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Determination the Toxicity of Poly Aromatic Hydrocarbons Using HPLC in Diyala River Sediments

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Abstract

In this paper sixteen Polycyclic Aromatic Hydrocarbons (PAHs) are estimated in sediments in five locations along Diyala River where entering Baghdad city until flows into Tigris River, (PAHs) are organic compounds that contain two or more fused rings. Some of these compounds are classified as carcinogenic and mutagenic pollutants. Soxhlet technique is used in this work to extract PAHs from sediment samples with using methylene chloride as extraction solvent. High Performance Liquid Chromatography (HPLC) with UV-Vis detector is used for determination of PAHs. According to the results the maximum of total PAHs concentration in the sediment are found in the location No. ٤ with١٢١٣.٠٢mg/kg in summer and ٢٨٣,١٧ mg/kg in winter, while the minimum of total PAHs concentration are found in location No. ١ with ٤٦,٤٢ mg/kg in summer and ١٦,٧٠ mg/kg in winter. Locations numbers two three and five record values of total PAHs concentration with (١٤٨,٣٥, ٥٧٠,٤١ and ٩٥٨,٢٠ mg/kg) respectively in summer and (٥٥,٦٩, ١٠٠,٤٧ and ١٧١,٥٥ mg/kg) respectively in winter. Naphthalene, Fluorene, Anthracene and Fluoranthene are the most dominant compounds in summer, while Benzo [k] fluoranthene, Benzo [a] pyrene, Benzo [ghi] perylene, Dibenz [a,h] anthracene and Indino [١٢٣cd] pyreneare the least compounds presence in all sites.

Keywords: Poly Aromatic Hydrocarbons (PAH), High Performance Liquid Chromatography (HPLC), Soxhlet, Diyala River.

Introduction

Polycyclic aromatic hydrocarbons, also known polynuclear aromatic hydrocarbons, contain more than one fused rings without hC:\WINDOWS\hinhem.screteroatoms or substituents [١].United State Environment Protection Agency (USEPA) and the European Community [٢-٥]are classified sixteen of PAHs as priority pollutants Acenaphthene (Ace), Acenaphthylene (Acy), Anthracene (Anth), Benz [a] anthracene (B[a]anth), Benzo [a] pyrene (B[a]py), Benzo [b] fluoranthene (B[b]flan), Benzo [ghi] perylene (B[ghi]per), Benzo [k] fluoranthene (B[k]flan), Chrysene(Chy), Dibenz (a,h) anthracene (DB[a,h]anth), Fluoranthene (Flan), Fluorene (Fln), Indeno (١,٢,٣-cd)pyrene (In[١٢٣-cd]py), Naphthalene (Naph), Phenanthrene (Phe) and Pyrene (py)[١-٣]. PAHs classified as important environmental pollutants because they have high stability [٥], and their accumulation action in food chain in fatty tissues [٤]. High molecular weight of PAHs such as B[a] py have carcinogenic and mutagenic action [٣, ٦], low molecular weight PAHs also have toxic equivalence factor [٧]. PAHs introduced to the environment from natural and anthropogenic process essentially from incomplete combustion of fossil fuel such as (coal, petroleum) in power station process, vehicular emission and domestic heating [٣,٨]. Diyala River is important tributary of the Tigris River [٩]. Diyala River Enters into the city of Baghdad from the east passing through areas where the sewage water are thrown into River this increse the pollution, then the River enters the Rustumiya area, where that is the largest sewage treatment plant in Baghdad, the outlet of these big plant run into Diyala River after treatment, then Diyala River run into the Tigris River. The information in literature regarding PAHs levels in Iraqi environment are very rare. The aim of this paper is to determine the concentrations of priority sixteen PAHs in Diyala River sediment in Baghdad at several locations.

Experimental Sample collection and preparation

Samples are collected from five selected points in summer (between ٢٠-٣٠ July) and in winter (between ٢٠-٣٠ January) along Diyala River sediment in Baghdad according to

APHA [١٠] The samples sites are distributed in such a way that three of them before Al-Rustamiya sewage treatment plant and two sites after the plant, as shown in Fig. (١).

