

# Polycyclic aromatic compounds (PAC) in leaf lettuce

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## Polycyclische aromatische Verbindungen im Pflücksalat

**Zusammenfassung.** 23 Pflücksalatproben (*Lactuca sativa* var. *crispa*) aus südfinnischen Hausgärten wurden auf polycyclische aromatische Kohlenwasserstoffe (PAH) und deren Derivate untersucht. Die Gesamt-PAH-Gehalte variierten zwischen 4,8 und 94 µg/kg Frischgewicht. PAH-Nitroabkömmlinge wurden lediglich in fünf Proben festgestellt. Eine der Salatproben wurde auch auf PAH-Sauerstoffdrivate untersucht. An der Probe wurden 9H-Fluoren-9-on (1,3 µg/kg) und 6H-Benzo(cd)pyren-6-on (0,3 µg/kg) festgestellt.

Die PAH-Profile der Salatproben wurden an PAH-Profilen der freien Luft verglichen. Auf diese Weise konnte festgestellt werden, daß die PAH-Verbindungen von PAH-Verbindungen enthaltenden Partikeln der freien Luft stammen.

**Summary.** Twentythree samples of leaf lettuce (*Lactuca sativa* var. *crispa*) cultivated in private gardens in southern Finland were analyzed for polycyclic aromatic hydrocarbons (PAH) and derivatives. The total PAH concentrations varied from 4.8 to 94 µg/kg fresh weight. Nitro derivatives of PAH were detected in only five samples. One lettuce sample was also analyzed for PAH oxy-derivatives. 9H-Fluoren-9-one (1.3 µg/kg) and 6 H-benzo[cd]pyren-6-one (0.3 µg/kg) were found.

The profiles of PAH in lettuce were compared with those in ambient air. It was concluded that PAH in lettuce originate from deposition of PAH-containing particles present in ambient air.

## 1 Introduction

Leafy vegetables have been shown to be a significant source of polycyclic aromatic hydrocarbons (PAH) in human diets [1, 2]. The main source of PAH in the environment are anthropogenic combustion processes, e.g. heat production, vehicles and various industrial pro-

cesses. The PAH emitted into the atmosphere are mainly bound onto small particles. The deposition of these particles is the main origin of PAH in leafy vegetables [1]. Biosynthesis and absorption is believed to be of minor, if any, importance.

Mutagenic studies [3] have revealed that polycyclic aromatic hydrocarbons are not the only agents responsible for the mutagenic activity found in extracts from environmental sources. Pitts [4] first discovered that PAH react with nitrogen oxides present, e.g. in the exhaust gases of vehicles to form nitro-PAH, some of which are highly active mutagens in the Ames test [5]. Nitro-PAH have been found in vehicle particulate emissions [6] and in urban air [7]. Dennis et al. [8] analyzed 24 samples of various foodstuffs for nitro-PAH and found 9-nitroanthracene in peated malt and 1-nitropyrene in two tea samples. The concentrations were of the order of 0.2–2 µg/kg.

Capillary gas chromatography coupled with a selective detector is usually used in the analysis for nitro-PAH. Flame ionization detectors [9], nitrogen sensitive detectors [10], electron-capture detectors [11], thermal-energy analyzers [8], electron-impact mass spectrometry [7] and negative-ion chemical-ionization mass spectrometry [7] have all been mentioned in the literature as the means of detection.

In addition to the nitro-derivatives of PAH, various oxygenated PAH derivatives have also been found in environmental samples, such as airborne particulate matter [12] and in emissions from diesel engines, wood and coal combustion and municipal incineration [13]. The oxy-PAH are formed in the atmosphere by oxidation of PAH induced by light or ambient oxidizing agents, e.g. ozone. Incomplete combustion of organic material can also produce oxy-PAH. The compound types usually formed include ketones, aldehydes, quinones, anhydrides and coumarins [12]. The contribution of the oxygen-containing PAC<sup>1</sup> to the total mutagenicity of air particulates has not yet been established. Gas chromatography/mass spectrometry techniques have mainly been used in the analysis for oxy-PAH [12, 13].

The aim of this study was to provide information regarding the presence of PAC in the environment by analyzing lettuce samples. Lettuce has been considered as a good indicator crop for contaminants present in atmospheric fall-out particles [2].

