

## Levels of Polyaromatic Hydrocarbons in Egyptian Vegetables and Their Behavior During Soaking in Oxidizing Agent Solutions

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**Abstract:** The residues of persistence potentially toxic organic compounds polyaromatic hydrocarbons (PAHs) were measured in 13 species of edible vegetables collected from Great Cairo governorate (Egypt). And the efficient role of washing by oxidizing agents hydrogen peroxide ( $H_2O_2$ ), potassium permanganates ( $KMnO_4$ ) and midribs of cabbage leaves solutions on the elimination of PAHs from contaminated vegetables was investigated. Vegetable samples were all Soxhlet-extracted in triplicate, cleaned up by open chromatography and analysed using gas chromatography with mass selective detection. It was observed that all tested vegetables have been contaminated with PAHs. The total content of 16 PAHs ranged from 1.22 to 12.63 ppb with the highest PAHs found in leafy vegetables. PAHs (3-4 rings) were dominant in all vegetable samples. The results also indicate the efficient role of washing by oxidizing agent in elimination of PAHs from naturally contaminated carrots. Midribs of cabbage leaves solution is the most efficient in removing PAHs followed by  $H_2O_2$  and  $KMnO_4$ . The removal percentages of the total PAHs ranged from 65.5 to 97.8% and from 79.4 to 99.9% at 4 and 8% concentrations of oxidizing reagents, respectively.

**Keywords:** PAHs %oxidizing agents % Vegetables

### INTRODUCTION

Polyaromatic hydrocarbons (PAHs) refer to a large group of organic chemicals containing two or more fused aromatic rings made up of carbon and hydrogen atoms as a by-product from the incomplete combustion or pyrolysis of organic materials. Gaseous and particle-bound PAHs can be transported over long distances before deposition and may accumulate in vegetation. This could indirectly cause human exposure to PAHs through food consumption and, thus might pose a human health threat [1]. PAHs are ubiquitous in the environment being present in air, soil, water and food [2, 3]. The effect and fate of PAHs in nature are of great environmental and human health concerns due to their widespread occurrence, persistence in terrestrial ecosystems and carcinogenic properties as well as to have cardiovascular, bone marrow or liver toxicity [4, 5]. Since carcinogenicity is the critical endpoint of toxicity of PAHs and some PAHs e.g. benzo[a]pyrene, benzo[a]anthracene and dibenzo[a,h]anthracene are genotoxic, it is not possible to define a level of intake which is without possible [6, 7].

It has been established that there are two major sources of PAHs formation in foods the first source is mainly due to the method of food preparation. The other major source of contamination of foodstuffs is by contact with either petroleum products or coal tar products. Because of the geochemical process and atmospheric deposition of air pollution particulate on the crops, it would be possible to generate these naturally occurring PAHs in foods [8]. As PAHs are ubiquitous in the environment, it is not surprising that they present in almost all foods [7]. For example, it has been reported that PAHs are present in cereals, grains, flour, bread, vegetables, fruits, fish, meat, processed or pickled foods and contaminated cows milk or human breast milk [9-11]. Cereals and cereal products, milk, vegetables and fruits are the highest contributors to total PAH intake. Since these products are the most important dietary component. In Egypt until now studies dealing with the detection of PAHs residues in vegetables are still lacking. Therefore this study was performed to determine the levels of PAHs grown in Great Cairo Governorate and provide a method for efficient, economical and rapid for decontamination of fresh vegetables naturally contaminated with PAHs using some of oxidizing agent solutions.

## MATERIALS AND METHODS

**Samples:** Total of 130 vegetables samples belonging to 13 different species (lettuce, leak, green onion, Spinach, spearment, pepper, squash, eggplant, cucumber, tomatoes, sweet potatoes, potatoes and carrot) were collected randomly from different regions in Great Cairo Governotote (Cairo, Giza and Kalubia). All samples were collected during the period of January, 2003 up to July 2004 to determine the concentrations of PAHs. Twenty kilogram of carrots were collected from the local market in Great Cairo. A representative sample of about 2 kg was examined for PAHs and washing treatments were carried out on carrot samples previously naturally contaminated with PAHs. All of the samples were stored in amber bottles in a freezer until analysis.

