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DETERMINATION OF ORGANIC COMPOUNDS FROM DIFFERENT TYPES OF COFFEE BY HPLC AND GC-ECD ANALYSIS

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Abstract

A study was conducted to investigate the organic compounds residues in different types of coffee from market in Romania. High pressure liquid chromatography with fluorescence detector (HPLC), respectively gas chromatography with electron capture detection (GC-ECD) was used for the determination of 7 polycyclic aromatic hydrocarbons (PAHs) and 9 organochlorine pesticides (OCPs) in coffee samples. It was found that concentrations of PAHs in coffee samples varied from 0.001 µg/Kg to 90.732 µg/Kg. B[a]P was absent in the green coffee samples. The detection limits ranged from 0.02 ng/kg to 0.04 ng/kg and quantification limits were 0.2 ng/kg. Calibration and recovery studies gave satisfactory results. Concentrations of OCPs in coffee samples varied from 0.001 mg/Kg to 0.007 mg/Kg. The methods provide a rapid and accurate determination of these organic compounds in coffee samples.

Key words: coffee samples, HPLC, GC-ECD, PAH, OCP

1. Introduction

It was about a decade ago that scientists started to pay great attention to the significance of vegetation in environmental pollution processes (Paterson et al., 1990). Polycyclic aromatic hydrocarbons (PAHs) are well-known environmental pollutants, even at low concentrations. PAHs are included in the European Community (EC) and in the Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties (Cooke and Dennis, 1986). On the other hand organochlorine pesticides (OCPs) are ubiquitous and persistent pollutants. They have been worldwide contaminants in 1969, but nowadays their use has been prohibited in most countries. Among the OCPs that have become globally enriched in food chains are included the insecticides DDT, lindane (γ-HCH), aldrin, dieldrin and the fungicide hexachlorobenzene (HCB). The latter is also a product of rapid, high temperature combustion.

Vegetables are essential constituents of the human diet. Monitoring of PAHs and OCPs in vegetables is nowadays a priority objective in order to get an extensive evaluation of vegetable quality to avoid possible risks to human health.

Numerous research papers have been published on PAHs and OCPs in environmental samples, such soil (Kulhanek et al., 2005), plants (Ho and Hsieh, 2001; Soceanu et al., 2006), water (Dobrinas et al., 2008; Reddy and Quinn, 2001; Dobrinas et al., 2002; Reddy and Quinn, 2001), marine sediments (Gong et al., 2007; Zaghden et al., 2007), organisms (Carls et al., 2006) and zooplankton (Covaci et al., 2002). Although few foods have been analyzed to date, PAHs have been detected in vegetable oils, fruits, sea food, tea, coffee, potato, toasted bread (Nieva-Cano et al., 2001; Stall and Einsenbrand, 1988; Vinas et al., 2007; Vo-Dinh,

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1989) and OCPs have been detected in fish and fish oil, vegetable oil, vegetables, fruits, baby food and meat (Colume et al., 2001; Chuang et al., 2001; Di Bella et al., 2006; Garrido-Frenich et al., 2006; Zawiyah et al., 2007). Food pollution is due to the deposition of PAHs from air or water, or as a result of preservation, drying and cooking procedures (Vo-Dinh, 1989).

OCPs are nonsystemic, as they cannot penetrate into the plant. They form a deposition on the surface of the leaf or other plant organ; their potential absorbtion depends on the formulation, lipophilicity and concentration of the active ingredients and therefore the extraction of these pesticides is rather simple compared to the systemic organophospateand carbamate pesticides. Most of the methods applied to the determination of PAHs and OCPs in vegetables are based on solvent extraction (the extract is cleaned up in a silica, alumina or florisil column) followed by gas chromatography with various detectors.

The studies for the contamination of food are enclosed on the JECFA (Joint FAO/WHO Expert Committee on Food Additives) list and the European Commission limited the Benzopyrene content in food (Report 1996; EC 2005).

Since only a few research studies on the PAHs and OCPs content in food samples, and in vegetables in particular, have been undertaken, in this paper we report a simple method for the determination of 7 PAHs and 9 OCPs in different types of coffee, by HPLC and GC-ECD.

