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Microwave-assisted solvent extraction — a new method for isolation of polynuclear aromatic hydrocarbons from plants

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Abstract

The efficiencies of polynuclear aromatic hydrocarbons (PAHs) transfer into various solvents from pollen and spruce needles by two alternative extraction techniques — sonication and a microwave enhanced process — were compared. The effects of extraction mixture composition, temperature, time and number of repeated extractions on the recoveries were studied. Microwave extraction — realised at 140°C for 20 min with *n*-hexane–acetone (3:2, v/v) as the extraction solvent — was identified as the most effective extraction procedure for isolation of PAHs from biotic matrices (pollen and spruce needles). Gel permeation chromatography on Bio-Beads S-X3 with chloroform as a mobile phase was used for clean-up of extracts. HPLC with programmable fluorescence detection was employed for identification and quantification of analytes. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Pollen; Spruce needles; Microwave-assisted solvent extraction; Polynuclear aromatic hydrocarbons

1. Introduction

Polynuclear aromatic hydrocarbons (PAHs) are widespread environmental contaminants mostly originating from various emission sources. Due to the mutagenic and carcinogenic potential of some representatives of this group, the content of PAHs has been monitored in a variety of environmental matrices including air, water, soil/sediments and plant tissues.

Most of plant surfaces exposed to air are covered with waxy or lipidic layers [1]. Air contaminants may be absorbed and accumulated on these surfaces,

thus they act as economic, very convenient passive samplers [2,3].

Based on PAHs levels determined in vegetation sampled at a particular site, local pollution point sources can be identified as well as regional/global environmental contamination patterns characterised [1,4]. For this purpose pollen, plant needles/leaves and/or mosses have been often utilised as bioindicators of environmental pollution by PAHs [2].

Different extraction techniques employing organic solvents such as methanol, acetone, chloroform, dichloromethane, *n*-hexane and cyclohexane are widely used for extraction of PAHs from plant matrices [1]. The use of supercritical carbon dioxide for supercritical fluid extraction has also been re-

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ported [5–8]. Various experimental techniques have been applied for isolation of PAHs by means of “classic” solvent-based procedures. Soxhlet extraction represents a common method of choice [9–12]; however, it is rather time consuming with the additional drawbacks of high solvent consumption as well as energy demands. To improve the PAHs extraction efficiency and to reduce the time needed, extraction enhanced by sonication [13,14] is nowadays the preferred technique in many laboratories. Recently a commercial instrument enabling temperature and pressure controlled microwave heating — microwave extraction system (MES) [15–17] — has become attractive for automated extraction of organic compounds. The possibility of fine tuning extraction conditions as well as good reproducibility allow accurate determination even of less stable analytes. Consequently MES represents a rapid sample preparation technique that enables extractions with reduced amounts of common laboratory solvents at elevated temperatures (under higher pressure the solvent mixture can be heated above its boiling point). Focused microwave assisted extraction (FMW) [18,19] is another technique which used microwaves for extraction, proceeds under atmospheric pressure with varying intensities of microwave irradiation and times of extraction setting. Recently, an accelerated solvent extraction (ASE) has also been utilised for extraction of PAHs (mainly from soil [20,21]).

The aim of our study was to compare the extraction efficiencies of the recently introduced pressurised microwave-assisted solvent extraction (MASE) with the conventional method — extraction enhanced by sonication — for the isolation of PAHs from pollen and spruce needles. Within the validation process conducted in our laboratory the MASE method was shown to provide results comparable to the Soxhlet procedure. In further experiments we focused on comparison of faster sonication and pressurised MASE, on the assumption that MASE will be a less time consuming and more efficient technique than sonication. The influence of various parameters both on the efficiency of PAHs extraction and the amount of co-isolated matrix components potentially interfering within the determinative step was examined.

2. Experimental

2.1. Materials

The samples of pollen and spruce needles (*Picea abies*) were collected within the monitoring project at the surrounding Prague-West region (Jíloviště), Czech Republic. Prior to further processing the samples of pollen were cryogenically milled to yield a powder. The samples of spruce needles were dried at 35°C for 48 h. Homogenised pollen and dried spruce needles were stored in amber glass jars at 4°C.

