

**The Concentrations, Distribution and Sources of Benzo (a) Pyrene in
Vegetables from Al-Diwaniya Province, Iraq.**

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) occur as pollutants in different types of food predominantly from environmental contamination, food packaging and food processing and the levels found depend on the source of the pollution. PAHs emissions from automobile traffic and industry activities are the main sources influence the PAHs levels and profiles in vegetables and fruits grown nearby. The present study was carried out to determine the levels of Benzo (a) Pyrene compound in samples of lettuce, tomato and cabbage and use these data to further estimate the dietary exposure of population how live in Al-Diwaniya province to Benzo (a) Pyrene compound. Samples of each vegetable were collected in six different places in Al-Diwaniya Province and analysed for Benzo (a) Pyrene compound. The analytical method involved saponification with methanolic KOH, liquid-liquid extraction with cyclohexane, clean-up on silica gel column and determination by gas chromatography. The maximum levels of Benzo (a) Pyrene were 9.32 $\mu\text{g/kg}$ in lettuce, 2.37 $\mu\text{g/kg}$ in tomato, 6.81 $\mu\text{g/kg}$ in cabbage.

Key words: Benzo (a) Pyrene, pollution of vegetables, Al-Diwaniya pollution, extraction of Benzo (a) Pyrene, PAHs

Chemistry classification : QD 450-801

Introduction:

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of chemical pollutants, which are being found throughout the environment in: air, water, soils and sediments [1,2]. Several studies were proved that existence of PAHs in food [3,4]. These compounds are represent group of highly lipophilic compounds containing fused aromatic rings ranged between two to

seven rings [5]. PAHs as pure compounds have white or yellowish crystalline appearance. They are belonging to group of the most dangerous contaminants occurring in environment because of anthropogenic activities. Their origin connected with burning of fossil fuels and organic matter, mining and or processing as the group of the most important sources of these compounds [6]. Manufacturing of paint and petrochemical industries represent the

second source important source of PAHs [7]. Natural sources, such as volcanic eruptions and forest fires contribute also to PAHs releasing into the environment [8, 9]. PAHs are worldwide distributed in air, soils, waters, but also in river and ocean sediments and sludge[10]. According to the abundance of these compounds in environment, it is not surprising that they are able to entry food chain in every level [11]. These compounds are highly toxic for plants [12] as well as for animals [13, 14]. Due to existence of these compounds in low levels therefore to determine and evaluate of impact of PAHs on the environment, robust analytical method are needed. An extraction of PAHs from different environmental mixtures belongs to the one of the most important step in PAHs analysis. Soxhlet-based extraction, supercritical fluid extraction together with microwave-assisted extraction, ionic liquid based dispersive liquid-liquid, dispersive solid phase extraction and pressurized liquid extraction are the most commonly used techniques [15-17]. As detection techniques, high performance liquid chromatography with UV and/ or fluorimetric detection is frequently used for determination of PAHs[18,19], gas chromatography coupled with mass spectrometry, adsorptive stripping voltametric determination or electro chromatography with mass detection and various electrochemical biosensors are also frequently used for PAHs determination [20, 21].

Low molecular weights of PAHs which consist of two and three rings are occurring in the atmosphere in the vapor phase whereas the largest molecular weights (five rings) of PAHs are bound to particles. Intermediate molecular weight (four rings) of PAH are partitioning between the particulate phases and vapor depending on the temperature of atmosphere [22]. Benzo

(a) Pyrene is consisting of four fused aromatic rings as shown in Figure 1.

The objective of this study to determine the level of Benzo (a) Pyrene in samples of vegetables in AL-Diwaniya province such as: lettuce, tomato and cabbage and the main sources of this compound.