2. Experimental

2.1. Chemicals and reagents

There were used the following PAHs and OCPs analytical standards: benzo[a]anthracene ((B[a]An), benzo[a]anthracene; benzo[k]fluoranthene (B[k]Fl), benzo[b]fluoranthene (B[b]Fl), (B[a]Py), benzo[a]pyrene; (B[ghi]P), benzo[ghi]perylene; (dB[α,h]An), dibenzo[α,h]anthracene; (I[1,2,3–cd]Py), indeno[1,2,3–cd]pyrene) from Supelco (Belefonte PA, SUA) and HCB, Lindane, heptachlor, p,p'- DDT, p,p'- DDE, p,p'- DDD, Aldrin, Dieldrin, Endrin from International Atomic Energy Agency, Monaco laboratory.

As sorbent materials: silica gel, florisil and aluminium oxide were assayed for preconcentration step. Silica gel 60 (0.2 – 0.5 mm) and aluminium oxide 90 (0.063 – 0.200 mm) were obtained from Merck, Darmstadt, Germany. Florisil (60 – 100 mesh) was obtained from Fluka (packed in Switzerland) and was activated overnight (12h) at 130^{o}C before use. Silica gel and aluminium oxide were activated at 420^{o}C for 4h before use. Anhydrous sodium sulphate (granulated for residue analysis – Merck) was activated at 200^{o}C for 2h before use.

There were used the following solvents: n-hexane and acetonitrile, supplied by Merck, Darmstadt, Germany, petroleum ether and ethylic ether supplied by J.T. Baker.

From the PAH mix solution (PAH Calibration Mix – Supelco), a new PAH solution at levels from 10 μg/ml to 100 μg/ml was prepared into acetonitrile and this solution was diluted to construct calibration lines for the PAHs.

2.2. Sampling

Samples of green coffee bean, roasted coffee bean, instant coffee granules and coffee without caffeine (all being registered to the same producer), all available at supermarkets were analyzed. No indication as regards the country of origin was given on the label.

2.3. Sample extraction and clean-up

Approximately, 10 g of each coffee type was used for Soxhlet extraction over a period of 4 h with hexane as solvent. Coffee samples were extracted in triplicate. The solvent was removed under reduced pressure at 40^{o} C. The lipid extracts were mixed with petroleum ether and the mixture was transferred to a separatory funnel where was shaken with acetonitrile and then was separated the layers.

The extracts were then evaporated under vacuum using a rotary evaporator and then the concentrated extract was purified by column chromatography. A home-made glass column containing a piece of glass wool on a glass frit was filled with 5 g of activated aluminium oxide, 5 g of activated silica-gel and about 1 g of anhydrous sodium sulfate on the top to fractionate the aliphatic and aromatic fractions. For OCPs fractions was used a home-made glass column containing a piece of glass wool on a glass frit filled with 5 g of Florisil and about 1 g of anhydrous sodium sulfate on the top. The PAHs and OCPs residues were eluted with petroleum ether: ethylic ether (94:6) mixture and the eluate was collected in a conical evaporating flask. The sorbent was not allowed to dry during the conditioning and sample loading steps. The eluate was finally concentrated in a Kuderna–Danish concentrator to approximately 1 mL and the concentrated aliquots were blown down under a gentle stream of nitrogen gas.

2.4. Instruments

2.4.1. HPLC

All HPLC measurements were assayed using a Varian device equipped with ProStar 240 Quaternary pump, a ProStar autosempler and ProStar 363 fluorescence detector. The optimized instrumental parameters were as follows: injection loop: 20μL; a ChromSep HPLC Column SS 100x4.6 mm; elution conditions: 32 min linear gradient elution from 70:30 acetonitrile/water to 93:03 acetonitrile/water, followed by 3 min linear gradient to 100% acetonitrile and held for 5 min; elution temperature:

30 °C; fluorescence detection: 16 min $\lambda_{ex.}$ at 274nm and λ_{em} at 414 nm, followed by 7 min λ_{ex} at 296 nm and λ_{em} at 406nm followed by 9 min at λ_{ex} at 300nm and $\lambda_{em\ at}$ 470 nm.

2.4.2. GC-ECD

A Varian gas chromatograph equipped with an electron capture detector (ECD) and a fused–silica capillary column HP–5, 30m×0.32mm×0.25μm film thickness were used for OCPs analysis. Operating conditions were as follows: initial temperature 60°C (1 min), increased at a rate of 20°C/min to 300°C and finally held for 10 min; injector temperature: 250°C; carrier gas: He; column flow-rate: 1.36mL/min; detector temperature: 300°C; make-up gas: N_2; operation mode: splitless (electronic pressure control); purge off time: 2 min; injection volume: 1 μL.

3. Results and discussion

Limits of detection (LOD) and limits of quantitation (LOQ) for PAHs were evaluated on the basis of the noise obtained with the analysis of blank coffee samples. LOD and LOQ were defined as the concentration of the analyte that produced a signal-noise ratio of three and ten, respectively.

