



POLYCYCLIC AROMATIC HYDROCARBONS IN VEGETABLES GROWN IN URBAN AND RURAL AREAS

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Abstract

Vegetables are an important part of a healthy diet because of their nutritional value. Contamination in vegetable may prove hazardous for population. Polycyclic aromatic hydrocarbons (PAHs) are compounds widespread in the environment, many of them showing carcinogenic effects. These compounds can reach the food chain by different ways and, therefore, the analysis of PAHs in food is a matter of concern.

The present work provides data regarding levels of PAHs: acenaphthene (Ace), acenaphthylene (Acy), fluorene (F), naphthalene (Np), anthracene (An), fluoranthene (Fl), phenanthrene (Ph), benzo[α]anthracene (B[α]An), benzo[k]fluoranthene (B[k]Fl), chrysene (Chry), pyrene (Py), benzo[ghi]perylene (B[ghi]Pe), benzo[α]pyrene (B[α]Py), dibenzo[α , β]anthracene dB[α , β]An, indeno[1,2,3-cd]pyrene (I[1,2,3-cd]Py) in different vegetables (potato, celery, dill, parsley, carrot, cucumber, onion, garlic, cabbage, spinach). Bulb, stem, leaves and fruit were taking into account. Samples were collected from rural and urban areas from Romania, belonging to Dobrogea region.

The RSD values were less than 4.7%, indicating that the GC-MS method is precise. The calculated R^2 values were above 0.998, indicating the linear relationship between targeted spiking levels and mean introduced concentrations, within the working range of concentrations. LOD and LOQ values were determined using calibration standards.

An analytical procedure was used, based on extraction and clean-up step, followed by the injection of concentrated extracts in gas chromatograph Hewlett- Packard 5890. A look at total PAH contents reveals that leafy vegetables showed higher values while chrysene was below the limit of quantification for most studied samples. Sample location appears to be one important factor affecting vegetables PAH contents. The obtained results show that PAHs were detected at higher concentrations in urban areas than in rural areas.

Key words: GC-MS; polycyclic aromatic hydrocarbons, vegetables

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1. Introduction

PAHs are a large group of widespread organic compounds of high environmental concern. Even though PAHs occur naturally, the highest concentrations are mainly due to human activities that cause a continuous increase of PAH levels in the environment. Many of the PAHs are toxic and exposure can result in mutagenesis and carcinogenesis in humans and animals (Larson, 2009).

The compounds containing five or more aromatic rings are known as "heavy" PAHs, whereas those containing less than five rings are named "light" PAHs. Both kinds of PAHs are non-polar compounds showing high lipophilic nature, therefore heavy PAHs are more stable and toxic than the other group. PAHs are ubiquitous environmental contaminants which are widespread in the air bonded to particulate matter. These compounds are produced during a variety of combustion and pyrolysis processes from anthropogenic and natural sources.

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In plants, low molecular mass PAHs can be adsorbed through surface adsorption and high molecular mass PAHs can contaminate the surface due to atmospheric fallout. A possible pathway from contaminated soil to plant roots was observed by direct relationship between soil and plant PAHs concentrations (Ashraf et al., 2013).

The 64th Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed all relevant information related to the toxicology, epidemiology, intake assessment, analytical methodology, formation, fate, and occurrence of PAHs in food (FAO/WHO, 2005). Overall, the Committee concluded that PAHs are clearly genotoxic as shown by *in vitro* and *in vivo* assays, and include benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene (FAO/WHO, 2005).

Analytical procedures to detect PAHs after extraction and purification from vegetables are commonly followed by HPLC, LC-MS, LC-MS-MS, GC-FPD, GC-MSD, GC-ECD or GC-MS determination (Fenoll et al., 2007; Osman et al., 2010, Soceanu et al., 2009; Zhou et al., 2006).

The aim of this study was to investigate the levels and distribution of PAHs: acenaphthene (Ace), acenaphthylene (Acy), fluorene (F), naphthalene (Np), anthracene (An), fluoranthene (Fl), phenanthrene (Ph), benzo[α]anthracene (B[α]An), benzo[k]fluoranthene (B[k]Fl), chrysene (Chry), pyrene (Py), benzo[ghi]perylene (B[ghi]Pe), benzo[α]pyrene (B[α]Py), dibenzo[α ,h]anthracene dB[α ,h]An, indeno[1,2,3-cd]pyrene (I[1,2,3-cd]Py) in different vegetables (potato, celery, dill, parsley, carrot, cucumber, onion, garlic, cabbage, spinach). Bulb, stem, leaves and fruit were taking into account.