## 2 Experimental

### 2.1 Samples

Leaf lettuce (*Lactuca sativa* var. *crispa*) were taken in early September, 1984. All samples had been grown in the open air, except sample 1, which had been grown under glass in a cold-frame. The samples had been sown in the beginning of June and had thus been exposed to the ambient air for about two months. The sampling locations are shown in Fig. 1 and described in Table 1. Sample 23, not shown on the map, was taken from Pori, a small town with some in-

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<sup>1</sup> The term PAC is used to describe PAH including derivatives

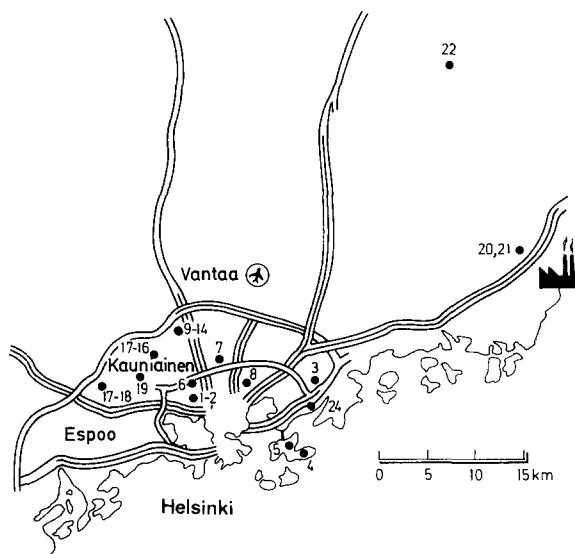


Fig. 1. Sites of sampling

dusty on the coast of the Bothnian Sea about 220 km north-west of Helsinki. The samples were stored deep-frozen until analysed.

## 2.2 Extraction and clean-up

The solvents were distilled before to use.

The extraction and clean-up of the extract was performed by a modification of the methods of Dennis et al. [8] and Grimmer and Böhnke [14]. The sample treatment scheme is presented in Fig. 2.

The final clean-up step using silica gel column chromatography was accomplished as follows: a glass column (10 mm i.d.) was filled with 10 g of silica gel (Merck, Art. 7754, heated to 380° for 4 h, 5% water was added and gently mixed for 1 h). A layer of anhydrous sodium sulphate (5 g) was placed on the top of the column and the column was washed with 40 ml of cyclohexane. The sample extract (1 ml) was added to the column, which was eluted first with 170 ml cyclohexane. The first 20 ml of eluate was discarded. The PAH were eluted with the next 150 ml of cyclohexane. The polar PAC fraction was finally eluted with 100 ml of dichloromethane. Both the frac-

100 g lettuce + 0.2 ml 10 mg/l 3,6-dimethylphenanthrene in ethyl ether (internal standard) + 200 ml acetonitrile

- homogenization (Ultra-Turrax)
- filtration with suction

acetonitrile extract

addition of water (420 ml)

acetonitrile-water mixture

extraction with cyclohexane (2 × 50 ml)

cyclohexane extract (100 ml)

washing with methanol/water (9+1, 50 ml); re-extraction of the washing solution with 50 ml cyclohexane; combination of the cyclohexane extracts

cyclohexane extract (150 ml)

- washing with methanol/water (4+1, 30 ml), methanol/water (1+1, 30 ml) and water (2 × 30 ml)

- extraction with 2 × 40 ml dimethylformamide/water (9+1)

dimethylformamide-water mixture

- addition of water (80 ml)

- extraction with cyclohexane (2 × 40 ml)

cyclohexane extract (80 ml)

- washing with water (2 × 30 ml)

- concentration (rotary evaporator, nitrogen)

sample in cyclohexane (1 ml)

silica gel fractionation (see text)

(1) PAH fraction

(2) nitro- and oxy-PAH fraction

concentration of the fractions to 0.1 ml (N<sub>2</sub>)

SIM analysis

Fig. 2. Scheme for sample extraction and clean-up

Table 1. Some characteristics of the lettuce samples

Sample	Site of growth	Remarks
1	Helsinki, Tali	About 1 km north of a highway, 200 m south of a road with dense traffic; cultivation allotment; grown under glass
2	As above	As above, but grown in open air
3	Helsinki, Vartiokylä	150 m north of a highway; about 1 km south of a coal-powered power plant; detached house area
4	Helsinki, Jollas	Close to a road with fairly dense traffic; detached house area
5	Helsinki, Laajasalo	As number 4
6	Helsinki, Pitäjänmäki	0.5 km from main road; detached house area
7	Helsinki, Länsi-Pakila	300 m north of the E3 highway; detached house area
8	Helsinki, Veräjämäki	1 km from highways E3 and E4; detached house area
9-14	Vantaa, Myyrmäki	About 1 km west of the E79 highway; about two km south of a coal-powered power plant
15-16	Vantaa, Hämevaara	1 km west of main road; detached house area
17-18	Espoo, Pihlajarinne	300 m west of main road; on the western edge of an area of sparsely populated countryside
19	Espoo, Rastaala	1.5 km from a busy road; sparsely populated detached house area
20-21	Porvoo mlk., Mickelsböle	1.5 km north of a highway; 4 km north of a large oil refinery; sparsely populated countryside
22	Pornainen, Halkia	40 km north-east of the city of Helsinki; 8 km from highway E4; sparsely populated countryside with no significant sources of pollution
23	Pori, Ruosniemi	220 km north-west of Helsinki; a small town with some industry; 50 m from a quiet road; detached house area
24	Helsinki, Marjaniemi	200 m south from a highway; cultivation allotment