The noise values were determined by measuring the amplitude signal by fluorescence analysis of a blank reagent (n = 7). The performance of the proposed method for the determination of PAHs in coffee samples is summarized in Table 1.

Table 1. Data on performance of the method

PAH$_s$	***Instrument linearity Range[μg/l]***	***r^2***	***LOD[a] [ng/kg]***	***LOQ [ng/kg]***
B[a}A	0.2-10	0.9993	0.04	0.2
B[b]F	0.2-10	0.9946	0.04	0.2
B[k]F	0.2-10	0.9968	0.02	0.2
B[a]P	0.2-10	0.9966	0.02	0.2
D[a,h]A	0.2-10	0.9977	0.04	0.2
B[ghi]P	0.2-10	0.9976	0.04	0.2
I[1,2,3-cd]P	0.2-10	0.9989	0.04	0.2

a- 7 determinations

A green coffee sample was enriched with PAH solutions. The theoretical concentration that enriched the sample was of 2 μg/kg. The recovery percent for the PAHs varied between 83-105 %.

Table 2 gives the results obtained from coffee samples and representative chromatogram of coffee samples are presented in Fig. 1.

In Table 2: (B[a]An), benzo[a]anthracene; benzo[k]fluoranthene (B[k]Fl), benzo[b]fluoranthene (B[b]Fl), (B[a]Py), benzo[a]pyrene; (B[ghi]P), benzo[ghi]perylene; (dB[α,h]An), dibenzo[α,h]anthracene; (I[1,2,3–cd]Py), indeno[1,2,3–cd]pyrene.

Table 2. PAHs concentrations from coffee samples

	Concentrations (μg/kg)			
	Green coffee bean	Roasted coffee bean	Instant coffee granules	Coffee without caffeine
B[a]An	0.017	0.742	0.473	0.014
B[k]Fl	0.002	0.085	0.495	0.010
B[b]Fl	0.001	0.219	0.104	0.028
B[a]Py	ND	0.293	0.857	0.119
B[ghi]P	LOQ	LOQ	LOQ	LOQ
dB[α,h]An	ND	0.040	0.045	0.011
I[1,2,3-cd]Py	0.003	0.113	0.142	0.014

ND- not detected; LOQ- below quantification limit

Benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and indeno[1,2,3–cd]pyrene were found in the most of the samples. Their contents are at μg/Kg level. Benzo[ghi]perylene were found under the quntification limit in all samples.

The least contaminated sample was the green coffee bean, with a mean content of 0.023 μg/Kg and the most contaminated sample was instant coffee granules, with a mean content of 2.116 μg/Kg.

Benzo[a]pyrene and dibenzo[α,h]anthracene were not detected in the green coffee beans. So, the possibility of contamination by environmental pollution, storage etc. is eliminated. But this PAH was found in the coffee without caffeine, in roasted and soluble coffee. Its presence can be explained by the processing of the coffee (important to produce marketable coffee), due probably to the roasting of the coffee. Roasting process is fundamental to obtain a coffee of a good quality, but is harmless to human health. This hypothesis is supported by the fact that B[a]P contamination of coffee is due to the kind of roasting process utilized (Badolato et al., 2006). The values obtained for B[a]Py concentrations were from 0.119 to 0.857 μg/Kg for roasted coffee samples. These values are comparable with those obtained by Badolato and others in ground roasted coffee (0.47-12.5 μg/kg) (Badolato et al., 2006). Also, B[a]Py levels measured in the studied types of coffee samples are lower than those measured in roasted coffe, cofee brews, or dryed coffee brew by other authors (Garcia-Falcon et al. 2005; Kayali-Sayadi et al.,1999]. Bishnoi and others measured the total PAH content (16.47-18.24 μg/L) in coffee samples and it can be observed that the total PAH content in our studied coffee samples is lower then that (Bishnoi et al., 2005).

The proposed method was used to determine nine OCPs in coffee sample and the representative chromatogram obtained are shown in Fig. 2, respectively the typical chromatograms of standards are shown in Fig. 3.

The levels of organochlorine pesticides detected in coffee samples are summarized in Table 3. The table shows that the OCP residue detected in all samples was lindane and the highest concentration was detected for pesticide aldrin 0.007 mg/Kg (this pesticide was detected in only one sample of coffee).

