A study of Potamogeton crispus L. and P.pectinatus L. plants as accumulative bioindicator of PAHs compounds pollutants in Diwaniyah River

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Abstract:

The current study intended to identification and measurement of polycyclic aromatic hydrocarbons (PAHs) concentrations and their bioaccumulation in two species of Potamogetonaceae ; potamogeton crispus and P.pectinatus from three stations in Diwaniyah River during growth period from Jan.-Jun.2012.The results of study showed that the total PAHs concentrations ranged between (1.1-52.4 and 2.05-13.99 µg/g dry weight) and 4-rings PAHs group was recorded high concentration of (0.418-4.124) and (0.318-8.072 µg/g DW) for both plants respectively. Therefore, the study investigated dominance in compositional pattern for this group of (63.3 and 60.1%) at first site during the growth period for both plants respectively. The statistical analysis showed no significant differences between plants while the study stations were significantly different especially in south Diwaniyah city. The present study showed that the main sources of low molecular weight is incomplete combustion of oil and derivatives (pyrogenic origin) at study region while the source of high molecular weight is an oil spill and municipal wastewater (pathogenic origin .(

دراسة نباتي الـ Potamogeton crispus L. والـ Potamogeton pectinatus L. كأدلة حياتية تراكمية للتلوث بالمركبات الهيدروكربونية الأروماتية متعددة الحلقات (PAHs) في نهر الديوانية

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الخلاصة:

هدفت الدراسة الحالية الى تشخيص وحساب تراكيز ستة عشر مركبا من المركبات الهيدروكربونية الأروماتية متعددة الحلقات(PAHs) وتراكمها الحيوي في نوعين من النباتات المائية هما Potamogeton crispus L. و Potamogeton pectinatus L. النامية في نهر الديوانية وجمعت عينات الدراسة من ثلاث محطات على النهر خلال فترة النمو ابتداء من شهر كانون الثاني لغاية حزيران 2012 . اظهرت نتائج الدراسة ان المجموع الكلي لتراكيز الـ PAHs تراوح بين (1.1- 52.4 و 2.05- 13.99مايكغم/غم وزن جاف) في النباتين على التوالي وسجلت مجموعة المركبات ذات الاربع حلقات بنزين اعلى تركيز(0.148- 4.124) و(0.318-8.072) مايكغم/غم وزن جاف للنباتين على التوالي ولذلك كانت هذه المجموعة من المركبات هي السائدة عند تحليل الطرز التركيبية لمجموع المركبات وسجلت اعلى النسب المئوية 63.3%) و 60.1%) خلال فترة النمو في المحطة الاولى لكلا النباتين على التوالي . لم تظهر نتائج التحليل الاحصائي اية فروقات معنوية بين النباتين طيلة فترة الدراسة الا ان التراكيز كانت

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مختلفة معنويا في المحطة الثالثة(جنوب المدينة) عن المحطتين الآخرتين. بينت نتائج تحديد مصادر التلوث بهذه المركبات ان ذات الاوزان الجزيئية الواطئة منها كانت من مصادر احتراق النفط ومشتقاته اما المركبات ذات الاوزان الجزيئية العالية فكان مصدرها من حوادث السكب المتعمد للمنتجات النفطية او مع مياه مجاري البلدية التي تطرح الى الانهار مباشرة.

1.Introduction :

Polycyclic aromatic hydrocarbons (PAHs) in the ecosystems are becoming increasingly prevalent contaminants due to increasing of urbanization and industrial contamination (NRC, 1983). Shallow water near urban and industrial areas receives large quantities of hydrocarbons from many sources, especially surface runoff, wastewater discharges, and chemical refineries (Connell, 1982). PAHs are generated from anthropogenic activities such as industrial processing, from incomplete combustions of fossil fuels, and spillage of petroleum, PAHs may enter the aquatic ecosystem either directly, by effluents or oil spills, or indirectly by terrestrial runoff or atmospheric deposition. PAHs may accumulate in vegetation due to contaminant affinity for lipids and such accumulation is important in terms of PAHs fate in the environment (Barber et al., 2003a, b .(