2.2. Chemicals

Chloroform and acetone (analytical reagent grade, Lachema Brno, Czech Republic) were redistilled in glass before use. Methanol and acetonitrile (Merck, gradient grade, for chromatography, Germany), water (Merck, for chromatography) and *n*-hexane (Merck, for organic trace analysis) were used as supplied. Sodium sulphate anhydrous (Penta Praha, Czech Republic) was heated at 500°C for 4 h and stored in a tightly capped bottle. All glassware was washed with detergent, rinsed with distilled water and then with acetone before use.

2.3. Standard compounds

A mixture of 16 priority PAHs (PAHs, Mix 9): acenaphthene (Ace), acenaphthylene (Acy), anthracene (Ant), benzo[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), benzo[*b*]fluoranthene (B[*b*]F), benzo[*g,h,i*]perylene (B[*g,h,i*]P), benzo[*k*]fluoranthene (B[*k*]F), chrysene (Chr), dibenz[*a,h*]anthracene (DB[*a,h*]A), fluoranthene (Flt), fluorene (Flu), indeno[1,2,3-*c,d*]pyrene (I[*c,d*]P), naphthalene (Nap), phenanthrene (Phe) and pyrene (Pyr) dissolved in acetonitrile at the concentrations 10 µg/ml was supplied by Dr. Ehrenstorfer (Germany). Stock solutions of PAHs standard mixtures were made up in acetonitrile from the received commercial standard mixture ($c=10$ µg/ml).

2.4. Apparatus

An ultrasonic bath (Tesla, Slovak Republic) and the microwave extractor MES-1000 (CEM Corporation, USA) were used to carry out the extraction step.

The automated GPC (gel permeation chromatography) system Gilson, consisting of a pump Gilson (305 Master pump), a fraction collector, an automatic regulator of a loop Aspec XLI (Controller keypad via RS232C), a microcomputer (software 731 PC via RS232C), a dilutor Gilson 401C (Gilson, France) and a stainless steel column 50×0.8 cm I.D. (Tessek, Czech Republic) packed with Bio-Beads S-X3, 200–400 mesh (Bio-Rad, USA) was used for clean-up of crude extracts.

The high-performance liquid chromatographic system (HPLC) was composed of a Hewlett-Packard 1050 series quaternary pump system, a HP 1050 series autosampler, a HP 1046 A fluorescence detector (FLD) (HP, USA) and a column LiChroCART 250-4 (250×4 mm I.D.) with the sorbent LiChrospher-PAHs (Merck, Germany).

2.5. Isolation

The survey of tested extraction procedures used for isolation of PAHs from pollen and spruce needles is shown in Table 1.

2.5.1. Extraction enhanced by sonication

A 5-g amount of sample was transferred into an Erlenmeyer flask with 50 ml of extraction solvent. The flask, covered with an aluminium foil to prevent photodegradation, was placed into an ultrasonic bath for a time depending on the procedure. The extract was carefully filtered through a layer of anhydrous sodium sulphate into a 250-ml round-bottomed flask. The extraction was repeated as described in Table 1 (50 ml of extraction solvent for each further extraction). The solvent was evaporated by a rotary vacuum evaporator at 40°C just to dryness and quantitatively transferred into a 10-ml volumetric flask by chloroform.

