9). The current work aimed to evaluate the root efficiency in PAHs accumulation and determine to root concentration factor (RCF) and translocation factor (TR) of PAHs in *Phragmites australis* and *Typha domingensis* plants grown in fresh water sediment of Euphrates River branches. In addition, a compositional profile of LMW-PAHs and HMW-PAHs compounds in root of both plants during four seasons were investigated as well.

### MATERIALS AND METHODS:

1-Sample Collection and Extraction:

Two species of aquatic plants (Typha domingensis Pers.) and (Phragmites australis(Cav.) Trin ex Steudel) as the dominant species in Iraqi fresh water were selected in this study, they were classified according to flora of Iraq (44). Plants were collected with their roots from five sites on Diwaniyah and Daghara rivers (Fig.1). Shoots and roots were washed, and aerial dried in the dark and cool cabin. Plants were grounded and homogenized. Five grams of each sample were extracted by ultrosonication for 1 h in 150 ml of acetone and hexane mixture 1:1 (v/v). The solvent was decanted, collected and replenished. Twice solvent fractions were combined and passed through an anhydrous  $Na_2SO_4$  column with election in 1:1 (v/v) acetone and hexane. The solvents were then evaporated and exchanged to 2 ml hexane, followed by filtration through 2 g silica gel column with elution by 11 ml of 1:1 (v/v)hexane and dichloromethane. Then the organic solvent mixture was evaporated and exchanged to cyclohexane with a final volume of 2 ml (26).

2-PAHs analysis in root:

An external standard method was used in quantifications of 16 PAHs based on fivepoint calibration curves for individual compounds. Plant samples were analyzed by high performance liquid chromatography Schimadzu (UV/vis. Detector 2500, and supelcosil LC –PAHs column (50 \*4. 6 mm, C18, 3µm particles) at the college of Pharmacy lab. / Kufa University. Acetonitrile

and water (both HPLC-grade) as mobile phase in a linear gradient program, (60% acetonitrile (0.3 minute) to100% (over 2.7 min) with flow rate 2ml/min, Detector (UV, 254) 5 µl of the sample was injected into a capillary column. Peaks in the chromatogram have identified by comparison of the retention time and spectra of standard with those in the sample (1, 16). Standard 16 PAHs were obtained from Aldrich Chemical Co. they are included Nephthalen( Nep) Acenaphthylene (Acpy), Acenaphthene (Acp), Fluorine (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Fla), Pyrene (Pyr), Benzo(a)anthracene (B[a]A), Chrysene (Chr), Benzo(b)fluoranthene (B[b]F),Benzo(k) fluoranthene(B[k]F), Benzo(a)pyrene (B[a]P), Dibenzo(a,h) anthracene (D[ah]A), Indeno (1,2,3-c,d)pyrene (InPy), Benzo(g, h,i)perylene (B[ghi]P). Sixteen PAHs have been divided into two different groups, low molecular weight LMW-PAHs have 2-3rings (Naphthalene to Anthracene) and high molecular weight HMW- PAH have 4-6 rings (Fluoranthene to Indeno(1,2,3-cd) pyrene) (11,10, 35). Root concentration factor (RCF) and translocation factor (TF) were calculated according to the following equations:

RCF= con. of PAH in root  $(ng.g^{-1} dw)/con.$  of PAHs  $(\mu g/g)$  in sediment. (8).

TF=SCF/RCF (33).

Where: SCF is shoot concentration factor.

PAHs concentration data of sediment and shoots of both plants were taken from other work.

3-Statistical Analysis:

Microsoft Excel statistical software and SPSS program were used for analyzing current data in determination of the least significant differences LSD values. Paired T-test were performed at P < 0.05 to compare other results.



Fig.1. Map of study region in Al-Qadissyah governorate.

#### **RESULTS AND DISCUSSION:**

1-PAHs Concentrations in Plants:

