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Purposeful scientific research is a basic pillar for the advancement of Iraq

Separation and determination of polycyclic aromatic hydrocarbons in fruits and vegetables by gas chromatography technique, Baghdad city, Iraq

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ABSTRACT
Polycyclic aromatic hydrocarbons (PAHs) are polluting compounds in various types of vegetables and fruits. The levels of these compounds depend on the source of the pollution It has been shown that the high percentage of PAHs in water, air and soil has a significant impact on the levels of PAHs in fruits and vegetable .The current study was conducted to detect and determine the levels of polycyclic aromatic hydrocarbons in samples of basil, mint, radish leaves, spinach, orange, mandarin, yellow apple, red apple, bitter orange, sweet lemon .Fruit and vegetable samples were collected from three different places and analyzed for thirteen polycyclic aromatic hydrocarbons .The analytical method included the use of gas chromatography technique to separate and quantify the PAHs compounds, under optimal separation conditions, and extract samples with the Soxhlet device. The concentrations of PAHs in vegetables ranged from (1.6262-5.3506 ppm) and in fruits (0.4203-3.6294 ppm). We also note the presence of chrysene in the basil sample with a higher concentration than all the compounds (2.2167ppm), while in the fruits, benzo as fluoranthene showed a higher concentration than all the compounds in the fruits with a concentration of (1.4034ppm). Among the mutagenic and carcinogenic polycyclic aromatic hydrocarbons, benzo(a) anthracene was found in high percentage in Dora region basil with (1.5538ppm), while acenaphthylene was not detected in all vegetable samples, acenaphthene and acenaphthylene were not detected in all fruit samples.

Keyword: Poly aromatic hydrocarbons, Fruit, Vegetables.

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants produced mainly during the incomplete combustion of organic compounds (such as wood, oil, coal and gasoline). These emissions result from human activities. Also, some PAHs originate in the environment from natural sources such as oil spills, coal deposits, open burning, and volcanic activities. The primary route of exposure to PAHs in the general population is food intake and air intake [1]. In addition to the important role that climate plays in the absorption and accumulation of PAHs in plants, a study conducted in China on spinach, to analyze four PAHs, showed that the concentration of these compounds increases in spinach roots 60.8-111.5% with increasing temperature from15/10 to 21/16 °C due to increased solubility of PAH compounds in the soil [2]. A study conducted in Brazil showed that the total levels of PAHs in vegetable samples (4.38-17.93 lg/kg) were higher than in fruit samples (3.77-4.05 lg/kg) [3]. It is necessary to assess the health risks posed by PAHs in vegetables exposed to soil and air that may be contaminated with these compounds. When studying the migration and transfer of PAHs from the soil to the roots of vegetables, it was found that the concentrations of PAHs in vegetables will increase when the concentrations of PAHs in the soil increase, the atmosphere is also another important source of PAHs in vegetables. Studies have shown that the concentrations of PAHs in the above-ground plant parts were greater than in the underground parts [4]. Vegetables link the environment and the human food chain. Contamination of various foods with polycyclic aromatic hydrocarbons is present everywhere because these compounds are lipophilic [5]. Pollution is the largest environmental cause of disease and premature death in the world today. In the worst affected countries, pollution-related diseases result in more than one in four deaths[6] .The lack of awareness by humans of the forms of pollution leads them to carry out activities that lead to the production of harmful by-products in unimaginable quantities and forms .The environment can no longer be balanced [7]. There are more than 500 polycyclic