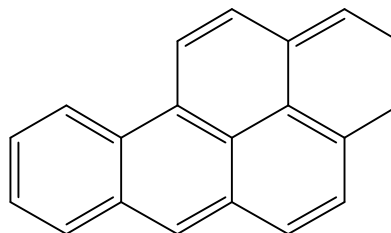


Figure1. Structure of Benzo (a) Pyrene

Materials and methods

Laboratory equipment

All sample containers and glassware were thoroughly washed with hot detergent solution followed by rinsing with purified water and acetone (analytical grade), respectively. These were finally kept in the oven at 120 °C overnight. To avoid contaminations of samples, different syringes and glassware were used for standard and for solutions extracted from samples.

Sampling

Samples of lettuce (2 kg), tomato (5 kg) and cabbage (7.5 kg) were collected from six different places from Al-Diwaniya markets. All samples for each type of vegetable were combined, and the mixed samples were homogenized with no addition of fluids. The homogenates were kept frozen at -15 °C until analysis [23].

Chemicals and reagents

The standard solution of Benzo (a) Pyrene was purchased from Supelco. This standard was stored at 4°C, protected from light. Solvents such as Cyclohexane, methanol and acetonitrile (HPLC grade) were purchased from Sigma Aldrich. All chemicals were used without further purification.

Extraction procedure

A homogenized portion of 25 g of each sample of vegetable (lettuce, tomato and cabbage) was boiled under reflux with 100 ml of a 2 M solution of potassium hydroxide in methanol for 5 h. The saponified material was transferred into separating funnel. The flask was rinsed with 100 ml methanol/distilled water (9:1, v/v). The mixture was extracted twice for 2 min for every time with 150 ml cyclohexane. The organic layer was first washed with 100 ml methanol/water (1:1, v/v) and then with 100 ml of distilled water. Then the organic layer was transferred into round bottom flask. The volume of the samples was reduced to 50 ml at 40 °C using a vacuum rotary evaporator. The cyclohexane was extracted with three aliquots of N,N-dimethylformamide–water (9:1, v/v) (50, 25 and 25 ml), then the combined dimethylformamide extract was diluted with 100 ml of a 1% sodium sulfate solution and re-extracted again with aliquots of 50, 35 and 35 ml cyclohexane. The combined solution was washed twice with 40 ml distilled water, dried with anhydrous sodium sulfate (2.5 g) and concentrated on a rotary evaporator to 5 ml at 40 °C[24].

Clean up

A glass column (200 x 10 mm i.d.) was packed with 5 g deactivated silica gel (15% water) and 2.5 g anhydrous sodium sulfate on the top. The Benzo (a) Pyrene extract

was applied to the top of the column and eluted with 85 ml of cyclohexane. The first 10 ml was discarded and the 10–85 ml fraction was concentrated to about 1 ml, and dried under a flow of nitrogen. Finally, the residue was dissolved in 2 ml acetonitrile.

Analysis conditions

The final concentrated extracts were analyzed using Gas Chromatography Shimadzu/2010 equipped with a split less injector, HP-5 capillary column: Hp5 (30m x Internal Diameter 0.28mm x Film thickness 0.25µm) and FID detector. The carrier gas was nitrogen at flow rate 1 ml/min, while oven temperature was programmed to increase from 50 °C (2 min) to 200 °C (2 min) at a speed of 20 °C/min, to 240 °C (2 min) at 5 °C/min, and to 290 °C at 3 °C/min and then held for 15 min. The injector and detector temperatures were 275 °C and 300 °C, respectively.

Results and discussion

Polycyclic aromatic hydrocarbons are representing a group of serious contaminants of the environment with significant impact on animal and human health. In addition to the fact that have ability to be accumulate in organisms including plants, which may serve as potential bio accumulators of these organic contaminants. According to this fact of plants to accumulate organic contaminants, negative effect of plants containing such pollutants on organisms must be carefully be in consideration. Several PAHs compounds, such as Benzo (a) Anthracene, Benzo (k) Fluoranthene, Benzo (a) Pyrene, di Benzo (a, h) Anthracene and Indeno (1,2,3-cd) Pyrene were classified as potential human carcinogens [47]. Figure2. illustrate the mean concentrations of Benzo (a) Pyrene in several vegetables (Lettuce, Tomato and Cabbage).