**Standards and chemicals:** The mixed standard solution of 16 PAHs (naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthrathene, fluorothene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3,c,d]pyrene were purchased from Qmx Laboratories Limited, UK.

All solvents and chemicals used were obtained from E. Merck Company (Germany). Midribs of cabbage leaves solutions were prepared by cutting it into small species and soaking in water at 4 and 8% concentrations.

**Extraction, clean-up and analysis:** PAHs were quantified from Soxhlet extract of each vegetable sample. In brief, 30 g each sample was mixed with 90 g of anhydrous sodium sulphate and placed in pre-extracted Whatman extraction thimbles (43 mm x 123 mm). The thimbles were placed into a 500 mL Quiekfit Soxhlet unit and extracted for 24 h with 300 mL of hexane:acetone (1:1). Extracts were then reduced to near dryness on a rotary film evaporator (Buchi R-124), taken up in 10 mL hexane and transferred into pre-washed and baked glass vials. The samples were then reduced further on a Techne Dri-Blok under a gentle stream of N<sub>2</sub> to 2 mL. The extracts were cleaned by passing through Bakerbond PCB-A SPE cartridges pre-conditioned with 10 mL hexane. They were again reduced under N<sub>2</sub> to near dryness, transferred to clean glass vials and made up to 1 mL for analysis. One µL of each clean sample extract was injected into an Hewlett Packarel 6890 gas chromatograph fitted with a 30 m HP5-MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) and connected to an Hewlett Packard 5973 mass selective detector. The carrier gas was

helium, maintained at a flow rate of 1.0ml/min by electronic pneumatic control. The injection port temperature was 230°C with electron energy of 70 eV. The quadrupole temperature was 280°C. The instrument was tuned on PFTBA. The oven programme for PAHs was as follow: 90°C for 2 min, 10°C/min to 240°C, 3°C/min to 310°C for 5 min. The mass spectrometer was operated in selective ion monitoring (SIM) mode using separate ions to identify and confirm compounds.

**Treatment of contaminated carrots:** The contaminated intact carrot samples were separately soaked for 10 min in potassium permanganate, hydrogen peroxide and midribs of cabbage leaves solutions at concentration of 4 and 8%. The samples were packed in amber glass bottles and stored in a freezer until analysis.

## RESULTS AND DISCUSSION

The results in Table 1 shows that all tested vegetables have been contaminated with PAHs with different levels ranging from 1.22 to 12.63 with a mean value 6.23 ppb. It was notice from the results that the 3-4 rings PAHs were predominates in all samples. However phenanthrene and flouranthene are the most abundant individual PAH compounds in vegetables under investigation. These compounds are more water-soluble than the higher molecular weight PAHs and so may be more susceptible to uptake from soils as well as deposition from polluted air. The higher PAHs content in green leafy vegetables such as lettuce 12.63 ppb, leak 10.71 ppb, green onion 10.09 ppb and spinach 8.87 ppb could be explained by their greater contact surface to the ambient air during growth. Lin *et al.*, [1] reported that tea leaves possess high surface area, so they may accumulate PAHs, especially from air. Lodovici *et al.*, [12] found that all vegetables except tomatoes investigated in Italian study exposed to polluted air, contained high levels of carcinogenic PAH. In addition, in a selection of reported benzo[a]pyrene concentrations in food by Greenberg *et al.*, [13] the level in kale was in a range of 12.6-48.1 ng/g. Kazerouni *et al.*, [8] found that the highest level Bap was found in collards and kale with levels of 0.48 and 0.47 ng/g, respectively. This indicate that PAH levels in uncooked food largely depend on the origin of the food and can be subject to regional variations.