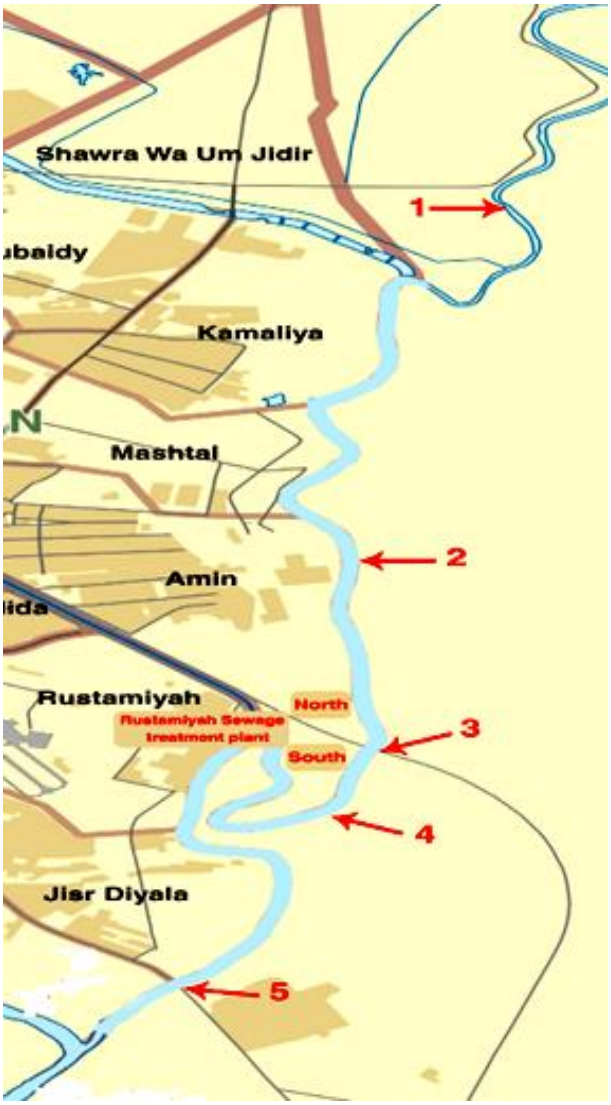

Fig.(١) Sites of Samples at Diyala River in Baghdad.

٢kg of sediments took from both River coast in each site, mix for homogenization of samples, then the samples dried in oven at ٦٠°C overnight. Weight ١٠ g from dried sediment as a test sample, mill the sample enter passing through ٤٥ micron particle size sieve. Extract the samples by soxhlet with ٢٥٠ ml of methylene chloride as an extraction solvent [١١].

Concentration the sample by using rotary evaporator to less than ١ ml, complete volume to ١ ml with methylene chloride. Samples now are ready to test in HPLC.

Materials

PAHs Standard Kit is used in this study was supplied from SUPELCO Analytical and Sigma-Aldrich GmbH/ Germany. The mobile phase solvents used in this study is a mixture of (Acetonitrile, Methanol, and Water), all solvents are HPLC grade supplied from (sigma-Aldrich, Hi Media Laboratories (England) & J.T Baker (Netherland) respectively.

Instruments

High performance liquid chromatography (HPLC); type Shimadzu (LC–٢٠ AD) was used for separating and detecting PAHs compounds with Column type (EC ١٦٠/٤ NUCLEOSIL ١٠٠-S C١٨ PAH) Stainless steel made, Length ١٥٠mm, I.D ٤,٦ mm, the column specially designed for PAHs analysis according to USEPA. Specification of the Stationary phase are as follows, support Silica, polymeric coated, spherical Particle shape, ٥µm Particle size, bonded phase with Special high-purity octadecyl-modified silica phase, and Pore size ١٠٠°A.

UV-Vis detector are used (Shimadzu SPD-٢٠A Prominence).

A special syringe made for HPLC type (M. SYRINGFE, ١٠٠F-LC) was used for injection ٢٠ µl of samples into the mobile phase to the column.