tions were concentrated to 100 µl using a rotary evaporator and a stream of nitrogen.

## 2.3 Determination

The PAC were identified and determined by the gaschromatography/selected-ion-monitoring technique described previously by Tuominen et al. [15].

## 3 Results

The PAH concentrations found in the lettuce samples are presented in Table 2.

The following nitro-PAH were determined in samples 1-23: 5-nitroindane, 1-nitronaphthalene, 2-nitronaphthalene, 2-methyl-1-nitronaphthalene, 1-amino-4-nitronaphthalene, 2-nitronaphthol, 2-nitrobiphenyl, 3-nitrobiphenyl, 4-nitrobiphenyl, 2-nitrodiphenylamine, 2-nitrofluorene, 3-nitro-9-fluorenone, 1,3-dinitronaphthalene, 1,5-dinitronaphthalene, 1,8-dinitronaphthalene, 2,2'-dinitrobiphenyl, and 2,7-dinitrofluorene. The concentrations were however below the detection limit of 0.5 µg/kg fresh weight in all but six cases, which are listed in Table 3.

In sample 24 the following PAC were determined: acenaphthylene-1-carboxaldehyde, acenaphthylene-1-carbonitrile, carbazole, anthraquinone, anthrone,

Table 2. Concentrations of PAH compounds in lettuce samples ( $\mu\text{g}/\text{kg}$  fresh weight)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Phenanthrene	2.4	5.4	2.0	3.2	5.1	3.7	1.7	9.8	3.1	4.3	12	11	1.1	3.6	6.5	1.1	0.47	3.0	1.2	0.56	5.2	6.1	1.6
Anthracene	nd	nd	0.09	nd	nd	nd	nd	0.19	nd	nd	nd	nd	nd	0.10	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acridine	0.17	0.29	0.14	0.38	0.38	0.28	0.10	0.49	0.12	0.21	0.24	0.60	0.14	0.51	0.37	nd	0.09	0.08	0.09	0.08	0.20	0.35	0.14
Dibenzofluorene	0.28	1.6	0.56	nd	nd	0.81	0.17	1.3	nd	nd	nd	0.17	0.17	0.91	0.76	0.10	0.23	0.21	0.29	nd	0.15	nd	nd
2-Methylphenanthrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Methylanthracene	0.39	1.2	0.51	0.41	1.0	1.2	0.34	1.3	0.43	0.84	2.1	1.7	0.17	1.2	1.0	0.10	0.58	0.70	0.28	nd	0.32	0.98	nd
1-Methylphenanthrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
9-Methylanthracene	1.5	20	4.9	8.3	15	6.1	4.8	24	2.5	6.3	28	15	1.1	3.8	10	2.7	6.8	7.5	3.4	1.3	7.5	14	5.5
Fluoranthene	1.1	8.3	3.2	6.7	11	6.3	3.0	10	1.7	2.9	18	14	0.89	3.4	10	2.9	6.0	7.1	2.9	1.3	3.2	8.2	6.6
Pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
9,10-Dimethylanthracene	0.08	1.9	0.42	0.57	1.2	0.89	0.29	1.6	0.25	0.36	2.5	1.5	0.13	0.43	0.96	2.6	0.63	0.69	0.45	0.22	0.22	1.4	0.87
Benzo[a]fluorene	0.12	0.48	0.26	0.67	1.4	0.61	0.22	0.98	0.28	0.28	2.8	1.6	0.11	0.24	1.1	0.26	0.49	0.51	0.30	0.13	0.11	0.84	1.6
Benzo[a]fluorene	0.06	0.68	0.17	nd	nd	0.82	0.16	0.52	nd	nd	nd	nd	0.05	0.17	0.81	0.14	0.28	0.40	0.18	0.14	0.07	nd	nd
1-Methylpyrene	0.47	8.4	1.1	2.9	4.6	3.3	1.7	7.9	1.1	0.72	11	7.0	0.50	2.1	4.0	1.8	4.0	5.1	3.3	0.98	1.3	4.3	3.4
Sum of benzo[a]anthracene, chrysene and triphenylene	nd	nd	nd	nd	nd	nd	nd	0.29	nd	nd	nd	nd	nd	0.16	nd	0.18	nd	nd	0.25	nd	nd	nd	nd
Naphthalene	nd	1.9	0.25	3.1	6.2	2.5	nd	1.5	1.6	0.68	11	4.3	nd	1.7	0.84	0.85	2.0	1.3	nd	0.60	nd	6.3	3.1
Sum of benzo[b]-, -[j]-, and -[k]fluoranthenes	nd	1.0	0.11	0.97	1.6	0.60	0.18	0.89	0.40	0.07	2.2	1.2	0.08	0.73	0.31	0.22	0.81	0.58	0.90	0.37	0.27	1.3	0.74
Benzo[e]pyrene	nd	0.68	0.11	0.70	0.95	0.36	0.18	0.65	0.28	nd	1.4	0.90	0.05	0.63	0.19	0.12	0.67	0.23	0.57	0.26	0.19	1.0	0.59
Benzo[a]pyrene	nd	0.26	nd	0.06	0.12	0.11	0.11	0.29	0.09	nd	0.36	0.40	nd	0.15	nd	0.07	0.11	0.75	0.33	0.13	0.05	nd	0.12
Pyrene	nd	0.51	nd	nd	0.54	0.30	0.32	0.32	0.32	nd	nd	nd	0.14	0.63	0.22	nd	0.72	0.41	0.66	0.25	0.37	nd	nd
Indeno(1,2,3-cd)pyrene	nd	0.08	nd	0.36	0.08	nd	nd	nd	0.17	nd	0.18	0.30	nd	0.15	nd	0.19	0.13	0.07	nd	nd	nd	0.10	0.09
Sum of dibenzo[a,c]- and [a,h]anthracenes	nd	0.44	nd	0.61	1.1	0.45	0.21	0.29	0.32	0.06	2.1	1.2	0.13	0.60	0.19	0.20	0.60	0.33	0.79	0.19	0.24	0.96	0.35
Benzo[ghi]perylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Anthanthrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total PAH	6.6	53	14	29	50	29	13	62	12	17	94	61	4.8	21	37	13	25	29	16	6.5	19	46	25