Potamogetonaceae, a rooted submerged plant, grows in freshwater lakes, ponds, rivers, and streams all over the world. It is a fast growing plant, which produces high biomass and has shown potential to accumulate considerable amounts of heavy metals. According to the characters which are identified by (Molfetas and Blandin, 1981) Potamogetonaceae plant measured as good bio-indicator species because of sedentary, of ecological importance, widespread and widely studied also sensitive to environmental variations especially organic and inorganic pollution. In Euphrates river, PAHs compounds were found in aquatic plant with concentrations several times higher than those in the water column and the distribution of PAHs in water, sediment and aquatic organisms may have the origin from different sources (Pyrogenic, Petrogenic and urban air) (Mohamed, 2007; Al-azawey, 2012; Alwan, 2013). Therefore, plants can be used as sentinel species and bioindicators for the detection of PAH contamination in the environment (Greenburg, 2003). Accumulation of PAHs by plants represents a starting point of hazardous compounds into the food web particularly in co-contaminated state with heavy metals, initiating a biomagnification process (Jones et al. 1989; Salanitro et al. 1997; Thomas et al. 1998 .(

Exposure to low doses of PAHs can stimulate the plant growth, but high doses of PAHs hamper and eventually inhibit plant growth (Ma, et al., 2010). The inhibition of photosynthesis is a key mechanism of toxicant action in plants and chlorosis (Huang et al. 1997; Marwood et al., 2001). Further, reactive oxygen species induction, plasma membrane damage. So under low concentrations, plant uptake is also one approach involved in phytoremediation for organic contaminated sites, and information about plant PAH

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concentrations is essential to predict the effectiveness of a phytoremediation operation (Sung et al., 2002; Gao et al., 2003; Joner and Leyval 2003; Gao and Zhu 2003, 2005). In the current study, we investigated the accumulation of Polycyclic aromatic hydrocarbons in Potamogetonaceae plants at Euphrates river branches (Diwaniyah River) and calculated compositional pattern in these plants and determination of PAHs compound sources in the study region.

.2Materials and methods:

.1-2Samples collection and pretreatment :

Two species of aquatic plants of Potamogetonaceae ; potamogeton crispus and P.pectinatus were classified according (Haynes and Nielsen, 2003). The plants were collected from three stations on Diwaniyah river (figure 1), It washed with river water then wrapped with aluminum foil. All samples will be labeled by station number, date, plant type. On return back to the laboratory (Environment Lap. Department of biology. College of Science/ Babylon Univ.), Plant samples were ground and homogenized, and ten grams were extracted by ultrasonication for 1 h in 100 ml of acetone and hexane mixture (1:1). The solvent was then decanted, collected and replenished. The sample was then sonicated for an additional hour. This process was repeated. The solvent fractions were combined and passed through an anhydrous Na2SO4 column with elution with 1:1 (v/v) acetone and hexane. The solvents were then evaporated and replaced with 2 ml hexane, followed by filtration through 2 g silica gel column, then eluted with 11 ml of 1:1 (v/v) of hexane and dichloromethane. The samples were then evaporated and exchanged to cyclohexane with a final volume of 2 ml (Kipopoulou et al., 1999 .(

-2-2PAHs analysis and calculation:

External standard method was used in quantifications of 16 PAHs based on five-point calibration curves for individual compounds. Plant samples were analyzed by high performance liquid chromatography Schimadzu (UV/vis detecter2500, and supelcosil LC –PAHs column(50 *4.6mm, C18, 3µm particles) at the college of Pharmacy /Kufa University. The mobile phase was acetonitrile and water (both HPLC-grade) in a linear gradient program 60% acetonitrile(0.3 minute) to100% (over 2.7 min)with flow rate 2ml/min ,Detector(UV,254) five µl of the sample was injected into a stationary phase capillary column. The peak in the chromatogram was identified by comparison of the retention time and spectra of standard with those in the sample (APHA, 2003; EPA, 2012). Standard 16 PAHs were obtained from Aldrich Chemical Co. they are included Naphthalene(Nap) 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-