aromatic hydrocarbons scattered in the atmosphere; Reactions between them and different types of chemical substances such as nitrogen oxides, ozone, and sulfur dioxide lead to the formation of many toxic chemicals [8]. PAHs consisting of two or three rings are considered low molecular weight and therefore non-carcinogenic, while PAHs consisting of more than three rings are considered to have high molecular weight and are carcinogenic [9]. The amounts of PAHs can vary greatly depending on the aromatic hydrocarbon, the area around the crops, or even the product itself [10]. In Iran, a study was conducted to evaluate the concentration of PAHs in vegetables, where these compounds were found in spinach, ,parsley and dill at concentrations of 49.8, 496.4, and 442 µg/ kg, respectively. Naphthalene, fluorine, anthracene, chrysene, phenanthrene, benzo[a]anthracene were discovered. In all vegetables, the compound benzo[a]anthracene was present in all samples, and the highest concentration of it appeared in the dill sample, and it is considered one of the most dangerous carcinogenic compounds [11]. Gas chromatography (GC) is usually the preferred method for separating, identifying and largely quantifying PAHs, in general gas chromatography provides greater accuracy, selectivity, and sensitivity than liquid chromatography [12]. Gas chromatography was used to detect the presence of polycyclic aromatic hydrocarbons (PAHs) in samples of vegetables grown near industrial areas in Shanghai, China, at concentrations (65.7-458.0 ng/g), Which exceeded the limit allowed by the US Food Protection Agency (USFDA)[13]. The current study dealt with the detection and estimation of PAHs in fruits and vegetables in different regions of Baghdad in Iraq using gas chromatography technique and under optimal separation conditions, the presence of quantities of PAHs that exceeded the permissible limit was detect According to the standards of the British Food Standards -UK, which confirmed that the sum of any four polycyclic aromatic hydrocarbons does not exceed (0.14ppm)[14].

2. Materials and methods

2.1 Samples

Samples were collected from parsley, spinach, mint, radish leaves, basil, yellow apple, red apple, bitter orange, sweet lemon,mandarin, and orange for the sake, taken (50 g) from each sample from three different places around Baghdad region in Iraq, in November 2021. Samples of vegetables and fruits were taken from areas near oil refineries and other types planted near industrial places, in addition to other samples collected from the side of the road and rural areas.

2.2 Materials used in the study

1- A mixture consisting of thirteen standard polycyclic aromatic hydrocarbons with a very high purity of not less than (99.9%) was selected prepared by the German company (D-86926 Greifenberg am ammersee, Flurstrasse).

1- Acenaphthene (Ace)
2- Acenaphthylene (Acy)
3-Fluorene (Flu)
4-Naphthalene (Np)
5- Benzo[ghi]perylene (B[ghi]Pe)
6-Phenanthrene (Ph)
7-Chrysene (Chry)
8-Pyrene (Py)
9- Benzo[α]anthracene (B[α]An)
10-Benzo[α]pyrene (B[α]Py)
11- Benzo[k]fluoranthene (B[k]Fl)
12- Benzo[a]fluoranthene (B[α]Fl)
13- Dibenz[ah]anthracene (Di[α,h]An)

2. Solvent used

Acetone of purity (99.0%) from Switzerland-fluka-chemika

n-Hexane of purity (99.0%) from GCC-UK

Acetonitrile of purity (99.9%) from BDH-UK

2.3 Preparing the solutions

A mixture of thirteen standard aromatic hydrocarbons with a concentration of 15 ppm was taken and from the standard stock solution a mixture of aromatic hydrocarbons was prepared by dilution with acetonitrile and different concentrations were obtained to construct the calibration curve (5,10,15) ppm.

2.4. Method:

1- Fruits and vegetables samples were collected from different agricultural areas around the city of Baghdad.

2- The samples were dried for each sample separately, but in different ways. Vegetable samples were dried after being thoroughly washed with sterile water under sunlight for a period of (6-7 days). As for the fruit samples, they were washed with sterile water and then cut with peels into small pieces using a knife and placed in an oven from J.P. type. SELECTA, s.a. at a temperature of (60 °C) for a period of (10) hours until it dries completely

3- Each sample was ground by a special mill for grinding to a very fine size (Lµ (100.

4- Take 50gm of the powder of each sample and put it inside the cellulose thumble finger, then put it in the part designated for it in the extraction device. Soxhlet Extraction

5- A mixture of normal hexane and acetone in a volume of (200 mL) and at a ratio of 1:1 was used as solvents for extraction, and the extraction process continued for (20) hours at the boiling point of the solvent mixture (50 °C).

6- The extracted material for each sample was placed on the Vortex Mixer, where it mixed the samples quickly to the point of homogeneity.