2.5.2. Microwave-assisted solvent extraction

A 5-g amount of sample was placed into the lined extraction vessel for microwave sample preparation (designed with material that is relatively inert to solvents and microwave transparent — PTFE PFA — perfluoroalkoxy inner liner was used in this case), covered by 50 ml of solvent mixture, placed to the microwave extraction system MES-1000 and extracted for 20 min at a temperature depending on the procedure. After extraction, the vessels were allowed to cool to room temperature before opening. The extract was carefully filtered through an anhydrous sodium sulphate layer into a 100-ml round-bottomed

Table 1
Characterisation of procedures tested for isolation of PAHs from pollen and spruce needles

Matrix	Type of procedure	Code	Extraction conditions		
			Solvent	Time (min)	Temperature (°C)
Pollen	Sonication	P-SO-CH-1	Chloroform	1×20	Ambient temperature
		P-SO-CH-2		3×20	
		P-SO-HA-1	<i>n</i> -Hexane–acetone (3:2, v/v)	1×20	
		P-SO-HA-2		3×20	
	Microwave extraction	P-MW-50	<i>n</i> -Hexane–acetone (3:2, v/v)	20	50
		P-MW-80			80
		P-MW-110			110
		P-MW-140			140
Spruce needles	Sonication	N-SO-CH	Chloroform	2×20	Ambient temperature
		N-SO-HA	<i>n</i> -Hexane–acetone (3:2, v/v)		
	Microwave extraction	N-MW-50	<i>n</i> -Hexane–acetone (3:2, v/v)	20	50
		N-MW-80			80
		N-MW-140			140

Table 2
FLD settings for PAHs determination

PAHs	λ_{ex} (nm)	λ_{em} (nm)	Time table (min)
Phe, Ant, Flt, Pyr	235	420	0
B[a]A, Chr	264	384	13
B[b]F, B[k]F, B[a]P, DB[a,h]A, B[g,h,i]P	295	405	17
I[c,d]P	300	500	24.5

flask. The solvent was evaporated by rotary vacuum evaporator at 40°C just to dryness and quantitatively transferred into a 10-ml volumetric flask by chloroform.

2.6. Clean-up

The clean-up procedure was carried out by GPC employing gel Bio-Beads S-X3. The mobile phase (chloroform) flow-rate was 0.6 ml/min; the injection volume was 1 ml. The first 15.5 ml of eluate were discarded and the next 15.5 ml were collected into a 50-ml round-bottomed flask. The purified extracts were subsequently subjected to concentration by rotary vacuum evaporator at 40°C just to dryness. The residue was immediately dissolved in 0.5 ml of acetonitrile and the solution was transferred into an amber vial and capped.

2.7. HPLC determination

The HPLC–FLD determination was carried out under following conditions: gradient elution [A= methanol–acetonitrile–water (50:25:25, v/v/v), B= acetonitrile; 0–1 min, 100% A, 22 min, 100% B, 30 min, 100% B], injection volume 20 μ l, column temperature 40°C. FLD settings are shown in Table 2.

3. Results and discussion

The extracts obtained by either of the procedures examined were not applicable to direct HPLC analysis. Plant pigments, waxes and other compounds soluble in organic solvents were coextracted from respective matrices together with target analytes. In the case of spruce needles the bulk coextracts were wax components consisting mainly of long chain

esters, polyesters and paraffins [22]; coextracts of pollen were represented mainly by lipids composed of mono-, di- and tri-acylglycerols, free fatty acids and sterols [23]. Clean-up procedure for crude extract utilising GPC and quantitation based on HPLC–FLD was optimised within the first phase of our experiments. In Table 3 data for extracts obtained by chloroform extraction (see procedure P-SO-CH-2, Table 1) are summarised. Because of workplace hazard related to the chloroform use, experiments aimed at its replacement by less toxic solvent mixture were conducted.

The recovery of clean-up step (GPC) together with the limit of detection (LOD) of HPLC–FLD is shown in Table 3.

The relative efficiencies of the extraction methods both for spruce needles and pollen are listed in Tables 4 and 5. The recovery obtained by sonication with chloroform as an extraction solvent, i.e. extraction method routinely performed in our labora-

Table 3
Performance characteristics of clean-up and determinative steps in PAHs analysis

PAHs	Recovery of GPC clean-up \pm R.S.D.s ^a (%)	LOD ^b (μ g/kg)
Phe	100.9 \pm 0.2	5.0
Ant	95.9 \pm 0.3	0.4
Flt	102.1 \pm 0.2	0.7
Pyr	102.9 \pm 0.2	0.2
B[a]A	99.7 \pm 0.3	0.3
Chr	98.1 \pm 0.2	0.2
B[b]F	96.5 \pm 0.2	0.8
B[k]F	97.0 \pm 0.2	0.1
B[a]P	97.3 \pm 0.2	0.2
DB[a,h]A	97.6 \pm 0.4	0.2
B[g,h,i]P	99.4 \pm 0.3	0.3
I[c,d]P	95.3 \pm 0.4	4.0

^a Spiking level of crude extract, 5 ng/g of sample ($n=10$).