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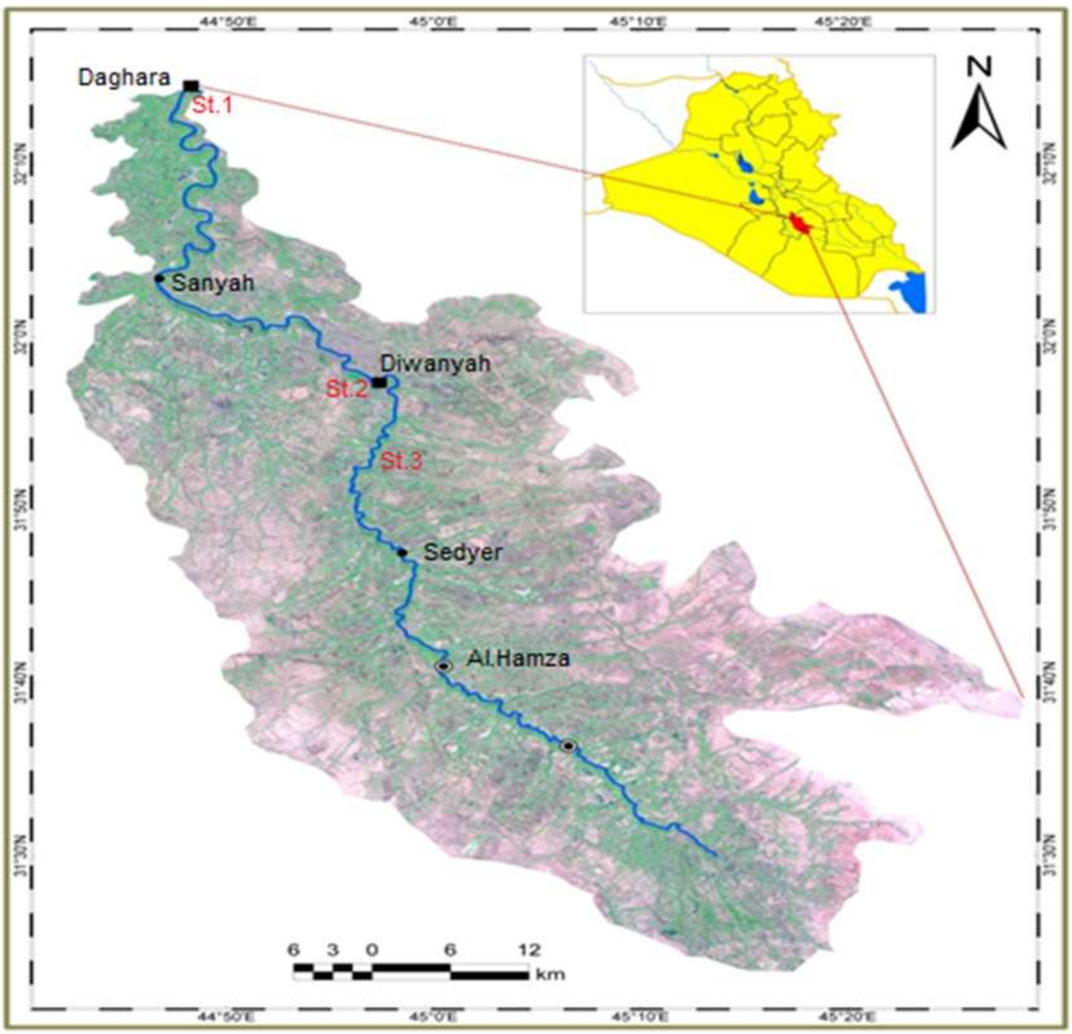
c,d)pyrene (IND), Benzo(g, h,i)perylene (B[ghi]P .(

According to the ratio values (Phen. /Ant, Flu/Py, BaA/Chr and low molecular weight LMW-PAHs to high molecular weight HMW-PAHs) the PAHs origin was determined, and the compositional profile was calculated by normalizing the individual PAHs in plant samples to the total amount of PAHs .