2. Materials and methods

2.1. Chemical and reagents

Standards of PAHs: Acenaphthene (Ace), acenaphthylene (Acy), fluorene (F), naphthalene (Np), anthracene (An), fluoranthene (Fl), phenanthrene (Ph), benzo[α]anthracene (B[α]An), benzo[k]fluoranthene (B[k]Fl), chrysene (Chry), pyrene (Py), benzo[ghi]perylene (B[ghi]Pe), benzo[α]pyrene (B[α]Py), dibenzo[α ,h]anthracene dB[α ,h]An, indeno[1,2,3-cd]pyrene (I[1,2,3-cd]Py) were supplied by International Atomic Energy Agency, Monaco laboratory.

Two usual sorbent materials of variable polarities (silica gel and aluminium oxide) were assayed for preconcentration step of PAHs. Silica gel 60 (0.2 – 0.5 mm) and aluminium oxide 90 (0.063 – 0.200 mm) were obtained from Merck, Darmstadt, Germany.

Two organic solvents were selected as eluents: n-hexane, supplied by Merck, Darmstadt, Germany and dichlormethane supplied by J.T. Baker.

Anhydrous sodium sulphate (granulated for residue analysis) was activated at 200°C for 2h before use. All glassware was washed with detergent, rinsed with deionised water and acetone before use.

2.2. Sample preparation

Samples were collected from rural (Slava Rusa, Greci, Ciobanu, Adamclisi) and urban (Constanta, Medgidia, Tulcea) areas from Romania, belonging to Dobrogea region. Different parts (bulb, stem, leaves and fruit) of vegetables (potato, celery, dill, parsley, carrot, cucumber, onion, garlic, cabbage, spinach) were investigated.

One kilogram of each vegetable sample was taken to the laboratory and stored in a freezer until the analyses were performed.

2.3. Sample analysis

To determine the PAHs from studied samples an analytical procedure described in previous paper (Soceanu et al., 2012) based on extraction and clean up step, followed by the injection of concentrated extracts in gas chromatograph was used.

Validation was made taking into account the document SANCO No 3131/2007 (SANCO 2007). LOD and LOQ values were determined using calibration standards and the values for LODs were in the range 0.07 and 1.29 µg/kg. The RSD values were less than 4.7%, and the calculated R^2 values were above 0.998. Recovery experiments were carried out by spiking studied samples with three different concentrations of PAHs standard solution. Recoveries were calculated from the differences in total amounts of each PAH between the spiked and unspiked samples and were between 80-100%.

3. Results and discussions

The sum of 15 PAHs varied in vegetables grown in rural areas from 0.753 to 8.216 µg/kg and in vegetables grown in urban areas from 0.360 to 10.062 µg/kg. Maximum PAH level was shown by cabbage (1.56 µg/kg). Results are given in Tables 1, 2 and 3. The mean sum of 15 PAHs was 4.887 µg/kg.

A look at total PAH contents reveals that leafy vegetables showed higher values while chrysene was below the limit of quantification for most studied samples. Sample location appears to be one important factor affecting vegetables PAH contents. The obtained results show that PAHs were detected at higher concentrations in urban areas than in rural areas. To determine if the differences between samples from urban and rural areas were significant a statistical analysis by ANOVA test (one-way variance analysis) and homogeneity variance test (F_{max} test) were performed. All statistical analyses were carried out at the 95% confidence level. According to these tests, statistically significant differences were found between vegetable samples

from urban and rural areas, respectively, among vegetables samples within each botanical families ($p<0.01$).

Therefore, it can be concluded that vegetables collected from rural areas are less contaminated with

PAHs than samples collected from urban areas. Contamination of Romanian vegetables by different levels of PAH may be due to pollution of the environment and deposition in plants.

Table 1. PAHs concentrations in parsley, dill, celery and carrot from urban and rural areas