Results and Discussion

HPLC Analysis

Analytical chromatography was performed with a flow rate of ١,٠ ml/min at room temperature. The injection volume was ٢٠ µL. The column was stabilized for at least ٢ hours until uniform zero baseline before tests. Many conditions are carried out and studied for best separation. Best separation condition found with isocratic mixture mobile phase prepared from acetonitrile, methanol and water (٦٥, ١٥ and ٢٠ %) respectively at ٢٦٠ nm wavelength. The output signal from the UV-Vis detector was displayed continuously on the computer. Each PAH are tested alone to detect retention time then test mixture of sixteen PAHs together for five concentration (١, ٢, ٣, ٤, ٥ mg/l).Calibration curve was constructed from the relation between the concentrations of each PAHs and its absorbance area of

HPLC chromatogram using UV-Vis detector, equations of calibration curves are listed in Table (١).

Chromatogram of sixteen PAHs shown in Fig. (٢).

Retention time (t_R) found from HPLC Chromatogram while Capacity Factor (K'), Separation Factor (α), Resolution (Rs), and number of Theoretical Plates (N), all these factors are calculated from HPLC chromatogram peaks according the following equations [١٢], PAHs concentrations (mg/kg) results are calculated according the equation below and listed in Table (٢).

Table (١)
Equations of Calibration curves of PAHs.

PAH	*Cal. Curve equation*	*R^2*	*LD × ١٠$^{-٣}$ mg/mL*	*SD*
١-Naph.	**y = ٠,٢٦٤X + ٠,٠٧٨**	٠,٩٧٠	١,٠٦٠	٠,٠٨٥
٢-Ace.	**y = ١,٩٠١X - ٠,٨٥٠**	٠,٩٩٥	١,٣٢١	٠٢٥١
٣-Acy.	**y = ٠,٢٠٦X + ٠,٢٣٦**	٠,٩٧٦	٠,٩٣٦	٠,٠٥٨
٤-Flo.	**y = ٠,٠٧١X + ٠,٠٦٧**	٠,٩٩٣	٠,٥١٤	٠,٠١١
٥-Phe.	**y = ٠,١٥٩X + ٠,٠٧٧**	٠,٩٩٦	٠,٤٠٤	٠,٠١٩
٦-Anth.	**y = ٠,٠٥١X + ٠,١١٣**	٠,٩٧٢	١,٠٢٦	٠,٠١٦
٧-Flan.	**y = ٠,٠٧٩X + ٠,٠٧٦**	٠,٩٩٥	٠,٤٢١	٠,٠١٠
٨-Py.	**y = ٠,١١٣X + ٠,٠٨٦**	٠,٩٩٦	٠,٣٨١	٠,٠١٣
٩-Chry.	**y = ٠,٩١١X + ٠,٠١٨**	٠,٩٩٧	٠,٣٥٧	٠,٩٨
١٠-B[a]anth.	**y = ٠,٤٦٠X + ٠,٠١١**	٠,٩٩٦	٠,٣٩٦	٠,٠٥٥
١١-B[b]flan.	**y = ٠,٣١٦X + ٠,٠٢٨**	٠,٩٨٦	٠,٧٢٨	٠,٠٧١
١٢-B[k]flan	**y = ٠,٣٩٨X + ٠,٠٩١**	٠,٩٥٧	١,٢٧٦	٠,١٥٤
١٣-B[a]py.	**y = ٠,٣٢٨X - ٠,٠٤٩**	٠,٩٨٢	٠,٨٣١	٠,٠٨٣
١٤-B[ghi]py.	**y = ٠,١٨٥X - ٠,٠٦٤**	٠,٩٩٥	٠,٤١٥	٠,٠٢٣
١٥-DB[a,h]anthr.	**y = ٠,١١٩X - ٠,٠١٩**	٠,٩٨٠	٠,٨٦٩	٠,٠٣٢
١٦-In[١,٢,٣-cd]py.	**y = ٠,٨٢٢X + ٠,٠٩٧**	٠,٩٨٣	٠,٧٩٣	٠,١٩٧

Conc. of PAH (mg/kg)= [Conc. from HPLC (mg/l) × Vol. extracted (l)]/ Weight of sample (kg).

$$K'_A = \frac{t_R - t_M}{t_M}$$

$$\alpha = \frac{K_B}{K_A}$$

$$R_S = \frac{2[(t_R)_B - (t_R)_A]}{W_A - W_B}$$

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$

Each prepared sample were collected are carried out with HPLC at the best conditions to separate and detect concentrations of sixteen PAHs.