-4-2statistical analysis :

Statistical analysis was performed by Microsoft Excel statistical software to determine significant differences among individual PAHs for stations and growth period (6 months). SPSS program used by one-way analysis of variance (ANOVA) to analyze current data and LSD values.



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-Results and discussion:

The chemical analysis of 16 PAHs in Potamogeton pectinatus and P.crispus samples indicated that significant increase of total PAHs concentrations (13.99 µg/g dry weight DW) in P.pectinatus samples were collected from St.3 (5 km after Diwaniyah city center) during April, while low concentration (2.06 µg/g)was detected at St.2 during

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Feb. (table 1) .The concentration of total PAHs in P.crispus (table 2) ranged (1.1-52.4µg/g DW) at St.1 during Mar. and St.3 through Jun. respectively. These results may be pointed out that the increase in bioavailability of PAHs during Apr. with increasing of concentration in downstream of the Diwaniyah River by many sources such as wastewater effluent and industrial activities (many point sources of municipal wastewater were discharged directly into these rivers and several vehicles washing places randomly distributed along rivers, as well as textile and tire factories at south Diwaniyah city). The morphological changes shape, area, thickness of the cuticle and total lipid of leaves are important factors in accumulated of PAHs because their lipophilic, they tend to accumulate in plants, especially in membrane bilayers (Duxbury et al. 1997; Thomas et al. 1998).Therefore, the current results showed the significant variation of PAHs accumulation between both plants .

The high and low concentration of 2 ring-PAHs(Naphthalene) in P. pectinatus and P. crispus plant were(0.2-4.6 µg/g dry weight)during Jun. and Jan. for (St.2 and St.3) and (0.12-3.3 µg/g DW) through Apr. and Jun. for (St.1and St.3) for both plants respectively. As related to 3ring–PAHs(Acenaphthalene, Acenaphthene, Fluorene, Phenanthrene and Anthracene) in P.pectinatus, ranged between(0.272-3.232µg/g DW) were recorded during Mar. at St.1 and Apr. at St.3 respectively. In P.crispus plant, the concentrations ranged(0.28-13.786 µg/gDW) during Apr. and Jun at St.2 and 3 respectively. The concentrations of 4 ring-PAHs (Fluoranthene, Pyrene, Benzo(a) anthracene and Chrycene,) in P.pectinatus plant ranged between((0.318-8.072 µg/gDW) were detected of during Jan. at St.2 and St.3 while this compound ranged between (0.418-4.124 µg/g) in P.crispus plant at St.1 during Mar. and Jun. respectively. As related to the 5 ring-PAHs (Benzo(b) Fluoranthene, Benzo (k) Fluoranthene, Benzo (a) pyrene) and Dibenzo(a)anthracene high concentration (1.356 and 0.819 µg/g) was recorded for during Jun. at St.3 and St.1 for both plants respectively, while this compound not detected during some months also other high molecular weight compound (6 rings) Benzo(ghi)perylen and Indeno(1,2,3-c,d)pyrene (IND) was not detected at different stations. While high concentrations (0.38 and 0.84 µg/g) of 6 rings compound for both plants also respectively. The statistical analysis showed no significant capability of uptake potential of total PAHs in concentrations of individual compounds between plants (T-test) while the study stations were recorded significantly differently ($p < 0.05$); especially at south Diwaniyah city (St.3) for both plants because of similarity in growth styles and nearby of pollution sources. The statistical analysis referred to significant differences in the concentration of HMW- PAHs compound only ($p < 0.05$) were recorded during months' growth with those LMW-PAHs due to low water solubility and hydrophobicity, most PAHs in aquatic environments are associated with dissolved or suspended

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particulate materials as well as physical dissipation of PAHs such as volatilization, photoxidation and biodegradation processes.