Investigation into the source of PAHs have been used the molecular ratios of some specific hydrocarbons [1]. For instance a ratio of fluorothene to pyrene concentrations (Fluor/pyr) greater than 1.0 were

Table 1: Levels of PAHs in vegetables collected from Great Cairo governorate

Mean concentration of PAHs ppb <sup>a</sup>													
Vegetables													
Compounds	Lettuce	Leak	Green onion	Spainch	Spearmint	Squash	Cucumber	Eggplant	Pepper	Tomato	Carrot	Potatoes	Sweetpotatoes
Naphthalene	0.150±0.10	nd <sup>b</sup>	0.01±0.010	0.83±0.56	0.48±0.21	0.03±0.02	0.05±0.03	nd	0.06±0.48	nd	0.21±0.10	0.07±0.05	nd
Acenaphthalene	0.070±0.04	0.06±0.03	0.013±0.007	nd	nd	nd	nd	nd	0.04±0.03	nd	0.053±0.041	0.027±0.01	nd
Acenaphthene	0.810±0.36	1.30±0.81		0.6±0.5	1.01±0.8	nd	nd	nd	0.65±0.60	0.01±0.008	0.9±0.63	0.58±0.35	0.24±0.14
Fluorene	0.630±0.43	1.50±0.60	0.55±0.38	0.7±0.3	0.57±0.4	0.06±0.04	nd		0.43±0.50	nd	0.82±0.56	0.77±0.52	0.15±0.05
Phenanthrene	1.380±0.80	3.50±1.10	1.00±0.8	2.53±1.8	0.79±0.55	1.62±1.0	0.2±0.1	2.17±0.9	1.35±0.7	0.68±0.52	1.4±1.03	1.23±0.74	0.73±0.51
Anthrathene	1.050±0.90	0.96±0.58	0.83±0.53	0.74±0.61	0.45±0.27	0.13±0.10	0.03±0.02	0.01±0.01	0.056±0.03	0.03±0.02	0.35±0.42	nd	nd
Fluornthene	4.230±1.50	2.13±0.95	5.26±2.85	1.5±0.8	3.00±0.93	1.01±1.53	0.72±0.56	0.43±0.31	1.46±0.60	0.25±0.12	1.1±0.90	1.85±0.75	1.00±0.68
Pyrene	1.600±0.82	0.73±0.59	nd	1.02±1.00	0.20±0.08	1.2±0.72	0.05±0.04	0.09±0.05	1.07±0.9	0.13±0.07	0.78±0.81	1.00±0.65	0.56±0.37
Benzo[a]anthracene	0.520±0.51	0.41±0.28	0.19±0.08		0.03±0.019	0.17±0.09		0.01	0.1±0.09	0.4±0.31	0.06±0.04	0.16±0.2	0.34±0.28
Chrysene	0.110±0.05	nd	0.21±0.11	0.25±0.3	0.124±0.11	nd	0.08±0.05	0.12±0.05	0.13±0.10	nd	0.08±0.07	0.07±0.05	nd
Benzo[b]fluoranthene	0.240±0.17	0.01±0.01	0.35±0.30		0.15±0.08	nd	nd	nd	nd	nd	nd	nd	nd
Benzo[k]fluoranthene	0.198±0.08	nd	0.33±0.19	0.13±0.07	0.328±0.23	0.09±0.05	nd	0.01±0.008	nd	nd	nd	nd	nd
Benzo[a]pyrene	0.610±0.39	0.02±0.01	0.29±0.15	0.54±0.50	0.132±0.07	0.04±0.03	0.07±0.05	0.02±0.02	0.16±0.07	0.01±0.005	0.15±0.12	0.03±0.23	nd
Indeno[1,2,3-c,d]pyrene	0.280±0.12	nd	0.15±0.07	nd	0.07±0.06	nd	nd	nd	nd	nd	0.11±0.20	nd	nd
Dibenzo[a,h]anthracene	0.550±0.26	0.06±0.04	0.14±0.08	nd	0.035±0.013	0.05±0.02	nd	nd	nd	nd	0.02±0.013	nd	nd
Benzo[g,h,i]perylene	0.200±0.17	nd	0.267±0.20	nd	0.028±0.02	0.2±0.08	nd	nd	0.05±0.03	nd	0.34±0.33	nd	nd
∑E PAHs	12.628±1.025	10.705±1.006	10.095±1.258	8.87±0.694	7.402±0.732	4.625±0.972	1.245±0.248	3.035±0.539	5.876±0.501	1.215±0.174	6.483±0.446	5.997±0.515	2.753±0.311