7- Put the extract for each sample in a centrifugation device on the highest rotation for a period of (10) minutes until the filtrate separated from the sediment.

8- Concentrate the filtrate only for each sample by means of a rotary evaporator.

9-Filter the extract using a spring filter, the hole size is 0.45µL.

10- After that, each sample was placed in a small, dark vial with the name of the sample and the date of extraction written on it.

11- Each sample was injected into a GC gas chromatography device, according to the device's analytical program.

3- Results and discussions

Each mixture was injected with three injections with the same concentration for three mixtures of different concentration (5,10,15) A calibration curve was found for each polycyclic aromatic hydrocarbon by taking the area of the peak for each compound over time and from the straight-line equation the detection limit and the quantitative limit for standard PAHs were also calculated. Find the concentration of all the aromatic hydrocarbons in vegetables and fruits as shown below.

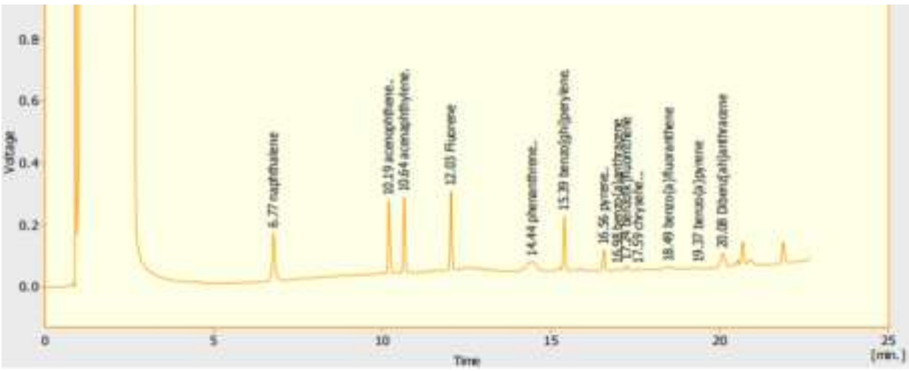

Fig. 1: chromatograms of a standard mixture containing thirteen polycyclic aromatic hydrocarbons at a concentration of 15ppm.

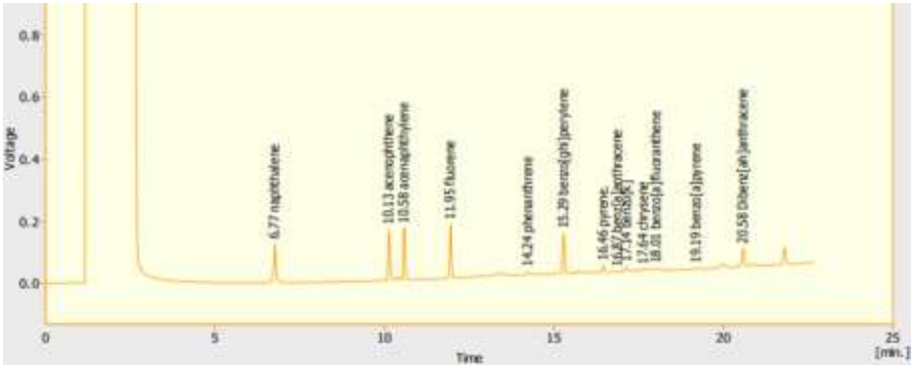

Fig. 2: chromatograms of a standard mixture containing thirteen polycyclic aromatic hydrocarbons at a concentration of 10 ppm