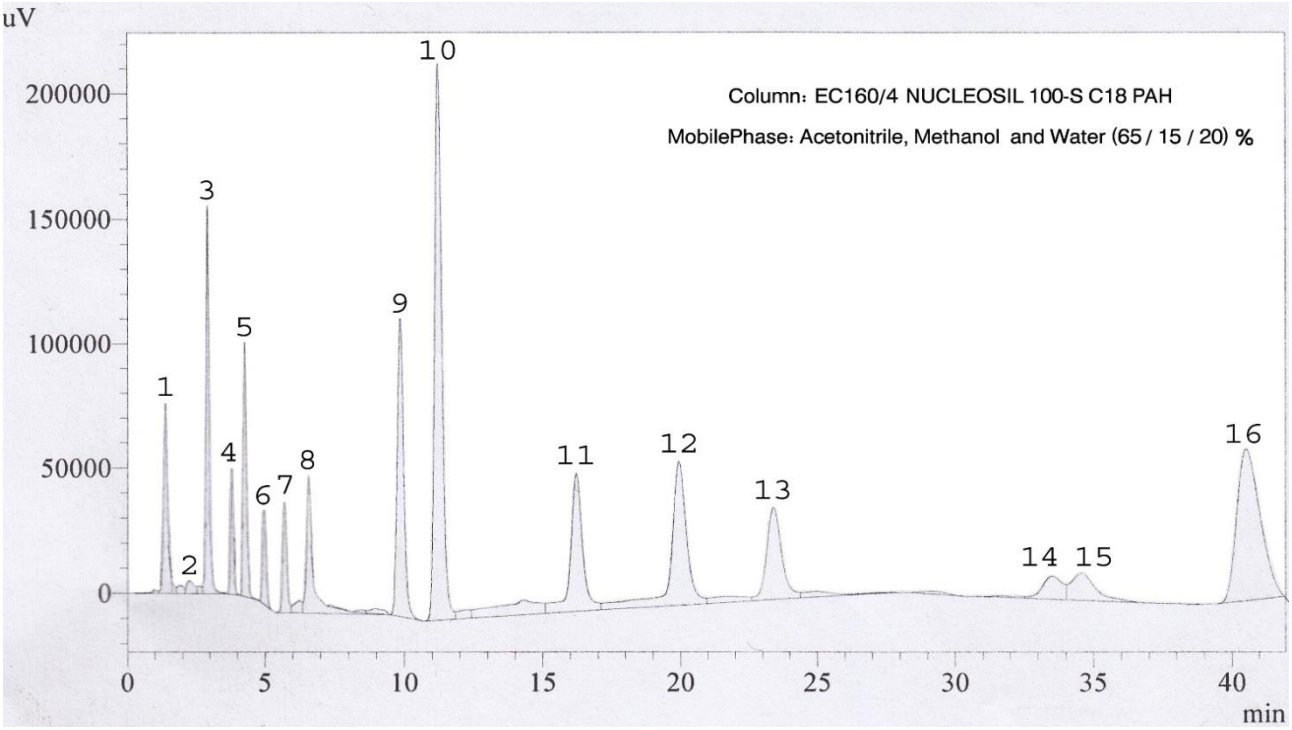

Fig. (٢) HPLC Chromatogram for ١٦ PAHs(٥mg/l) at ٢٦٠nm, flow rate ١ ml/min, mobile phase (Acetonitrile ٦٥٪, Methanol ١٥٪ and Water ٢٠٪)
١-Naph. ٢- Ace. ٣- Acy. ٤- Fln. ٥-Phe. ٦- Anth. ٧- Flan. ٨- Py. ٩- Ch. ١٠- B[a]anth. ١١- B[b]flan. ١٢- B[k]flan. ١٣- B[a]p ١٤- B[ghi]pyr. ١٥- D[a,h]an. ١٦- I[١٢٣-cd]py.

Table (٢)
Values of Retention Time (t_R), Capacity Factor (K'), Separation Factor (α), Resolution (Rs) and Number of Theoretical Plate (N).