PAH (ppb)	Parsley Urban area		Parsley Rural area		Dill Urban area		Dill Rural area		Celery Urban area		Celery Rural area		Carrot Urban area		Carrot Rural area	
	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Bulb	Leaves	Bulb	Leaves	Bulb	Leaves	Bulb	Leaves
I[1,2,3-cd]Py	0.369	0.240	0.533	0.577	0.851	0.371	0.842	0.250	0.230	< LOQ	0.390	0.301	0.570	0.560	<LOQ	<LOQ
Np	1.070	2.026	0.194	0.807	0.486	< LOQ	0.247	0.253	0.476	< LOQ	<LOQ	<LOQ	< LOQ	0.389	<LOQ	<LOQ
Acy	0.390	0.270	0.370	0.230	0.240	0.139	0.240	< LOQ	0.476	< LOQ	0.290	<LOQ	< LOQ	0.280	0.580	0.116
Ace	0.329	0.104	0.380	0.480	0.970	0.460	0.340	< LOQ	0.476	< LOQ	0.390	<LOQ	< LOQ	0.280	0.780	0.133
F	0.109	0.578	0.550	0.340	0.240	0.881	0.221	< LOQ	0.370	< LOQ	0.390	<LOQ	< LOQ	0.230	0.312	0.470
Ph	0.504	0.763	0.111	0.920	< LOQ	0.464	0.192	< LOQ	0.470	< LOQ	0.490	<LOQ	< LOQ	0.575	0.560	0.470
An	0.203	0.796	0.311	0.980	< LOQ	0.185	0.292	< LOQ	0.470	< LOQ	0.490	<LOQ	< LOQ	0.575	<LOQ	0.470
Fl	0.290	0.270	0.260	0.330	< LOQ	0.371	<LOQ	< LOQ	0.470	< LOQ	0.510	<LOQ	< LOQ	< LOQ	<LOQ	0.470
Py	0.207	0.270	0.280	< LOQ	< LOQ	< LOQ	<LOQ	< LOQ	0.370	< LOQ	0.390	<LOQ	< LOQ	0.280	<LOQ	<LOQ
B[α]An	0.519	0.560	< LOQ	0.390	< LOQ	< LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ	<LOQ	< LOQ	0.863	<LOQ	<LOQ
Chry	0.389	<LOQ	< LOQ	< LOQ	< LOQ	< LOQ	<LOQ	< LOQ	<LOQ	< LOQ	<LOQ	<LOQ	< LOQ	0.280	<LOQ	<LOQ
B[k]Fl	0.119	0.160	0.350	0.669	0.632	0.603	0.340	< LOQ	0.480	0.360	0.307	0.214	< LOQ	0.201	<LOQ	<LOQ
B[α]Py	0.207	0.160	0.950	< LOQ	< LOQ	0.324	0.276	0.250	0.530	< LOQ	0.490	0.230	0.214	0.258	<LOQ	<LOQ
B[ghi]P	0.958	0.270	0.380	0.170	0.480	0.460	<LOQ	< LOQ	0.470	< LOQ	0.490	<LOQ	0.214	0.280	<LOQ	<LOQ
dB[α .h]An	0.499	0.320	0.183	0.161	0.462	0.918	<LOQ	< LOQ	0.530	< LOQ	0.308	0.960	0.174	0.224	<LOQ	<LOQ
Σ PAHs	6.16	6.78	4.85	6.05	4.36	5.17	2.99	0.753	5.81	0.36	4.93	1.705	1.172	5.275	2.232	2.129

LOQ – limit of quantification; Values of means n=3

There are many sources of PAHs in the atmosphere and emitted PAHs should be mixed during their passage through the atmosphere away from the source regions to other locations where the vegetables was collected. The presence of PAH in foodstuffs depends on the environmental concentrations of these pollutants, as well as the physiological and ecological characteristics of the product (Martorell et al., 2010). In a study from Saudi Arabia (Ashraf and Salam, 2012) total PAH contents of the root vegetables like potato and carrot showed higher values (11 $\mu\text{g}/\text{kg}$) whereas turnip showed relatively lower contents at 9.26 $\mu\text{g}/\text{kg}$. For leafy vegetables, maximum PAH level was shown by cabbage 8.34 $\mu\text{g}/\text{kg}$, values that are higher than those found in the present study. Zhong and Wang (2002) have reported B[α]An levels in cabbage ($5.46 \pm 1.08 \mu\text{g}/\text{kg}$), cucumber ($2.33 \pm 2.02 \mu\text{g}/\text{kg}$) and eggplant

($2.39 \pm 1.82 \mu\text{g}/\text{kg}$) grown in China. In the present study levels of B[α]An are lower.

Because PAHs can be biotransferable and bioaccumulated in human bodies through dietary intake, we calculated the total daily dietary intakes (TDIs) of PAHs to evaluate the potential risk to the people through the intake of vegetables. The TDIs of PAHs for the people were estimated according to Eq. (1) (Wang et al., 2012):

$$TDI = \frac{I}{BW} \sum_{i=1}^L c_i \cdot (1 - w_i) \cdot DDI_i \quad (1)$$

where: BW is the body weight for an adult (assumed to be 60 kg); c_i ($\mu\text{g}/\text{kg}$) is the concentration of PAHs in vegetables; w_i (g/g) is the ratio of water content for vegetables (90%); and DDI_i (g/d) is the daily dietary intake of vegetables by the adult residents (215 $\text{g}/\text{day}/\text{person}$, according to the INS (2012)).