^b Calculated as three times the standard deviation of blank sample noise.

Table 4
Concentrations of PAHs in spruce needles extracts prepared by different procedures ($n=3-6$)

PAH	Analyte content ^c	Relative recovery (%)			
		N-SO-CH	N-SO-HA	N-MW-50	N-MW-80
Code of procedure ^a	N-SO-CH	N-SO-HA	N-MW-50	N-MW-80	N-MW-140
Residue ^b (mg, % of sample weight)	92 (1.8)	103 (2.1)	116 (2.3)	134 (2.7)	177 (3.5)
Phe	77.1	103	107	125	147
Ant	1.9	92	138	142	171
Flt	76.0	98	126	125	145
Pyr	81.3	97	108	94	112
B[a]A	9.2	100	102	108	104
Chr	47.4	102	118	111	118
B[b]F	10.0	99	114	107	108
B[k]F	5.0	101	100	103	103
B[a]P	3.2	95	96	93	92
DB[a,h]A	0.6	104	104	114	116
B[g,h,i]P	4.6	87	119	108	109
I[c,d]P	5.3	95	94	102	103
Sum PAHs (R.S.D.s, %)	321.6 (9)				
Average (R.S.D.s, %)		98 (12)	111 (4)	111 (4)	119 (8)

^a For account see Table 1.

^b Residue remaining after evaporation of extraction solvent; sample weight 5 g.

^c Content of PAHs was calculated on dry weight matter of sample (moisture 54%).

Note: Reagent blank samples were handled together with extracts of tested materials.

tory was 100% (see procedures P-SO-CH-2 and N-SO-CH, Table 1). The extraction mixture *n*-hexane–acetone (3:2, v/v) was found by preliminary experiments to be most applicable [the extraction efficiencies of *n*-hexane, acetone, *n*-hexane–acetone (1:1, v/v) and (3:2, v/v) were tested].

Comparing results obtained by conventional extraction sonication of spruce needles under various conditions it can be seen that *n*-hexane–acetone extraction mixture provided recoveries of PAHs almost equal to that of chloroform (see Table 4). Slightly lower amounts of matrix component potentially interfering within chromatographic analysis were extracted by this solvent mixture.

For extraction of PAHs from pollen by sonication (the composition of this matrix is very different from that of spruce needles) the replacement of chloroform by *n*-hexane–acetone mixture proved not possible; the PAHs recoveries when the latter solvent was employed were significantly lower (see Table 5). Based on the appraisal of data summarised in Tables 4 and 5 it can be concluded that good extraction efficiency is accompanied by higher coextract yields.

In the case of extraction employing MASE, chlo-

reform could not be used since this solvent does not absorb microwaves [24] thus no heating effect enhancing isolation of analytes from respective matrix is obtained. Therefore the solvent mixture *n*-hexane–acetone (3:2, v/v) which was tested in previous “sonication” was used. Acetone in this case is the component which absorbs microwaves. The extent of this phenomenon is related to dielectric constant — the higher is ratio of acetone in extraction mixture, the higher dielectric constant of a solvent mixture, the higher absorption of microwaves by this mixture and thus the higher the achievable temperature. Similarly to other procedures, the extraction time and number of necessary repetitions were important tested features as well. By preliminary experiments conducted at 100°C, acceptable recoveries of PAHs were obtained for both matrices after 20 min of extraction (times 3, 5, 10, 20 and 30 min were tested). The main parameter investigated in subsequent experiments was the extraction temperature which could be, unlike sonication, easily controlled. The highest values of PAHs recoveries were obtained at 140°C, at the maximum temperature, which allowed technical parameters of instrumen-