<sup>a</sup>Limit of detection is 0.005 ppb, <sup>b</sup>nd, not detectable

Table 2: Behavior of PAHs residues during soaking in oxidizing solutions for 10 min

Compounds	Treatments													
	Control	KmnO <sub>4</sub>				H <sub>2</sub> O <sub>2</sub>				MCL				
		4%	8%	4%	8%	4%	8%	4%	8%					
	Mean	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	Mean	Reduction (%)	
Naphthalene	0.23±0.05	0.09±0.03	60.9	0.07±0.023	69.6	0.06±0.017	73.9	0.05±0.01	78.3	nd	100.0	nd	100.0	
Acenaphthalene	0.06±0.015	0.02±0.005	66.7	0.014±0.005	76.7	0.009±0.001	85.0	<sup>b</sup> nd	100.0	nd	100.0	nd	100.0	
Acenaphthene	0.82±0.18	0.26±0.07	68.3	0.15±0.037	81.7	0.2±0.06	75.6	0.10±0.03	87.8	nd	100.0	nd	100.0	
Fluorene	0.53±0.12	0.22±0.08	58.3	0.14±0.03	73.6	0.19±0.05	64.2	0.08±0.03	84.9	0.05±0.01	89.2	nd	100.0	
Phenanthrene	2.17±0.45	0.81±0.23	62.7	0.56±0.19	74.2	0.85±0.25	60.8	0.51±0.13	76.5	0.023±0.005	96.9	nd	100.0	
Anthrathene	0.75±0.15	0.28±0.06	62.7	0.13±0.03	82.7	0.17±0.036	77.3	0.07±0.018	90.7	nd	100.0	nd	100.0	
Fluornthene	1.5±0.27	0.5±0.16	66.7	0.28±0.09	81.3	0.36±0.09	76.0	0.21±0.07	86.0	0.07±0.02	93.5	nd	100.0	
Pyrene	1.08±0.33	0.4±0.17	63.0	0.19±0.053	82.4	0.29±0.07	73.1	0.13±0.04	88.0	0.01±0.001	95.8	nd	100.0	
Benzo[a]anthracene	0.24±0.08	0.05±0.02	79.2	0.027±0.006	79.2	0.015±0.003	93.8	nd	100.0	nd	100.0	nd	100.0	
Chrysene	0.13±0.04	0.04±0.013	69.2	0.023±0.007	82.3	0.011±0.004	91.5	nd	100.0	nd	100.0	nd	100.0	
Benzo[b]fluoranthene	0.06±0.01	0.015±0.003	75.0	0.009±0.002	85.0	0.013±0.002	78.3	nd	100.0	nd	100.0	nd	100.0	
Benzo[k]fluoranthene	0.124±0.03	0.034±0.012	72.6	0.02±0.007	83.9	0.025±0.006	79.8	0.014±0.003	88.7	nd	100.0	nd	100.0	
Benzo[a]pyrene	0.548±0.14	0.161±0.035	70.6	0.089±0.03	83.8	0.12±0.03	78.1	0.05±0.013	91.0	nd	100.0	nd	100.0	
Indeno[1,2,3-c,d]pyrene	0.11±0.03	0.028±0.006	74.5	0.017±0.004	74.5	0.018±0.005	83.6	0.007±0.002	93.6	nd	100.0	nd	100.0	
Dibenzo[a,h]anthracene	0.06±0.03	0.013±0.013	78.3	0.008±0.003	86.7	0.015±0.005	75.0	0.006±0.001	91.7	nd	100.0	nd	100.0	
Benzo[g,h,i]perylene	0.376±0.09	0.112±0.08	70.2	0.085±0.021	77.4	0.09±0.018	76.1	0.062±0.015	83.5	0.04±0.01	89.4	nd	97.3	
E PAHs	8.788	3.033		1.812		2.436		0.283		0.193		0.010		
Reduction%			65.5		79.4		72.3		96.8		97.8		99.9	