The accumulation of PAHs compounds in plant tissues depends on several factors back to chemical and physical properties of this pollutant or eco-physiological factors in P. pectinatus such as growth season and plant length and age as well as current velocity and total particulate matter affect trapping suspended and particulate organic matter from plant body which work as adsorption surfaces for PAHs. PAHs may accumulate in vegetation due to a contaminant affinity for lipids and such accumulation is important in terms of the fate of PAHs in the environment (Barber et al., 2003a,b). The uptake process of semi-volatile organic compounds based on KOA (octanole-air partition coefficient) and solubility because of solubility is a basic parameter to study PAH bioavailability and potential accumulation in the organisms (McLachlan, 1999 ; DiToro et al., 2000). Therefore, With increasing number of rings, PAH becomes less water soluble and more lipophilic. So, bioconcentration of PAHs by aquatic plants increase with the partitioning of PAHs between the sediment and root system of plant and the movement of PAHs between the root and shoot (McGlynn and Livingston, 1997). In addition to the ambient water concentration and properties of PAH species, aquatic plant uptake of PAHs also depends on some factors of plants, including specific area of leaves, lipid content, surface hair, stomata density, cuticle structure and stomata pores.

Generally, high initial concentrations of PAHs in water and sediments in the Diwaniyah River (Alwan, 2013) corresponded to high PAHs uptake in plant tissues. Therefore, bioconcenteration factor was recorded high values in some aquatic plants which are growing in Euphrates River (Mohamed,2007; AL-Azawi,2012; Alwan, 2013). Irwin (1997) cited that the concentrations of PAHs in plants can be many times higher than those in water due to their low solubility in water and tend to accumulate in the sediment and biota depend on their content of organic matter also Potamogetonaceae grow in aquatic freshwater ecosystems, especially where the substrate has high organic content (Roper et al., 1997; Haynes and Nielsen, 2003). The results showed that significant differences between both plant in compositional patterns of PAHs accumulated in plant leaves, the individual PAHs in plants samples were normalized to the total amount of PAHs to calculate the mean percentage of 2,3,4,5,6 rings of total PAHs in potamogeton crispus and p.pectinatus plants during growth period in Diwaniyah River at study stations(figure 2). The high mean percentage was recorded 4 rings 63.3 and 60.1% at St.1 for both plants during Jun. And Apr. respectively, while 6 rings not recorded at St.1 or was low mean percentage which compared to other groups. In aquatic systems, PAHs exists in many forms freely dissolved, dissolved organic matter associated or

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suspended particulate matter associated, and sediment-associated phases. Therefore, the physical and chemical properties of the individual species, such as solubility, vapor pressure, and sorption coefficient. As well as the characteristics of the phase are primarily controlled in the distribution of PAHs among the phases of PAHs and bioavailability of PAHs in aquatic ecosystem(Readman et al., 1984; Zhou et al., 1999). The results of this study indicated that the efficiency of Potamogeton crispus and P.pectinatus plant to sorb PAHs from their surrounding environment, especially the high molecular weight 4 rings agreed with the results of (Hsu, 2004) whom demonstrated that great potential in application used for treating waste water containing PAHs by Ceratophylum demersum and Naja gramunea. Duxbury et al. (1997) indicated that the Lemna gibba plant had a high capacity for uptake and depuration kinetics of three representative Ant., Phe. and B[a]P. Mohamed et al., (2010) observed that high concentrations of HMW-PAHs were accumulated in two aquatic plants (Ceratophyllum demersum and Typha domingensis) which are collected from the Euphrates River and determined the possible sources of these compounds (pyrogenic, petrogenic and urban air), a similar conclusion was made by Al-Azawi, (2013) were studied four macrophytes are P.pectinatus, P.perfoliatus, Ceratophyllum demersum and Typha domingensis at Hilla river.

PAHs are introduced into the environment, neither via point sources such industrial discharge, fossil fuel combustion and petroleum spill or nonpoint sources, such as urban runoff and atmospheric fallout (Countway et al. 2003; Liu et al. 2008). Therefore, PAH isomer ratio calculations were designed for determination of PAHs origin, so Phe. /Ant. and Flu./Pyr. Were examined for the both aquatic plants according to (Hwang, et al.,2003). All sites of the plant samples were Phe. /Ant. < 10 and Flu/Pyr. < 1(table 3). In both plants pyrogenic sources have a high abundance of LMW PAH such as incomplete combustion processes and petrogenic sources for HMW Chr/BaA >1. While the LMW / HMW ratio was determined as pyrolytic sources in station 1 and petrogenic origin in two other stations. However, some sources are in-between pyrogenic and petrogenic profiles, e.g. used lubricating oil and asphalt (Brown and Peake,. 2006).