Results showed that high concentrations of HMW-PAHs in *P. australis* roots ranged from 0.2-69.9 ng.g<sup>-1</sup> dry weight (DW) during summer at Daghara and Diwaniyah River respectively, (table1) whereas LMW-PAHs are not detected during some seasons and stations whereas it had approached 4.22 ng.g<sup>-1</sup> during autumn at Diwaniyah River. The HMW and LMW in *Typha domingensis* roots were found to be 35.67 and 22.1 ng.g<sup>-1</sup> in samples collected in summer from Diwaniyah and in the spring at Hilla Rivers respectively, whereas they were not detected

at other stations. Concentrations of LMW ranged from 2.6-44.2 ng.g<sup>-1</sup> in *P.australis* shoots in the summer and spring at Hilla and Diwaniyah River respectively, whereas HMW ranged 0.27-21.8 ng.g<sup>-1</sup> in summer and Daghara at and Hilla River spring respectively. LMW-PAHs level increased from 1.2 to 98.6 in T. domingensis shoots whereas HMW concentrations decreased from 0.3-38.7ng.g<sup>-1</sup> during spring and autumn at Hilla and Diwaniyah River respectively, with significant differences between both groups of compounds (LSD= 15.2 and 3.2) in roots and shoots of Phragmites australis, respectively, whereas it was 7.1 and 2.4 in

roots and shoots samples of T.domingensis. These results referred to increasing of HMW-PAH concentration in root of both plant on Diwaniyah River, that might be related to either, the high values of total PAHs in sediment of this site which are located under different pollution sources (4) or accelerated biodegradation of LMW through root exudate and associated microorganisms occurred at the rhizosphere (5), or it could be absorbed through plant roots and transported to shoots whereas high molecular weight PAHs can be strongly adsorbed on the root epidermis because of high lipophilicity and resists the biodegradation (49, 26). Also (Günther et al. (21), Binet et al. (6,7) and Fang et al. (17) stated that ryegrass (monocotyledons) had often been selected for the remediation of HMW-PAH in polluted soil because of the increasing in rhizosphere microorganisms. However, PAHs adsorb to plant cell walls, and then they gradually diffuse into subcellular fractions of tissues. Lipid content of intracellular components limited the accumulation of lipophilic compounds, and diffusion rate is related the to the concentration gradient established between cell walls and cell organelles. Unique work showed that Phenanthrene (3 rings) entered the epidermis of the root maize or wheat cells radially and once in the cortex. the apoplasmic flow appeared to dominate the symplasmic movement (48). In addition, many concentrations of PAHs were found in plant roots than in leaves. This plant aided to dissipation of 5- and 6-ring PAHs in soil because of rhizosphere microorganisms activity (6). Generally, according to their molecular weight, plants absorb PAHs either as a gas through the leaves or as a particulate phase by their roots (15, 43). However, emergent plant could absorb semi volatile organic pollutant by two routes, atmospheric deposition, root absorption of PAHs were grown in polluted sediment, then PAHs accumulated in plant roots could transport to the shoots by the transpiration stream (40,19). However, the relative importance of the stomata and root uptake for contaminant fluxes to leaves is related to the watersolubility of individual PAHs, which increases with decreasing molecular weight. These conclusions might support our results in detecting high concentrations of LMW-PAHs in leaves (Table 1). Li et al. (30) was attributed dominance of LMW 2-3 ring, especially naphthalene, in the leaves of Anatolia. santolin for absorption of PAHs from air. However, the low concentration of LMW and HMW- PAHs were found during summer because of increasing of photoxidation and volatilization that were related to high temperature in the study region (4). A similar pattern was observed with increasing of HMW-PAH in Phragmites australis and Typha domingensis roots during the investigation period. This may be related to growing of both species in the same environment and expose to the same pollution sources, also a high accumulation of PAHs in the roots than in the shoot due to poor PAH transport from the roots to aboveground portions of the plant (19). So, Common reed plants might release organic materials by the roots to the surrounding medium (water and bottom sediment), which might encourage the huge amount of bacteria found on the surface of the root system (28). However, Typha spp. and Scirpus lacustris have been used in horizontal-vertical macrophyte based wetlands to treat phenanthrene.(31). Wei et al. (47) indicated that the remediation ability of PAHs attributed to leaching, volatilization, photodegradation, desorption and a biotic factors such plant species, plant metabolism, bioaccumulation, microbial degradation and plant microbial interactions.

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Table 1.	Seasonal	changes in	the concentration	s of 2, 3, 4, 5	and 6 rings	-PAHs con	mpounds (ng.g	<sup>1</sup> ) in roots a	ind shoots
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		of Phrag	<i>mites australis</i> col	lected from th	he studv sta	itions duri	ng (2011-2012)		