PAH	t_R	*K'*	*α*	*Rs*	*N*
١-Naph.	١,٣٩	٢,٦٦	١,٨٧	١,٧٣	٣٠٦
٢-Ace.	٢,٢٧	٤,٩٧	١,٣٤	١,٢٦	١٥٤
٣-Acy.	٢,٩٢	٦,٦٨	١,٣٤	٢,٠٥	٢٠٩٩
٤-Flo.	٣,٧٩	٨,٩٧	١,١٤	١,٤٧	٤٤٩٩
٥-Phe.	٤,٢٨	١٠,٢٦	١,١٨	١,٨٠	٥٧٣٧
٦-Anth.	٤,٩٧	١٢,٠٨	١,١٦	١,٦٢	٩٩٩٧
٧-Flan.	٥,٧١	١٤,٠٣	١,١٦	١,٥٤	٤٥١٦
٨-Py.	٦,٥٨	١٦,٣٢	١,٥٣	٤,٨٢	٤٤١٨
٩-Chry.	٩,٨٧	٢٤,٩٧	١,١٤	١,٧٢	٤٨٦٧
١٠-B[a]anth.	١١,٢٣	٢٨,٥٥	١,٤٦	٥,٠٣	١١١٧٩
١١-B[b]flan.	١٦,٢٢	٤١,٦٨	١,٢٤	٣,١٥	٦٢٤٨
١٢-B[k]flan	١٩,٩٥	٥١,٥٠	١,١٨	٢,٥٦	٦١٢٥
١٣-B[a]py.	٢٣,٤٠	٦٠,٥٨	١,٤٤	٦,٧٤	٥٨٠٣
١٤-B[ghi]py.	٣٣,٥١	٨٧,١٨	١,٠٣	٠,٦٦	٥٣٠٤
١٥-DB[a,h]anthr.	٣٤,٥٦	٨٩,٩٥	١,١٧	٣,٠٢	٥٠٠٣
١٦-In[١,٢,٣-cd]py.	٤٠,٥٢	١٠٥,٥٦			٩٤١٣

Acynaphthene has a very small area at ٢٦٠ nm chromatogram because it has very poor absorbance at this wave length as shown in Fig. (٢)

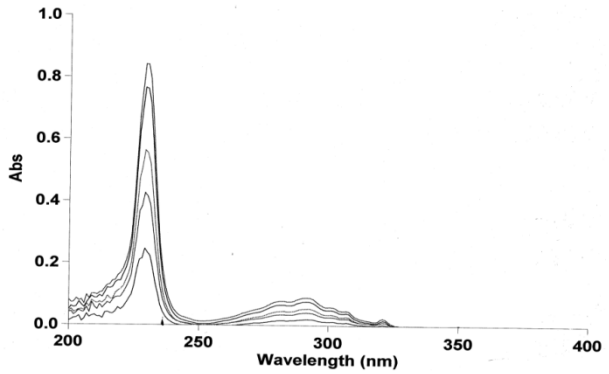

Fig. (٣) UV-Vis scanning spectrum for Acenaphthene (١, ٢,٣, ٤, ٥) mg/l, acetonitrile solvent.

Higher wave length absorbance area of Ace. was obtained at ٢٢٨ nm. So the HPLC detector set at ٢٢٨nm to detect Ace. as shown in Fig (٤).

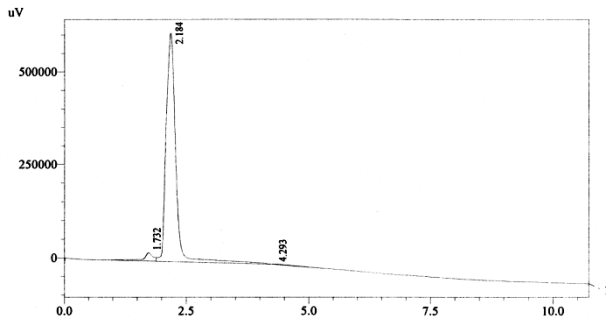

Fig. (٤) HPLC Chromatogram for acenaphthene at ٢٢٨nm

There are no enough information in the scientific literature for PAHs concentrations in Iraqi all environments for comparison with this study. The concentrations of the sixteen PAHs in sediment samples of Diyala River in summer and winter seasons are listed in Tables (٣).