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Table (1):Monthly changes in concentrations of 16 PAHs (µg/g dry weight) in *Potamogeton pectinatus* plant samples at study stations during growth period in Diwaniyah River (Jan.-Jun.2012).

Months	Stations	2 rings	3 rings	4 rings	5 rings	6 rings	Total PAHs
Jan.	St.1	1.2	0.343	1.468	0.18	0.024	3.22
	St.2	0.8	0.956	0.318	0.2	0.024	2.3
	St.3	4.6	2.05	2.54	0.41	0.218	9.82
Feb.	St.1	1.1	0.49	1.302	0.19	0.076	3.16
	St.2	1	0.478	0.35	0.22	0.011	2.06
	St.3	3.33	2.37	1.21	0.32	0.107	7.34
Mar.	St.1	1	0.272	2.02	0.21	0.097	3.6
	St.2	0.8	0.42	1.64	0.177	0.06	3.1
	St.3	1.2	1.16	0.61	0.21	0.092	3.23
Apr.	St.1	0.9	0.34	2.2	0.184	0.031	3.65
	St.2	1.2	0.394	1.184	0.236	0.031	3.045
	St.3	1.8	3.232	8.072	0.784	0.102	13.99
May	St.1	0.54	0.497	1.604	0.53	0	3.17
	St.2	1.53	0.288	0.52	0.243	0.041	2.62
	St.3	0.9	0.371	1.042	0.138	0.06	2.51
Jun.	St.1	0.33	1.906	4.41	0.8197	0.006	7.47
	St.2	0.2	2.01	3.69	0.32	0.06	6.28
	St.3	3.8	2.122	2.499	0.784	0.38	9.585

Table (2):Monthly changes in concentrations of 16 PAHs (µg/g dry weight) in Potamogeton Crispus plant samples at study stations during growth period in Diwaniyah River (Jan.-Jun.2012)

Months	Stations	2 rings	3 rings	4 rings	5 rings	6 rings	Total PAHs
Jan.	St.1	0.152	0.404	0.94	0.214	0.086	1.8
	St.2	0.84	0.79	2.87	0.35	0.08	4.93
	St.3	1.1	1.1	3.74	0.42	0.14	6.5
Feb.	St.1	0.165	0.276	0.4274	0.24	0.082	1.2
	St.2	0.3	0.66	1.88	0.29	0.088	3.22
	St.3	0.54	0.612	0.964	0.204	0.132	2.45
Mar.	St.1	0.14	0.292	0.418	0.142	0.07	1.1
	St.2	0.22	0.31	1.01	0.142	0.1	1.8
	St.3	0.44	0.77	0.57	0.486	0.07	2.34
Apr.	St.1	0.12	0.673	0.94	0.114	0.03	1.87
	St.2	0.2	0.28	1	0.132	0.049	1.66
	St.3	1.4	1.33	0.5	0.33	0.08	3.64
May	St.1	0.36	1.047	1.875	0.217	0.0482	3.55
	St.2	0.36	1.047	1.875	0.217	0.0482	3.55
	St.3	2.3	2.05	1.86	0.98	0.118	7.31
Jun.	St.1	0.764	1.09	4.124	0.394	0.142	6.51
	St.2	0.42	1.45	2.9	0.394	0.142	5.31