	PAHs	2 rings		3 rings		4 rings		5 rings		6 rings	
Stations		Ro	Shoo	Roo	Shoo	Roo	Shoo	Root	Shoo	Roo	Shoo
	Season	ot	t	t	t	t	t		t	t	t
	Spring	0.1	35.2	22.1	9.8	22.1	4.12	7.4	1.8	7.17	0.33
Hilla	Summer	ND	1.2	ND	2.03	ND	2.03	0.14	1.7	0.1	0.3
River	Autumn	0.2	17.3	0.1	14.8	0.1	10.4	1	12.9	1.2	2.56
	Winter	5.5	14.4	0.22	30.05	0.22	20.4	7.3	7.9	8.96	4.4
	Spring	0.24	6.32	3.5	52.38	0.2	4.79	0.43	4.8	0	0.86
Daghara	Summer	ND	2.3	1.1	3.07	6.48	0.97	8.4	0.97	12	0.81
<b>River St.2</b>	Autumn	1	21.3	1.2	71.24	10.3	10.64	2.24	10.67	2.5	3.14
	Winter	11.7	26	2.2	42.9	2.6	34.8	20.9	34.8	7.7	6.4
	Spring	0.1	6.3	ND	52.38	4.4	14.6	5.7	4.8	7.5	0.86
Daghara	Summer	ND	1	0.11	3.07	0.8	3.8	0.1	0.97	0.1	0.81
<b>River St3</b>	Autumn	13.2	21.3	12.6	71.2	2.4	21.17	0.2	10.6	0.4	3.1
	Winter	15.3	26	1.6	42.9	5.9	24.5	7.6	34.86	2.8	6.4
Diwoniyo	Spring	1.6	9.1	1.9	10.54	0.46	7.5	1.6	3.75	2.3	1.4
b Divor	Summer	0.2	1.8	0.4	4.37	1	3.8	0.1	2.2	1.2	0.97
II KIVEI St 1	Autumn	1.6	9.5	8.2	<b>98.6</b>	2.6	79.2	18.3	29.4	5.22	1.06
51.4	Winter	3.7	7.4	10.2	29.93	3.3	10.6	5.43	7.3	17.7	0.74
Diwoniyo	Spring	0.6	8.3	1.2	15.3	3.3	9.7	35.67	12.7	22.5	2.2
h Rivor	Summer	7.6	2.7	2	6.8	4.5	5.5	2.9	1.9	10.6	0.8
	Autumn	2.4	51.1	4.4	87.89	5.8	38.7	4.6	36.6	2.1	12.9
01.5	Winter	2.2	12.9	2.8	51.16	1.5	26	5.62	25.46	4.3	6.7

Table 2. Seasonal changes in the concentrations of 2, 3, 4, 5 and 6 ring-PAHs compounds (ng.g<sup>-1</sup>) in roots and shoots of *Typha domingensis* collected from the study stations during (2011-2012).

	PAHs	2 rings		3 rings		4 rings		5 rings		6 rings	
Stations		Ro	Shoo	Roo	Shoo	Root	Shoo	Root	Shoo	Roo	Shoo
	Season	ot	t	t	t		t		t	t	t
	Spring	0.1	6.2	ND	23.4	23.45	21.8	6.32	4.1	1.91	1.05
Hilla	Summer	0.08	2.6	ND	6.4	12.4	4.8	2.54	1.4	0.98	0.4
River	Autumn	0.8	7.8	2.2	24.4	26.6	17.2	4.8	7.2	1.1	2.7
	Winter	1.2	1.8	2.6	3.7	30.4	4.4	6.2	1.7	1.6	0.46
	Spring	0.4	11.7	1.8	16.3	22.4	5.8	6.8	2.02	2.2	0.75
Daghara	Summer	0.03	2.4	0.1	15.6	0.2	6.07	4.62	1.97	12.9	0.39
<b>River St.2</b>	Autumn	0.8	6.01	1.6	17.9	2.2	18.44	10.53	9.8	2.74	1.46
	Winter	ND	3.4	1.8	3.4	11.8	5.5	6.81	4.5	38.2	0.57
	Spring	ND	11.7	1.1	25.1	2.2	13.55	3.3	4.11	2.5	1.02
Daghara	Summer	0.4	2.3	0.8	4.6	1.2	3.8	28.22	1.2	3.3	0.27
<b>River St.3</b>	Autumn	1.8	6.1	ND	9.7	1.4	8.01	9.11	14.46	7.3	0.79
	Winter	1.8	3.4	0.8	7.3	1.8	4.9	11.4	3.6	3.2	1.8
Diwoniyo	Spring	0.08	6.5	0.2	2.2	12.3	4.86	3.7	3.13	10.3	2.31
b Divor	Summer	0.2	1.13	3.5	0.37	68.1	1.6	8.4	0.36	2.1	0.54
	Autumn	0.4	5	0.84	1.66	42.5	10.05	20.7	8.05	9	3.11
51.4	Winter	1.6	4	ND	1.3	69.9	3.79	17.2	4.9	15.2	2.9
Diwaniya	Spring	1.4	19.2	1.6	44.2	6.6	6.56	4.4	13.15	2.6	3.06
h River	Summer	1.1	6.4	0.9	7.66	1.3	7.9	2.2	3.6	2.1	0.36
St 5	Autumn	1.2	7.2	4.22	9.5	42.7	6.7	10.7	7.5	6	0.97
51.5	Winter	2.4	13	1.2	12.22	37.9	5.6	34.8	3.4	11.8	1.2