Table 5
Concentrations of PAHs in pollen extracts prepared by different procedures ($n=3-6$)

PAH	Analyte content ^c		Relative recovery (%)		
	Code of procedure ^a	P-SO-CH-2	P-SO-CH-1	P-SO-HA-1	P-SO-HA-2
Residue ^b (mg, % of sample weight)		260 (4.9)	353 (6.5)	257 (4.7)	193 (3.5)
Phe		22.9	72	78	75
Ant		0.8	69	68	74
Flt		14.8	77	89	86
Pyr		10.5	77	63	96
B[a]A		3.2	80	100	103
Chr		4.9	85	100	78
B[b]F		4.9	73	93	101
B[k]F		2.4	72	92	94
B[a]P		3.9	75	86	99
DB[a,h]A		0.5	73	102	91
B[g,h,i]P		4.2	77	55	85
I[c,d]P		1.7	51	47	60
Sum PAHs (R.S.D.s, %)		74.7 (14)			
Average (R.S.D.s, %)			73 (9)	81 (15)	87 (10)
		Relative recovery (%)			
Code of procedure ^a	P-MW-50	P-MW-80	P-MW-110	P-MW-140	
Residue ^b (mg, % of sample weight)	261 (4.6)	306 (5.4)	492 (9.8)	633 (11.9)	
Phe	88	78	179	185	
Ant	82	80	104	174	
Flt	81	97	111	148	
Pyr	69	100	131	161	
B[a]A	80	108	133	151	
Chr	103	128	189	192	
B[b]F	76	105	115	149	
B[k]F	64	93	99	126	
B[a]P	79	122	130	137	
DB[a,h]A	93	98	164	185	
B[g,h,i]P	57	91	105	119	
I[c,d]P	108	119	136	148	
Average (R.S.D.s, %)	82 (9)	102 (8)	133 (9)	156 (8)	

^a For account see Table 1.

^b Residue remaining after evaporation of extraction solvent; sample weight 5 g.

Note: Reagent blank samples were handled up together with extracts of tested materials.

tation used (leakage of solvent vapours was detected by sensor when higher extraction temperatures were set — automatic shut down of device followed). In any case, distinctly higher PAHs levels were determined by MASE at temperatures exceeding 100°C, probably due to increased diffusivity of solvent into the internal parts of the matrix under elevated temperatures. Nevertheless, simultaneously with increased PAHs extractability, increased amount

of matrix components was co-isolated at higher temperature (see Tables 4 and 5 and Fig. 1). However, no significant interference with peaks of target analytes within chromatographic analysis under the HPLC–FLD conditions used were found.

During repeated sonication of pollen, a significant increase of three-ring PAHs (Phe, Ant) and Flt was not recorded to compare with single sonication (see Tables 4 and 5). This implies the possibility of