<sup>a</sup>Mean = ppb±SD, Limit of dection is 0.005ppb, Values given are mean of five replicates, <sup>b</sup>nd, not detectable

characteristic of pyrolytic origin, whereas ratio less than 1.0 was characteristic of petroleum hydrocarbons [14]. And a ratio of phenanthrene to anthracene (Phen/An) less than 10 suggested combustion sources, while Phen/An greater than 10 implied petrogenic sources [15, 16]. From the calculation of Flur/pyr and Phen/An in vegetables tested in this study it was indicated that PAHs resulting from incomplete combustions products via pyrolytic process from industrial plants and from farm waste and home waste fires in addition to atmospheric fall out of automobiles exhausts in cities and town along the road of Great Cairo governorate.

The effect of washing by oxidizing agent solutions on the removal of PAHs were summarized in Table 2. The results indicate the efficient role of washing by oxidizing agents solutions, MCL of cabbage leaves (MCL), hydrogen peroxide ( $H_2O_2$ ) and potassium permanganate  $KMnO_4$  in reduction of PAHs from naturally contaminated carrots. On the other hand, washing with tap water not provided significant effective loss with the contamination by PAHs. It was noticed that reduction of these contaminants depends on the type and levels of PAHs individuals as well as the type and concentration of oxidizing agents. It appears from the results that MCL is the most efficient followed by  $H_2O_2$  and  $KMnO_4$  in removing PAHs contamination. MCL solution at 4% concentration completely eliminated acenaphthylene, acenaphthene, fluorene, fluoranthene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene and dibenzo[a,h]anthracene. And giving 89.2, 96.9, 93.5 and 95.8% reduction in phenanthrene, anthracene, pyrene, benzo[a]anthracene and benzo[ghi]perylene. At 8% concentration MCL eliminated PAHs residues completely except for benzo[ghi]perylene (97.3% loss). This may be explained on the basis that MCL has the ability to exert peroxidase enzyme [17]. It was found that peroxidase has the ability to break PCBs [18].

The results in Table 2 also show a significant decrease in the levels of PAHs under study by washing with  $H_2O_2$  and  $KMnO_4$  solutions at 4 and 8% concentrations. The levels of total PAH reduction were 65.5, 79.4, 72.3, 96.8%, respectively. The redox potentials of  $H_2O_2$  and  $KMnO_4$  are high (-1.736 and -1.507 v, respectively) and therefore are highly reactive towards PAHs. The pathway by which  $H_2O_2$  breaks down aromatic ring compounds involves highly free radicals (e.g.  $HO_2$  and  $HO$ ) [19].

It could be concluded from the above results that Egyptian vegetables contaminated by different levels of PAH individuals; this contamination may be due to

pollution of the environment and deposition in plants. So more efforts should be applied by the Ministry of Environment to find effective ways to control the environmental pollutions. Washing with oxidizing agent solutions show a significant effect on decomposition and removal of PAHs from contaminated vegetables and therefore they are necessary for kitchen use to decrease the intake of PAHs residues. And so, decrease from the possible health hazard arising from the toxic PAHs residues in food.

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