2-Root Concentration Factor and Translocation Factors:

Data of the current study indicated that there a significant increment of root was concentration factor (RCF) of HMW-PAHs compared to LMW-PAHs in P. australis (Fig.2) and *T. dominance* roots at five stations in the study region during four seasons (Fig.3). The high value of RCF was recorded for HMW to be (11.7 and 4.46) during the summer at Diwaniyah (St.4) and Daghara Rivers (St.2), whereas a low value of LMW to be (0.38 and 0.94) during the summer at Diwaniyah River St.4 and St. 5 for both respectively. plants Statistically, T-test indicated a significant increment in RCF values in P. australis and T. domingensis roots for LMW-PAHs and HMW-PAHs accumulation (*P*<0.05). These results manifested the different capability in dissipation efficiency of both groups in the roots of both plants and this may be related to the chemical solubility of these chemicals in water and in lipiphilicity of PAHs within the component of root cell which is known to be crucial in the transport within the plant shoots. Similar data were reported by Meng et al.,(33) in restricted uptake of 5-6 ring of the plant compared to 2-4 ring of PAHs while the uptake of pyrene was several times greater than of Phenanthrene (3 ring). Subsequently, increasing of RCF with increasing of contact time between root and solution and lipophilicity also the accumulation of these PAHs correlated with their concentrations in soil and plant composition (18, 25). Results of translocation factor (TF) showed that high values of LMW-PAHs were detected for both plants, especially in spring, which was ranged (0.4-48) in P. australis at St.4 and St.1 respectively (Fig.4). In T.domngenesis, translocation factor ranged 0.98-43) in

summer at St.5 and St.1 respectively (Fig.5). However, TF values decreased during the winter for all stations except St.5 that may be due to increased pollution level of PAHs in this site (4). Significant differences were detected ( $P \le 0.05$ ) of TF between HMW and LMW PAHs from roots to leaves of both plants respectively. This variation might be associated with different molecular weight and solubility of PAHs or related to plant species. In addition, low values of TF with HMW were found for all stations and seasons, whereas high values of them (0.62 and 16.7) were observed on both plants respectively in the spring and low factors of (0.03 and 0.1) noticed in the summer for both plants this can be due to increasing of biodegradation in rhizosphere zone. Also, seasonal variation of organic pollutants probably due to the increasing number of bacteria grown on the root system and bottom sediments. (28). Consequently, the observed between difference P.australis and T.domingensis in concentrations of PAHs can be linked to plant species characteristics, rhpizospheric properties including (root structure, root exudates, presence of rhizospheric microorganisms, rhizobia symbionts). In addition, the transpiration rate and the lipid content of root cell fractions are the main drivers of the subcellular partition of PAHs in roots (25). This work outlined the importance of aquatic plant in promoting the accelerated removal of PAHs in freshwater sediments either by degradation of LMW-PAHs with rhizosphere microorganism or restricted of HMW-PAHs in root tissues. P. australis and T. domngnesis in dicted high efficiency in dissipation of PAHs from sediment. These plants have a high ability to tolerate high levels of organic pollutants. So, it can be used as phytoremedidant.





Stations and Months Fig.2. Root Concentration Factor (RCF) of HMW-PAHs and LMW-PAHs in P. australis roots in study region during the four seasons (2011-2012). (P<0.05).



Fig.3.Root Concentration Factor (RCF) of HMW-PAHs and LMW-PAHs in T. domingnesis roots in study region during the four seasons (2011-2012). (P<0.05).



Stations and Months

Fig.4. Translocation Factor (TF) of HMW and LMW in P .australis roots in study region during the four seasons (2011-2012).



Fig.5.Translocation Factor (TF) of HMW and LMW in *T. domingnesis* roots in study region during the four seasons (2011-2012).

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