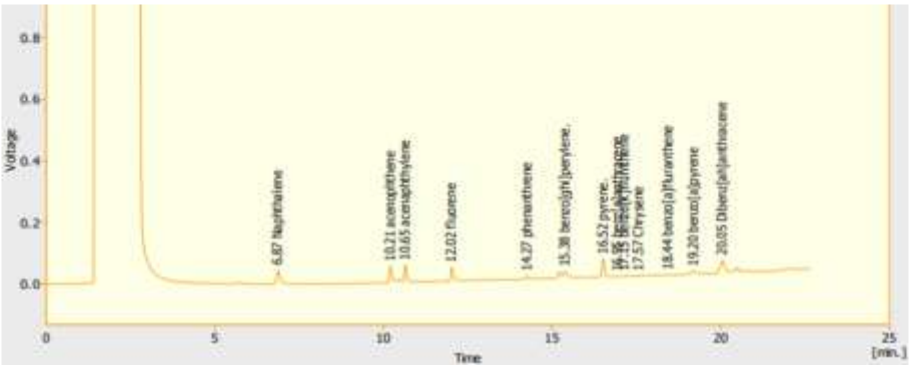

Fig. 3: chromatograms of a standard mixture containing thirteen polycyclic aromatic hydrocarbons at a concentration of 5 ppm.

The standard deviation, relative standard deviation, and relative error were also calculated from the equation of the straight line according to the equation.

$$S.D= \frac{\sqrt{\sum(Xi-\overline{X})^2}}{n-1} ....(1)$$

$$R.S.D = \frac{S}{\overline{X}} * 100 \;........... (2)$$

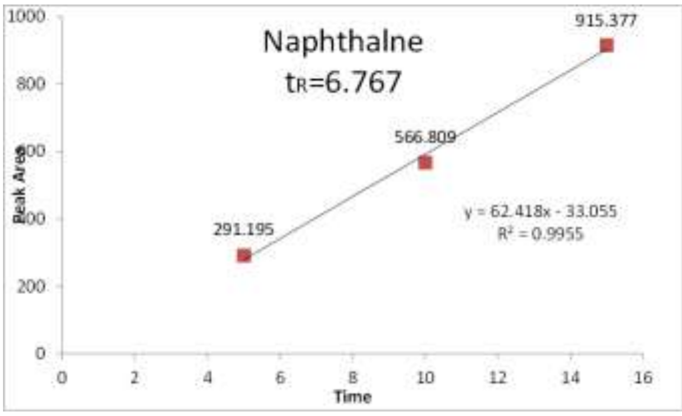

Fig. 4: represents the relationship between the concentration of Naphthalene against the area of the peaks in the standard mixture.

Table 1: Standard Concentrations of Naphthalene.

No	Name	Conc.(μg/mL)	Area	Median	S.D	R.S.D%	S.E
1	Cal-std1	5	291.195	291.184	0.019925	0.006842	0.006842
2	Cal-std2	10	566.7713	566.809	0.160843	0.028379	0.092863
3	Cal-std3	15	915.2667	915.257	0.105832	0.011563	0.061102

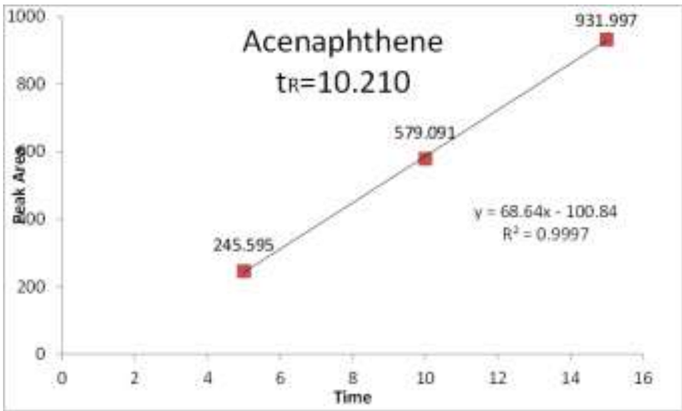

Fig. 5: represents the relationship between the concentration of Acenaphthene against the area of the peaks in the standard mixture.

Table 2: Standard Concentrations of Acenaphthene.