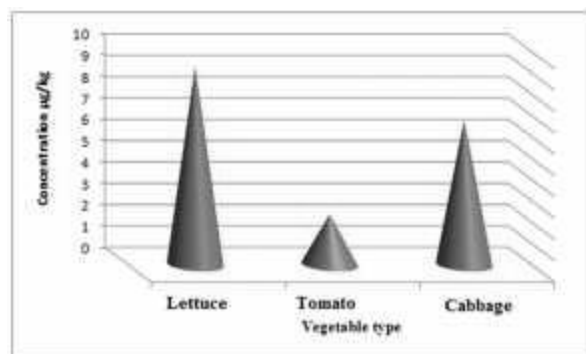


Figure2. Levels of Benzo (a) Pyrene in three types of vegetables

Table2, shows the concentrations and the mean of Benzo (a) Pyrene found in vegetables (Lettuce, tomato and Cabbage). Among the vegetables, Lettuce showed highest maximum concentration of Benzo (a) Pyrene 9.32 µg/kg with mean 8.32 µg/kg as compared with Tomato maximum concentration 2.27 µg/kg with mean 1.57 µg/kg and Cabbage maximum concentration 6.81 µg/kg with mean 6.00 µg/kg. These results are in agreement with (Camargo and Toledo, 2003) were expected due to the large surface area of lettuce in comparison to the other ones.

Table1. Mean and maximum value of Benzo (a) Pyrene in studied vegetables.

Vegetable type	Mean	Maximum
Lettuce	8.42	9.32
Tomato	1.57	2.37
Cabbage	6.00	6.81

Conclusions

The results of this limited study indicate that the highest level of contamination with Benzo (a) Pyrene in Lettuce compared with Tomato and cabbage for the samples taken from Al-Diwaniya markets.

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تراكيز وتوزيع ومصادر مركب البنزو اي بايرين لبعض الخضروات المتواجدة في مدينة الديوانية

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الخلاصة:

تنتشر مركبات الهيدروكربونات العطرية المتعددة الحلقات كملوثات بيئية وهي من المركبات المصنفة كمواضع مسرطنة والتي يمكن ان تظهر في الاغذية اثناء عملية التعبئة وعمليات التصنيع للاغذية. تتكون الهيدروكربونات الحلقية المتعددة الحلقات كناتج للاحتراق الغير منتظم من عوادم السيارات والمركبات العاملة بمختلف انواع الوقود وكذلك كنتيجة لانبعاث مخلفات الاحتراق الناجمة من الانشطة الصناعية المختلفة. تنتقل هذه الملوثات الى النباتات كنتيجة لترسب الغبار الملوث بهذه المواد وانتقاله للنبات بصورة مباشرة او عن طريق المياه الملوثة بهذه المركبات. في هذه الدراسة تم قياس مركب البنزو اي بايرين وهو احد مركبات الهيدروكربونات المتعددة الحلقات في عدة نباتات منتقاة من السوق المحلية لمدينة الديوانية ولعدة نباتات مثل الخس والطماطة والملفوف حيث جمعت العينات من ستة مواقع مختلفة لغرض تحليلها. حضرت العينات باستخدام الصوبنة بوجود هيدروكسيد البوتاسيوم والميثانول واستخلص مركب البنزو اي بايرين بطريقة استخلاص السائل-السائل باستخدام مذيب الهكسان الحلقي وتمت عملية التنظيف باستخدام تقنية كروماتوغرافيا الغاز لتقدير المركب اعلاه وقد اظهرت النتائج اعلى تركيز للمركب في نبات الخس هو 9.32 µg/kg وفي نبات الطماطة 2.37 µg/kg وفي نبات الملفوف كان اعلى تركيز هو 6.81 µg/kg.

الكلمات المفتاحية: التلوث في الديوانية, التلوث في الخضروات, البنزو اي بايرين, استخلاص البنزو اي بايرين من الخضروات, المركبات الهيدروكربونية العطرية المتعددة الحلقات.