Naphthalene, Acenaphthene, Fluorene, Anthracene, Phenanthrene and Fluoranthene. are the most dominant PAHs in summer season, while the concentration are less in winter season for all PAHs compounds. As can be seen in samples, location No. ٤ record the highest of total PAHs concentration in summer with ١٢١٣,٠٢ mg/kg, Anthracene (٣-ring PAH) record the maximum concentration in summer season in location No. ٤ with ٢٦٧ mg/kg. Naphthalene (٢-ring PAH) record high value with٢١٣,٧٣ mg/kgalso in location No.٤. Benzo [b] fluoranthene, Benzo [k] fluoranthene, Benzo [a] pyrene, Benzo [ghi] perylene., DiBenz [a,h] anthracene, and Indino [١٢٣-cd] pyreneare records the lowest concentrations in both seasons.

Table (٣)
Clay sample PAHs Conc. (mg/kg) in eight site at summer season.

Comp.	Summer Season					Winter Season				
	Site No. ١	Site No. ٢	Site No. ٣	Site No. ٤	Site No. ٥	Site No. ١	Site No. ٢	Site No. ٣	Site No. ٤	Site No. ٥
١-Naph.	١١,٦١	١٦,١٦	٨٥,١٢	٢١٣,٧٣	٢٠٦,٢١	٤,١٩	٥,٣٤	٥,٦٠	١٨,٣٥	٩,٣٤
٢-Ace.	ND	٤,٨٣	٢١,٧٩	٤٤,٦٢	٢٨,٧٠	١,٢٣	٢,٣٦	٥,٣٤	٦,١٥	٤,٩٨
٣-Acy.	٣,٦٢	٩,٤٨	٧٠,٧٦	١٠٥,٧٩	٣١,٣٧	٣,٤٦	٥,١٠	٨,٣٥	١١,١٥	٦,٤١
٤-Flo.	٦,٩٢	٢٣,٥١	١١٥,٢٦	١٠١,١٦	٢٥٨,٠٦	٣,٣٣	٧,٣٩	١٢,٧٠	٥٠,٠٢	٢٩,٧٥
٥-Phe.	٣,٢١	١٩,٢٧	٢٩,٦١	٤٣,١٢	٦٨,٢٥	٠,٨٢	ND	٢,٧١	١٠,٦١	٨,١٨
٦-Anth.	١٦,٨٧	٣٥,٨٠	٨٤,٥٤	٢٦٧,٧٥	٤٩,٨١	١,٠٨	١١,١٥	١٩,٣٨	٤٢,٣٥	١٤,٣٥
٧-Flan.	٢,٦٧	١٧,٨٤	٧٥,٣٦	١٩٩,٥٩	١٥٥,٠٢	١,٢١	١٣,٩٢	١٩,٧٧	٣٤,٧٧	٢١,٠٢
٨-Py.	١,٥٢	٤,٣٢	١٦,٠٢	٢٢,٥١	٦٩,٧٤	١,٣٨	٨,٠٣	١١,٤٧	١٦,٨٣	١٥,٢٦
٩-Chry.	ND	٢,٥٩	٣٢,٠٥	٦٧,٦١	١٩,٢٥	ND	٠,٣٤	٠,٨٥	١٣,٦٩	٥,٣٩
١٠-B[a]anth.	ND	١٢,٣٦	٢٤,٧٢	٧٣,٨٣	٢٠,٤٤	ND	٠,٢٨	١,٧٣	٢٥,٣٥	١٣,٨٢
١١-B[b]flan	ND	ND	ND	٧١,٧٣	٣,٥٤	ND	١,٧٨	١,٢٦	٢٥,١٣	ND
١٢-B[k]flan	ND	٢,١٩	ND	ND	ND	ND	ND	٢,٥٤	ND	٢٨,٩٩
١٣-B[a]py.	ND	ND	ND	١,٥٨	٢١,٦٢	ND	ND	٢,٠٢	٧,٧٣	٤,١١
١٤-B[ghi]per.	ND	ND	ND	ND	١,٧٨	ND	ND	٣,٥٢	١٣,٢٢	٣,٥٩
١٥-DB[a,h]anth	ND	ND	ND	ND	٥,٢٠	ND	ND	١,٧٤	٥,٢١	٤,١٩
١٦-In[١٢٣cd]py	ND	ND	١٥,١٨	١,٥٩	٠,٧٨	ND	ND	١,٤٩	٢,٦١	٢,١٧
Total PAHs	٤٦,٤٢	١٤٨,٣٥	٥٧٠,٤١	١٢١٣,٠٢	٩٥٨,٢٠	١٦,٧٠	٥٥,٦٩	١٠٠,٤٧	٢٨٣,١٧	١٧١,٥٥