nd not detected ( $<0.05 \mu\text{g}/\text{kg}$ ); - not analysed

Table 3. Concentrations of nitro-PAH detected in the lettuce samples

Sample	Nitro-PAH species found	Concentration $\mu\text{g}/\text{kg}$ fresh weight
5	2-Nitrobiphenyl	0.5
	2,7-Dinitrofluorene	1.0
6	1,8-Dinitronaphthalene	1.0
7	1,8-Dinitronaphthalene	0.5
22	2-Nitrobiphenyl	1.0
23	2-Nitrodiphenylamine	0.5

Table 4. Concentrations of oxy-PAH in lettuce sample 24

Compound	Concentration $\mu\text{g}/\text{kg}$ fresh weight
9H-fluoren-9-one	1.3
6H-benzo[cd]pyren-6-one	0.3

anthracene-9-carbonitrile, anthracene-9-carboxaldehyde, phenanthrene-9-carboxaldehyde, 7H-benz[de]anthracen-7-one (benzanthrone), 3-nitrofluoranthene, 6-nitrochrysene and dibenzocarbazole. The two positive identifications are shown in Table 4.

The concentrations of all the other compounds were below the detection limit of  $0.1 \mu\text{g}/\text{kg}$  fresh weight.

#### 4 Discussion

The fact that airborne particulates are the main source of PAH in lettuce is evident from a comparison of the results of samples 1 and 2. The total concentrations was greater by a factor of eight in the lettuce grown in the open air ( $6.6$  and  $53 \mu\text{g}/\text{kg}$ ).

The PAH results are in good agreement with those of corresponding studies in Germany [16] and Sweden [2]. On the other hand, there were no distinct differences between the concentrations with respect to the location of growth. Even in samples from the same area, e.g. samples 9–14, the concentrations sometimes differed by one order of magnitude. It is believed that the “trapping efficiency” of a plant, determined by the local rainfall conditions, the amount of irrigation, the exact form and structure of the plant etc., is a more important factor than the average PAH concentration in the surrounding air. As pointed out by Larsson [2], the boosting effect of a nearby highway on the PAH levels of leafy vegetables has a range of only some tens of metres at the sides of the road.