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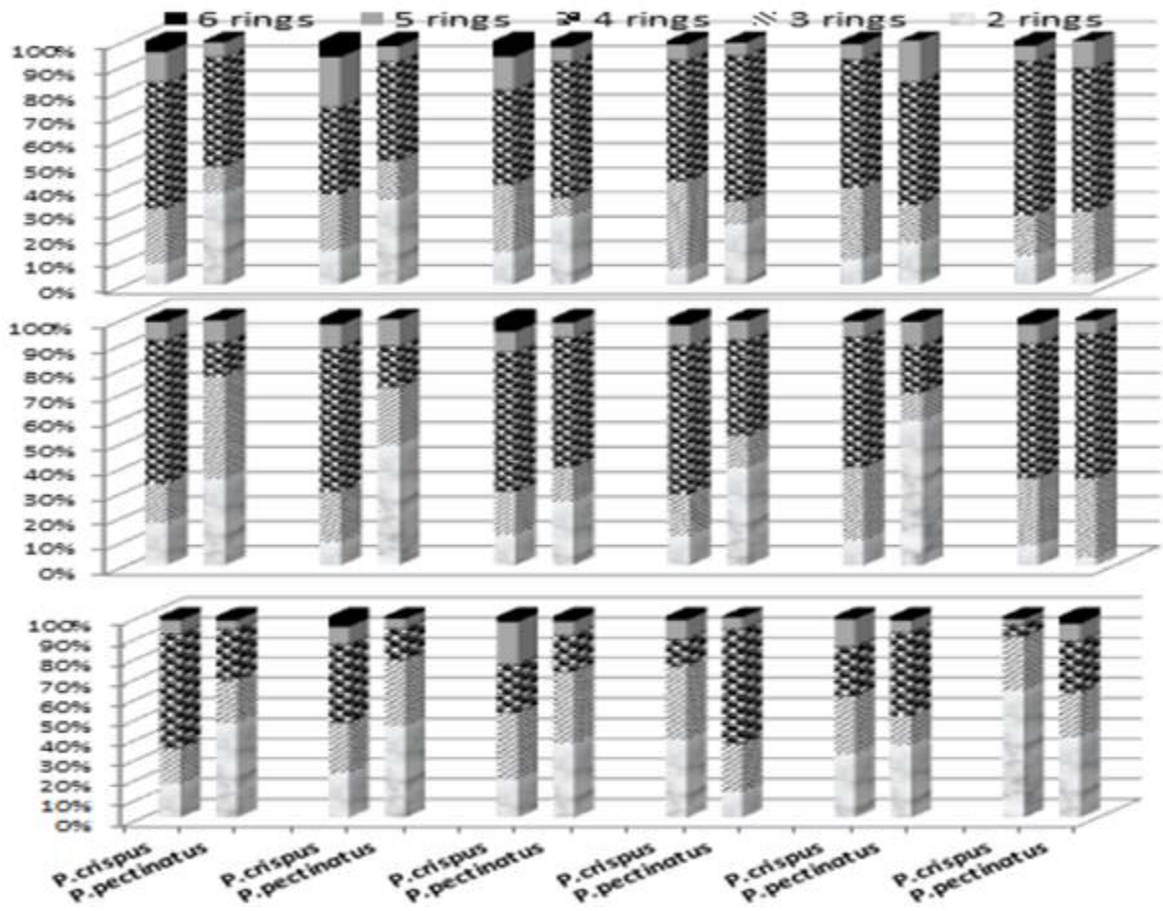

Figure(2): The mean percentage of 2,3,4,5,6 rings of total PAHs in *potamogeton crispus* and *p.pectinatus* plant during growth period in Diwaniyah River at study stations.

Table(3): Characteristic values of selected isomeric ratio for pyrogenic and petrogenic sources of PAHs in potamogeton crispus and p.pectinatus plant during study period (Jan-Jun 2012).

Site		Phen/Ant	Flu/Py	BaA/Chry	LMW-PAHs/HMW-PAHs
Pyrogenic origin		< 10	>1	< 1	< 1
Petrogenic origin		>15	< 1	>1	>1
St.1	*p.crispus*	0.45	0.1	1.3	0.6
	p.pectinatus	3.1	0.15	2.02	0.6
St.2	*p.crispus*	0.8	0.12	5.1	0.5
	p.pectinatus	1	o.4	1.3	3.2
St.3	*p.crispus*	0.9	0.6	1.15	2.5
	p.pectinatus	1.2	0.22	1.8	1.9

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