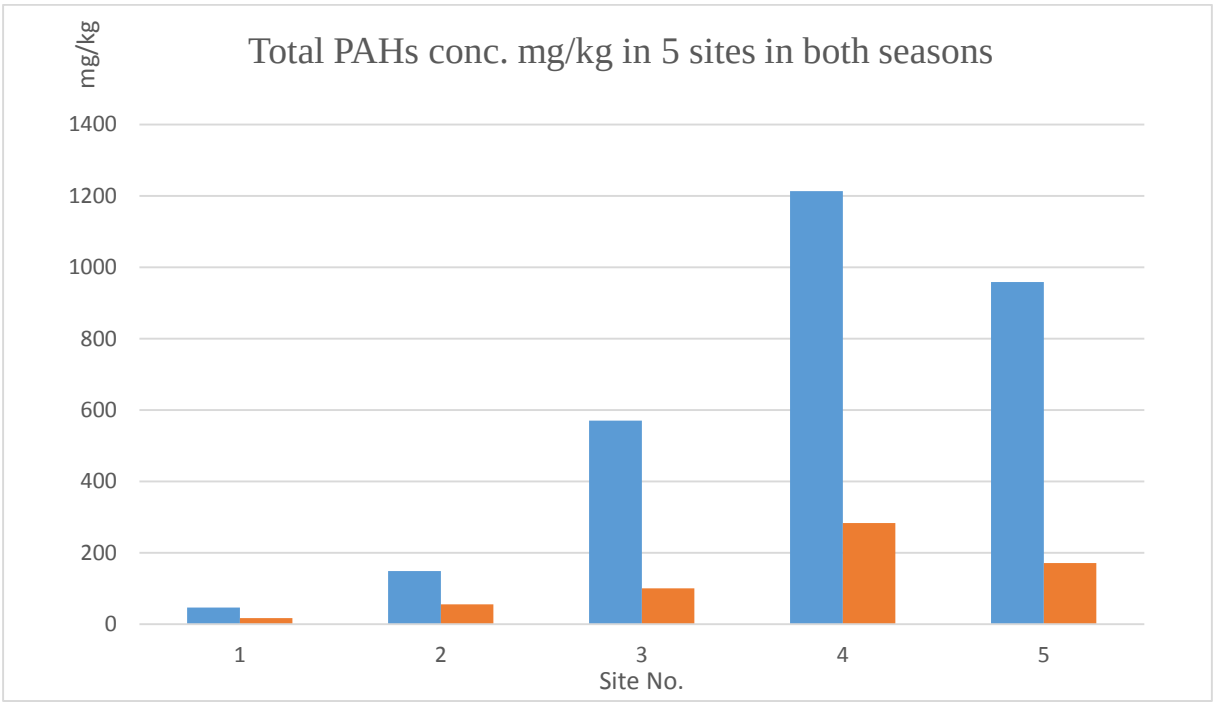

Fig. (٥) Total PAHs concentrations mg/kg in five sites studied at the Summer and Winter seasons.

Pollution of the river sediment due to deposition of pollutants on both sides from running water in the river. Accumulation of deposits gradually in the sediments leads to significant increase in the PAHs concentration because the half-life of PAHs compounds much longer than half life in the water because PAHs degradation mostly occur by radical mechanism depend on the access of ultra-violate from sun.

The results of PAHs in the summer sediment concentrations shows that the maximum of total concentrations values are founds in site No.٤, with total PAHs concentration of (١٢١٣,٠٢ mg/kg), this level of PAHs concentration is very high. Minimum of total PAHs concentrations values founds in site No.١ with (٤٦,٤٢mg/kg), then total PAHs concentrations increase to (١٤٨,٣٥mg/kg) in site No.٢, this increase due to thrown of untreated sewage and industrial water into river, a significant increase of total PAHs concentrations with (٥٧٠,٤١mg/kg) in site No.٣ before sewage treatment station, another increasing of total PAHs concentration in site No.٤ with maximum level concentration with (١٢١٣,٠٢mg/kg), then the total concentration decrease in site No.٥ to (٩٥٨,٢٠mg/kg).