No	Name	Conc.(μg/mL)	Area	Median	S.D	R.S.D%	S.E
1	Cal-std1	5	245.595	245.619	0.115879	0.047183	0.066903
2	Cal-std2	10	579.091	579.121	0.150263	0.025948	0.086754
3	Cal-std3	15	931.997	932.036	0.129965	0.013945	0.075036

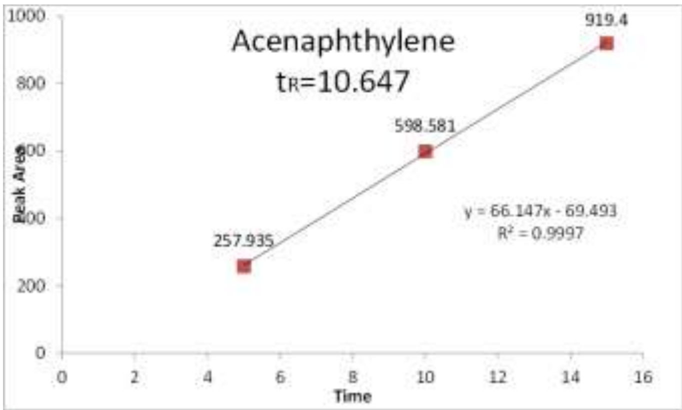

Fig. 6: represents the relationship between the concentration of Acenaphthylene against the area of the peaks in the standard mixture.

Table 3: Standard Concentrations of Acenaphthylene.

No	Name	Conc.(μg/mL)	Area	Median	S.D	R.S.D%	S.E
1	Cal-std1	5	257.935	257.981	0.084923	0.032924	0.049031
2	Cal-std2	10	598.581	598.621	0.125861	0.021027	0.072666
3	Cal-std3	15	919.4	919.383	0.062266	0.006772	0.035949

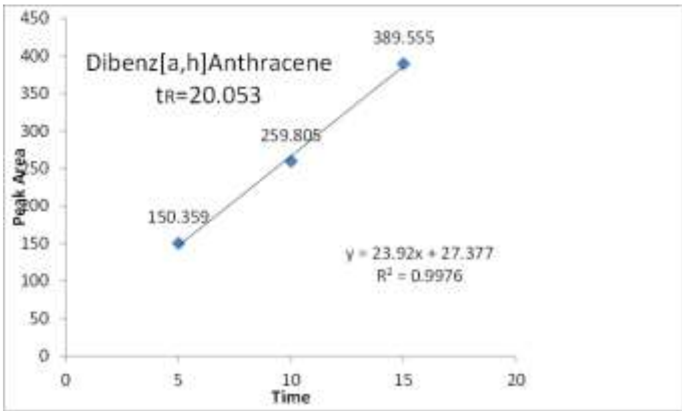

Fig. 7: represents the relationship between the concentration of Dibenz[a,h] anthracene against the area of the peaks in the standard mixture.

Table 4: Standard Concentrations of Dibenz[a,h]anthracene.

No	Name	Conc.(μg/mL)	Area	Median	S.D	R.S.D%	S.E
1	Cal-std1	5	150.3593	150.024	0.75785	0.504026	0.437545
2	Cal-std2	10	259.805	260.453	1.136253	0.437349	0.656016
3	Cal-std3	15	389.555	388.969	1.399272	0.359198	0.80787

The detection limit and the quantitative limit were calculated according to the following equation:

$$\text{Limit of Detection(LOD)} = \frac{3*S*Conc.}{a} \quad ..(3)$$

$$\text{Limit of Quantification(LOQ)} = \frac{10*S*Conc.}{a} \quad ...(4)$$

S :standard deviation
Conc.: is the lowest concentration of the compound in the titration curve
a :slope

Table 5: shows the number of rings, retention time, detection limit, and quantitative limit of thirteen standard polycyclic aromatic hydrocarbons detected using gas chromatography technique.

No.	PAH	N.of armatic rings	t_0 min	t_R min	LOD	LOQ
1	Naphthalene	2	0.8	6.770	0.00477	0.0159
2	Acenaphthene	3	-	10.193	0.0253	0.0844
3	Acenaphthylene	3	_	10.643	0.0192	0.0641
4	Fluorene	3	_	12.030	0.2229	0.743
5	Phenanthrene	3	_	14.443	1.3443	4.481
6	Benzo[ghi]perylene	6	_	15.387	0.2400	0.800
7	Pyrene	4	_	16.560	0.2154	0.718
8	Benzo[a]anthracene	4	_	16.977	0.4140	1.380
9	Benzo[k]fluoranthene	5	_	17.240	4.1204	13.7347
10	Chrysene	4	_	17.590	8.7183	29.061
11	Benzo[a]fluoranthene	5	_	18.487	12.5439	41.813
12	Benzo[a]pyrene	5	_	19.373	4.1265	13.755
5	Dibenz[ah]anthracene	13	-	20.083	0.4752	1.584