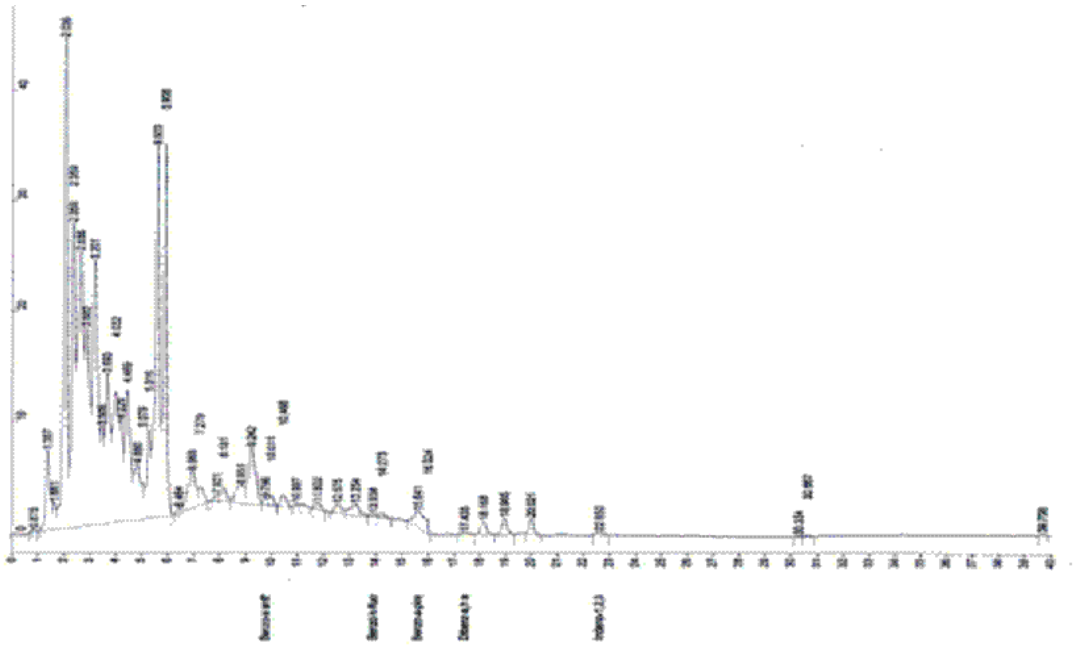

Fig.1. Representative HPLC chromatogram for coffee without caffeine samples

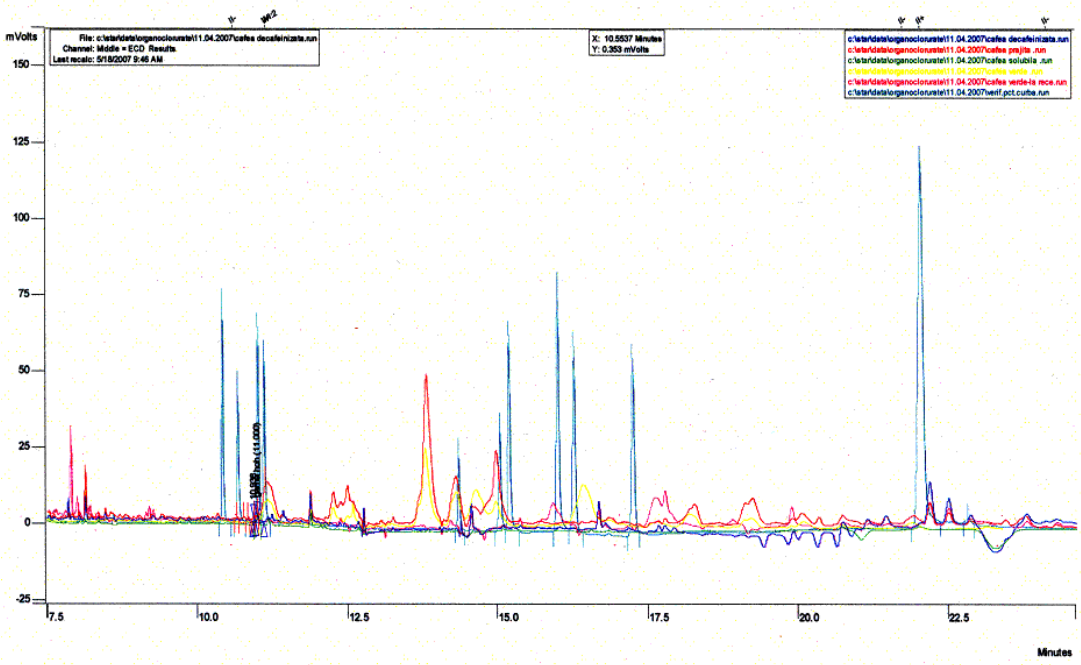

Fig.2. Representative GC-ECD chromatogram for coffee samples

Heptachlor was detected in 2 samples and HCB in one sample. DDT and its metabolites (which degrade very slowly under environmental conditions) were not detected in all studied samples.