Table 2. PAHs concentrations in cucumber, garlic and onion

PAH (ppb)	Cucumber Urban area	Cucumber Rural area	Garlic Urban area		Garlic Rural area		Onion Urban area		Onion Rural area	
	Fruit	Fruit	Bulb	Leaves	Bulb	Leaves	Bulb	Leaves	Bulb	Leaves
I[1.2.3-cd]Py	0.471	0.820	0.153	0.312	0.240	0.430	0.364	0.412	<LOQ	0.141
Np	< LOQ	<LOQ	0.323	0.463	<LOQ	<LOQ	< LOQ	0.427	0.820	0.870
Acy	0.123	0.390	0.920	0.220	0.112	0.125	0.360	0.430	0.790	0.360
Ace	0.472	0.830	0.940	0.103	0.920	0.136	0.109	0.152	0.790	0.435
F	0.235	< LOQ	0.189	0.234	0.610	0.940	0.560	0.135	0.111	0.733
Ph	1.063	<LOQ	0.230	0.520	0.280	0.380	0.304	0.412	<LOQ	0.410
An	0.420	< LOQ	<LOQ	0.520	0.602	0.680	0.316	0.504	0.446	0.429
Fl	0.693	0.690	0.920	0.350	0.570	0.350	<LOQ	0.390	<LOQ	0.490
Py	0.801	< LOQ	0.920	0.330	0.840	0.250	<LOQ	0.260	<LOQ	< LOQ
B[α]An	1.026	< LOQ	0.920	0.307	0.604	0.608	<LOQ	<LOQ	<LOQ	<LOQ
Chry	0.112	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ	0.560	<LOQ	<LOQ	0.830
B[k]Fl	<LOQ	0.905	0.394	1.364	0.540	0.107	0.182	0.203	<LOQ	0.849
B[α]Py	0.183	0.218	0.342	0.586	0.570	0.720	0.218	0.334	<LOQ	0.550
B[ghi]P	1.095	0.360	0.350	0.610	0.602	0.307	0.360	0.450	<LOQ	0.284
dB[α.h]An	<LOQ	0.410	0.448	0.382	0.113	0.192	0.240	0.342	<LOQ	0.935
ΣPAHs	6.694	4.623	7.049	6.301	6.603	5.225	3.573	4.451	2.957	7.316

LOQ - limit of quantification; Values of means n=3

Table 3. PAHs concentrations in potato, cabbage and spinach

PAH (ppb)	Potato Urban area	Potato Rural area	Spinach Urban area	Spinach Rural area	Cabbage Urban area	Cabbage Rural area
	Fruit	Fruit	Leaves	Leaves	Leaves	Leaves
I[1.2.3-cd]Py	0.521	0.364	0.689	0.547	0.540	0.450
Np	0.147	0.248	0.514	0.496	1.201	0.967
Acy	0.358	<LOQ	0.598	0.462	0.632	0.403
Ace	0.221	0.198	<LOQ	<LOQ	0.523	0.402
F	0.144	0.140	0.347	0.406	0.740	1.560
Ph	0.324	<LOQ	0.688	0.599	1.020	0.620
An	0.120	0.106	0.984	0.832	0.912	0.870
Fl	0.247	0.269	0.847	0.715	0.790	0.522
Py	0.306	0.247	0.932	0.912	0.871	0.669
B[α]An	0.247	0.155	0.715	0.625	0.669	0.479
Chry	<LOQ	<LOQ	<LOQ	<LOQ	< LOQ	<LOQ
B[k]Fl	0.401	0.336	0.865	0.749	0.549	0.426
B[α]Py	<LOQ	0.247	0.964	0.895	0.437	0.374
B[ghi]P	<LOQ	0.141	<LOQ	0.321	0.565	0.324
dB[α.h]An	0.116	<LOQ	0.732	0.657	0.613	0.467
ΣPAHs	3.152	2.451	8.875	8.216	10.062	8.131

LOQ - limit of quantification; Values of means n=3

In recent years, the number of international surveys carried out to estimate dietary intakes of PAHs has been small. In the present study, the *TDI* of ΣPAHs through vegetables was found to range from 0.12 µg/kg/d to 3.60 µg/kg/d. In Greece, the dietary intake of PAHs via vegetables was found to range from 1.6 to 4.5 µg per person per day (Voutsas and Samara, 1998), while in one study from South China (Wang et al., 2012) the *TDI* of PAHs via vegetables was 12.8 ng/kg/d. This remarkable difference found could be explained by the fact that in the China study all vegetables were obtained from an e-waste recycling site in South China.

The results of surveys carried out in The Netherlands (5-17 µg/kg/d) (De Vos et al., 1990) and Spain 6.33–8.42 µg/kg/d (Falco et al., 2003) indicate PAH intake levels higher than those obtained in the present study.

4. Conclusions

This study was conducted to reveal and draw attention to the great problem of environmental pollution, in particular by PAH residues in different vegetables from Romania, to ensure safety and quality.

The obtained showed a mean sum of 15 PAHs of 4.887 µg/kg and the tolerable daily intake of ΣPAHs through vegetables was found to range from 0.12 µg/kg/d to 3.60 µg/kg/d.

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