The analytical concentration of aromatic hydrocarbons in fruits and vegetables was found using the following equation

y =ax+b …….(5)
y =Peak area
a = Slope
x =Conc. (ppm) PAHs
b = Is the y intercept

And by multiplying the analytical concentration (ppm) by the volume of the solvent used (mL), divided by the weight (gm) of the sample taken, we get the actual concentration for vegetables and fruits.

Table 6: shows the concentrations of polycyclic aromatic hydrocarbons in separated vegetables using gas chromatography technique.

No.	PAH	Basil Doura	Spinach Doura	Mint Shuala	Radish leaves Shuala
1	Naphthalene	0.2639	0.1193	0.3727	0.6441
2	Acenaphthene		0.1531	0.2218	
3	Acenaphthylene				
4	Fluorene	0.7630	0.2011		
5	Phenanthrene	0.0550	0.1617		
6	Benzo[ghi]perylene				
7	Pyrene		0.1685	0.7700	
8	Benzo[a]anthracene	0.4987			
9	Benzo[k]fluoranthene	2.2162	0.7969	0.8144	
10	Chrysene			1.4817	
11	Benzo[a]fluoranthene				
12	Benzo[a]pyrene	1.5538			1.0418
13	Dibenz[ah]anthracene		0.0256		0.1014
14	Total	5.3506	1.6262	3.6606	1.7873

Table 7: shows the concentrations of polycyclic aromatic hydrocarbons in separated fruits using gas chromatography technique.

No.	PAH	Orange Rashidiah	Bitter orange Rashidiah	Mandarin Rahidiah	Yellow apple Shuala	Red apple Shuala	Sweet lemon Shuala
1	Naphthalene		0.4827	0.6677		0.6276	0.1039
2	Acenaphthene						
3	Acenaphthylene						
4	Fluorene						0.1578
5	Phenanthrene		0.4870		0.6724		
6	Benzo[ghi]perylene						
7	Pyrene	0.0845		0.9781		1.0500	
8	Benzo[a]anthracene		0.8258				
9	Benzo[k]fluoranthene						
10	Chrysene	0.3358		1.4034			
11	Benzo[a]fluoranthene			0.5802			0.2690
12	Benzo[a]pyrene			0.4561			
13	Dibenz[ah]anthracene	0.0840					
14	Totale	0.4203	1.7955	3.6294	0.6724	1.6776	0.5307

The concentration of PAHs in vegetables and fruits differed due to the different areas from which the samples were taken.

It is clear from the current study that the contents of PAHs in the fruits showed

Higher concentrations of leafy vegetables, where the concentrations of aromatic hydrocarbons ranged in vegetables (1.6262-5.3506ppm), while in fruits (0.4203-3.6294 ppm).

A similar study was conducted to the current study in Iran, where the results showed that the highest

Levels of PAHs appeared with naphthalene (5.0 ± 114.1 µg/kg) and acenaphthene.(7.1 ± 135.1 µg/kg), While it is noted that the lowest concentrations were from benzo[k]fluoranthene (not detected), Benzo[b]fluoranthene (not detected),(Indeno[l,2,3-cd]pyrene) (not detected) , Benzo[a]pyrene (not detected) and (Benzo[g,h,i] perylene (not detected)[104]. The location of the sample is considered one of the most important factors influencing the contents of vegetables and fruits from PAHs, therefore, we find that the highest concentrations were found in the basil area of Al-Dora (5.3506ppm) due to the presence of the oil refinery near the agricultural area from which the sample was taken. As for the fruits, Al-Rashidiyah region showed the highest concentration of PAHs, (3.6294ppm) due to the presence of an oil refinery near the agricultural area from which the fruit was taken.

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