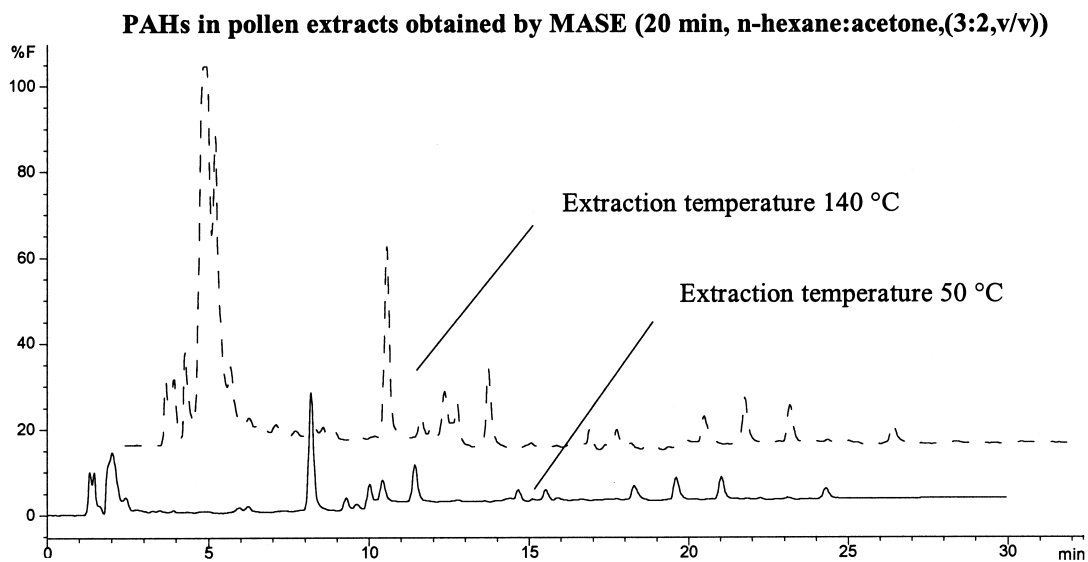


Fig. 1. Extraction of pollen matrix components by MASE at the lowest and the highest tested temperature as recorded by HPLC–FLD.

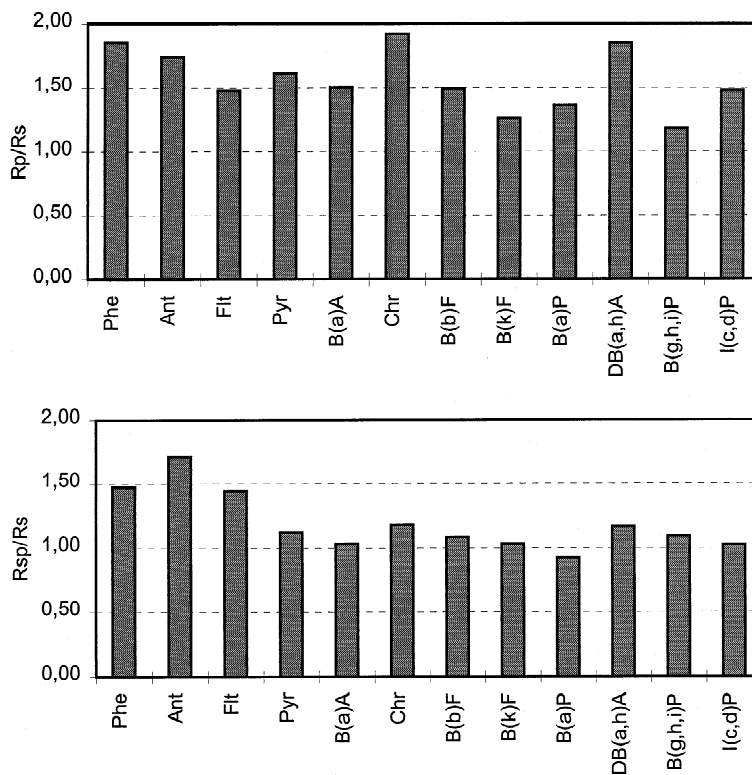


Fig. 2. Comparison of extraction by chloroform supported by sonication and by MASE at 140°C. (a) Pollen (Rs: procedure P-SO-CH-2, Rp: procedure P-MW-140, see Table 1). (b) Spruce needles (Rs: procedure N-SO-CH, Rsp: procedure N-MW-140, see Table 1).

evaporation of these PAHs under conditions of repeated sonication and indicates elimination of this problem by utilisation of pressure controlled microwave instrument with closed vessels. The composition of the matrix also influenced PAHs extractability, which is documented in Fig. 2. In the case of spruce needles practically all of “higher” PAHs (four, five and six-rings) were extracted by sonication, only the three-ring PAHs (Phe, Ant) and Flt needed the elevated temperature gained by MASE for sufficient extraction. For pollen, when PAHs can probably easily penetrate into the internal parts, only MASE with 140°C was established as the most efficient technique for extraction of all analysed PAHs.