The effects of emission from power plants and industry are furthermore strongly dependent on local meteorological conditions during the cultivation period. This can be seen, e.g. by comparing the results of samples 20–22. the samples 20 and 21 contained very low PAH concentrations although taken from a site

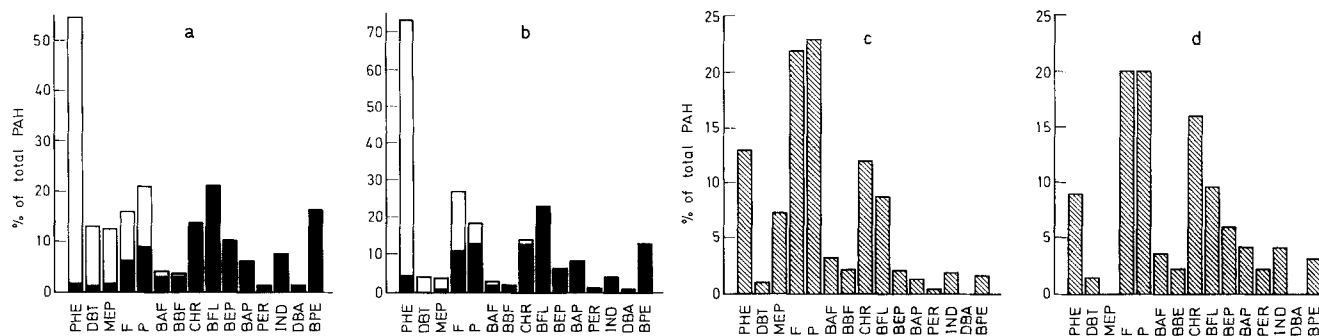


Fig. 3a-d. PAH profiles of ambient air [17] and lettuce. a ambient air from a busy street in the city of Helsinki. b ambient air from Kokkola, a small town on the coast of the Gulf of Bothnia. c lettuce sample 6. d lettuce sample 20. PHE phenanthrene; DBT bibenzothiophene; MEP sum of methylphenanthrenes and -anthracenes; F fluoranthene; P pyrene; BAF benzo[a]fluorene, BBF benzo[b]-fluorene; CHR sum of benzo[a]anthracene, chrysene and triphenylene; BFL sum of benzo[b]-, -[j]-, and -[k]fluoranthenes; BEP benzo[e]pyrene; BAP benzo[a]pyrene; PER perylene; IND Indeno(1,2,3-cd)pyrene; DBA sum of dibenzo[a,c]- and -[a,h]anthracenes; BPE benzo[ghi]perylene

only a few kilometers north of a large oil refinery with presumably significant PAC emissions. The levels were however appreciably higher in sample 22, taken from a countryside location with no apparent local source of pollution. Possibly the prevailing south-westerly winds in the area could explain this difference.

The profiles of PAH in lettuce, i.e. the relative concentrations of the components of the PAH mixture, were very similar in the different samples. This suggests a common source of the PAH compounds. The profiles of the most prominent PAH species in lettuce samples 6 and 20 are compared in Fig. 3 with two examples of PAH profiles of ambient air. The proportions of particle-bound PAH (filter) and gas-phase PAH (XAD) are indicated in the figure. If the re-evaporative effect (strongest for low-molecular-weight PAH species) of the air stream on the particle-bound PAH attached to the filter of the high volume sampler is taken into account, the profiles of the ambient air and of the lettuce samples are rather similar. This is further evidence for the earlier proposal that ambient air particulates are the major source of lettuce PAH.

Nitro-PAH were found in only a few samples, in concentrations just above the detection limits. Obviously, a more sensitive and selective method is needed for proper establishment of nitro-PAH levels in complex matrices. The PAH/nitro-PAH concentration ratio is likely to be similar in both lettuce and ambient air.

The oxy-PAH results also indicate that lettuce can be used as an indicator plant for particulate pollution in ambient air. The two oxy-PAH species detected in sample 24 have been shown to be the two most common contaminants of this type in ambient air particulate extracts [12, 13, 17].

The total consumption of lettuce in Finland is in the range of 1 kg/person/year. If the average total con-

tent is 30 µg/kg fresh weight, the total PAH intake from lettuce is 30 µg/person/year. This is a relatively low figure when compared, for example, with the estimated annual intake from fats and oils of 230 µg/person [18]. It should also be remembered that rinsing of lettuce and removal of the outer leaves appreciably reduces the PAH contamination level.

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