Anthracene, Fluorene, Naphthalene, and Fluoranthene are record very high level PAHs concentrations in sediment between (١٩٩,٥٩ to ٢٦٧,٧٥ mg/kg) in sites No.٤ and No.٥ respectively.

Benzo [k] fluoranthene, Benzo [a] pyrene, Benzo [ghi] perylene, DiBenz [a,h] anthracene and Indeno [١٢٣-cd] pyrene are mostly not detected or low concentration in all sites in both seasons.

Other PAHs record different values of sediment concentrations in all sites as shown in Table (٢) and Fig. (٥).

In winter the PAHs concentration of sediment is much less compared with their concentration in the summer season; due to the significance difference in flow rate of the River. Site No.٤ also records highest value of a the total PAHs concentration with ٢٨٣,١٧ mg/kg, while a minimum of a total PAHs concentration with ١٦,٧٠ mg/kg is record in site No.١; the rest sites record a total PAHs concentration between (٥٥,٦٩ to ١٧١,٥٥ mg/kg**).**

Conclusions

Diyala River is a toxic environment for more than ٢٠ years ago and this toxicity increased day by day.

The results of this paper indicate that Diyala River sediments show very high levels of PAHs pollution, total PAHs concentrations found high level before and after Al-Rustamiya sewage treatment plant in about ١٥ km, then concentration decreased in Diyala River sediments before entry into Tigris River. Al-Rustamiya sewage treatment plant thrown high level of water polluted with PAHs into Diyala River, so it is very important to study the coefficient of this plant to remove these pollutants and maintain the ability of sewage plant to remove the PAHs pollutants from thrown water into Diyala River.

Reference

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نسب المركبات. حسب النتائج المستحصلة كان الحد الاعلى لمجموع تراكيز المركبات الاروماتية متعددة الحلقات في الرواسب سجل في الموقع رقم ٤ مع ١٢١٣,٠٢ ملغ/ كغم في الصيف و ٢٨٣,١٧ ملغ/ كغم في الشتاء، بينما كان الحد الادنى لمجموع تراكيز المركبات الاروماتية متعددة الحلقات في الموقع رقم ١ مع ٤٦,٤٢ ملغ/كغم في الصيف و ١٦,٧٠ ملغ/ كغم في الشتاء. المواقع بالارقام ٢ و٣ و٥ سجلت قيم لمجموع تراكيز المركبات الاروماتية متعددة الحلقات هي ١٤٨,٣٥ ٥٧٠,٤١ و٩٥٨,٢٠ ملغ/ كغم على الترتيب في الصيف و ٥٥,٦٩ و١٠٠,٤٧ و١٧١,٥٥ ملغ/ كغم على الترتيب في الشتاء. تراكيز المركبات Naphthalene, Fluorene, Anthracene and Fluoranthene كانت الاعلى في الصيف، بينما المركبات Benzo [k] fluoranthene, Benzo [a] pyrene, Benzo [ghi] perylene, Dibenz [a,h] anthracene and Indino [١٢٣cd] pyrene هي الاقل تواجدافي كل المواقع.

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الخلاصة

في هذا البحث تم تقدير ستة عشر من المركبات الاروماتية متعددة الحلقات في رواسب نهر ديالى عند خمس مواقع على طول النهر عند دخوله مدينة بغداد حتى يصب في نهر دجلة، المركبات الاروماتية متعددة الحلقات هي مركبات عضوية تحتوي على اثنين او اكثر من الحلقات الاروماتية. يصنف بعضها كمسببة للسرطان والتشوهات الخلقية. الاستخلاص بتقنية السوكسليت استعملت لاستخلاص المركبات من الرواسب باستعمال كلوريد المثيلين كمذيب استخلاص. كروموتوغرافيا السائل ذات الاداء العالي استعملت مع كاشف الاشعة فوق البنفسجية–المرئية لتقدير