Fig. 3 shows the influence of temperature on MASE efficiency. During extraction of spruce needles only an increase of three-ring PAHs (Phe, Ant) and Flt was found; extraction efficiency of all

analysed PAHs from pollen increased with increasing temperature.

Considering calculated R.S.D.s, better repeatability of MASE compared to extraction sonication was recorded. Unfortunately, commercial pollen/needle certified reference material is not yet available, hence an unbiased assessment of results is not possible. Due to the technical limitations of the microwave system, which did not allow temperature increase to the maximum theoretical temperature (156–160°C at 1.2 mPa) [13] and due to the lack of CRM, it is not clear, whether maximum recovery was obtained. However, similar results to those in this work were obtained by Lopez-Avila et al. [15]. They were concerned with the analysis of PAHs in standard reference soils and sediments by MASE at various temperatures (80, 115 and 145°C) and times of extraction (5, 10 and 20 min). They found the best

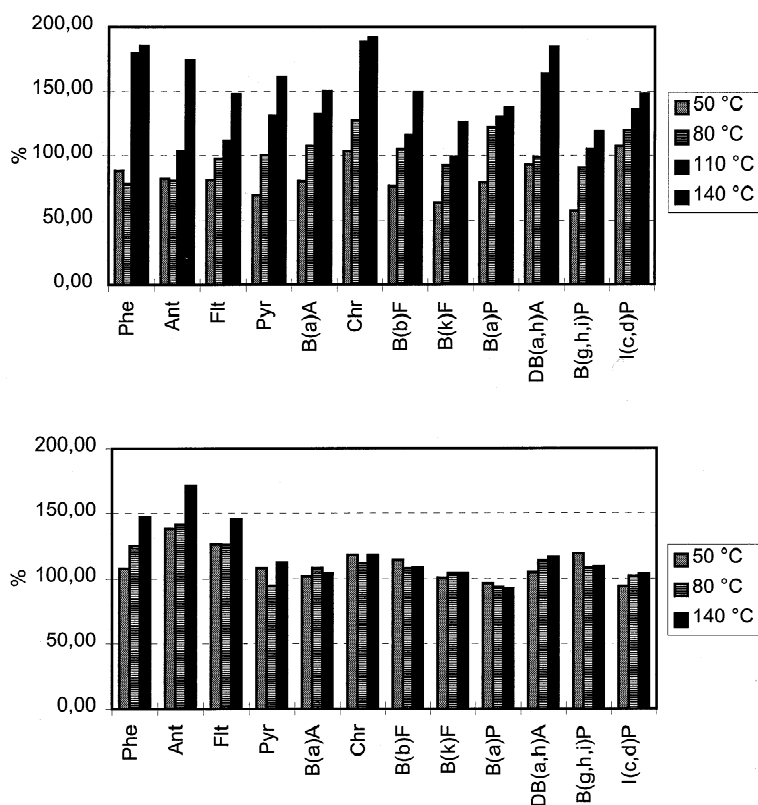


Fig. 3. Comparison of extraction efficiencies obtained by MASE conducted under different temperatures. (a) Pollen (100%=procedure P-SO-CH-2, see Table 1). (b) Spruce needles (100%=procedure N-SO-CH, see Table 1).

recoveries for the extraction solvent mixture *n*-hexane–acetone (1:1, v/v) at 115°C and 145°C and 20 min for both types of materials.

4. Conclusion

Two extraction techniques — conventionally used extraction enhanced by sonication and the newly introduced microwave-assisted solvent extraction — were compared for the purpose of PAHs determination. Replacement of chloroform by the less hazardous *n*-hexane–acetone mixture was possible for the isolation of PAHs from spruce needles and pollen. Microwave-assisted solvent extraction thus seems to be a viable alternative in particular cases.

Comparing both techniques examined with respect to performance parameters the main advantages of microwave extraction were recognized as: (1) reduced solvent use (2) higher efficiency of extraction achievable under optimised conditions simultaneously with (3) reduced extraction time (4) easy maintenance of extraction vessels (5) enhancement of analytical capabilities such as improvement of recovery and repeatability and (6) the possibility of simultaneously extracting up to twelve samples resulting in increased sample throughput over conventional extraction techniques. The drawback of MASE is the relatively high price of the apparatus; the capacity of the solvents to absorb microwave energy also plays a role in the efficiency of extraction. On the other hand, the apparatus for sonication is cheaper and its service is easier, but it consumes more solvents and has a longer extraction time.

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References

- [1] S.L. Simonich, R.A. Hites, *Environ. Sci. Technol.* 28 (1994) 939–943.
- [2] H. Kylin, E. Grimwall, *Environ. Sci. Technol.* 28 (1994) 1320–1324.
- [3] J. Jacob, G. Grimmer, A. Hildebrandt, *Sci. Total Environ.* 139–140 (1993) 307–321.
- [4] S.O. Baek, R.A. Field, M.E. Goldstone, P.W. Kirk, J.N. Lester, R. Perry, *Water, Air Soil Pollut.* 60 (1991) 279–300.
- [5] B. Wenclawiak, C. Rathmann, A. Teuber, *Fresenius J. Anal. Chem.* 344 (1992) 497–500.
- [6] N. Saim, J.R. Dean, M.P. Abdullah, Z. Zakaria, *J. Chromatogr. A* 791 (1997) 361–366.
- [7] B.W. Wenclawiak, T. Paschke, M. Krappe, *Fresenius J. Anal. Chem.* 357 (1997) 1128–1132.
- [8] C. Rodriguez, A. Bispo, J.L. Haag, M. Jauzein, F. Colin, *Polycyclic Aromat. Compd.* 9 (1996) 349–356.
- [9] J.M. Becnel, K.M. Dooley, *Ind. Eng. Chem. Res.* 37 (1998) 584–594.
- [10] U.L. Nilsson, C.E. Östman, *Environ. Sci. Technol.* 27 (1993) 1826–1831.
- [11] T. Spitzer, S. Kuwatsuka, *Environ. Pollut.* 62 (1989) 63–71.
- [12] E. Övervik, J. Gustavsson, *Atm. Environ.* 29 (1995) 1553–1558.
- [13] M.D. Burford, S.B. Hawthorne, D.J. Miller, *Anal. Chem.* 65 (1993) 1497–1505.
- [14] K. Cejpek, J. Hajšlová, Z. Jehličková, J. Merhaut, *Int. J. Environ. Anal. Chem.* 61 (1995) 65–80.
- [15] V. Lopez-Avila, R. Young, W.F. Beckert, *Anal. Chem.* 66 (1994) 1097–1106.
- [16] K. Ganzler, A. Salgo, K. Valko, *J. Chromatogr.* 371 (1986) 299–306.
- [17] Renoe B.W., *American Laboratory*, August, 34–40 (1994).
- [18] H. Budzinski, P. Baumard, A. Papineau, S. Wise, P. Garrigues, *Polycyclic Aromat. Compd.* 9 (1996) 225–232.
- [19] Letellier, M., Budzinski, H. Garrigues, P. Wise, S., *Spectroscopy*, 13 (1996/1997) 71–80.
- [20] J.A. Fisher, M.J. Scarlett, A.D. Stott, *Environ. Sci. Technol.* 31 (1997) 1120–1127.
- [21] O.P. Heemken, N. Theobald, B.W. Wenclawiak, *Anal. Chem.* 69 (1997) 2171–2180.
- [22] K.E. Percy, C.J. McQuattie, J.A. Rebbeck, *Air, Pollutant and Leafy Cuticle*, NATO ASI, Springer Verlag, Berlin, Heidelberg, 1994, Vol. G.36.
- [23] S. Kubišová, D. Titěra, *Pyl ve výživě včel (Pollen in bee nutrition)*, published by Český svaz včelařů, Státní zemědělské nakladatelství, Praha, 1988.
- [24] Lautenschläger W., Schweizer T., *LaborPraxis*, Mai, 376–381 (1990).