Green bean coffee is a material rich in nutrients that promotes the growth of microorganisms (Avallone et al., 2001). Bacteria isolated from defective green bean coffee with traditional enrichment techniques were able to grow on and to degrade DDT in liquid media (Barragan-Huerta et al., 2007). So, its possible that in coffee beans DDT and its metabolites to be degraded by bacteria. Also, DDT and its metabolites were not detected because of the restricted use in agricultural activity.

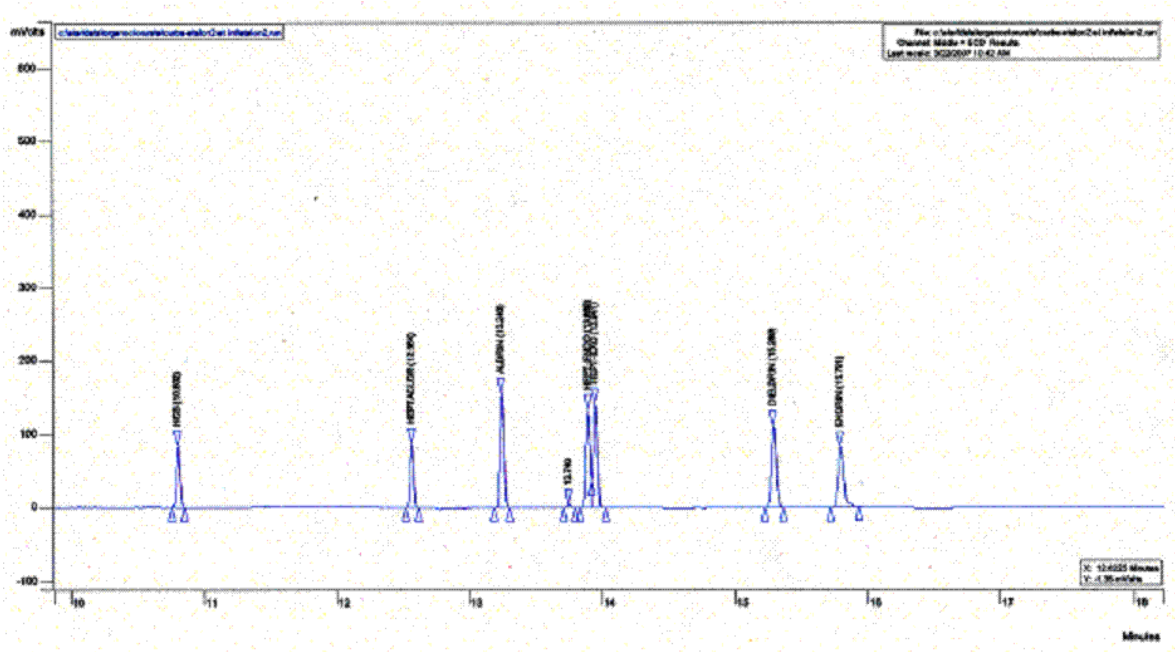

Fig.3. The chromatograms of organochlorine pesticides standards

Table 3. The levels of organochlorine pesticides in coffee samples

Pesticides	***Concentration (mg/Kg product)***			
	Green coffee bean	***Roasted coffee bean***	***Instant coffee granules***	***Coffee without caffeine***
HCB	ND	0.002	ND	ND
lindane	0.0014	0.001	0.004	0.001
heptachlor	0.011	ND	ND	0.004
DDT	ND	ND	ND	ND
DDD	ND	ND	ND	ND
DDE	ND	ND	ND	ND
aldrin	ND	0.007	ND	ND
dieldrin	ND	ND	ND	ND
endrin	ND	ND	ND	ND

The low residual levels of pesticides in coffee at concentrations between 0.001 and 0.007 mg/Kg indicate a situation without toxicological risks for the consumer of coffee. However, the problem of human impact connected to use of these compounds cannot be ignored.

4. Conclusions

HPLC and GC-ECD are ideal techniques for analytical quality control and research in the food and beverage industry**.** Detection and quantification limits for the PAHs in the coffee samples (green coffee bean, roasted coffee bean, instant coffee granules and coffee without caffeine, all being registered to the same producer) were found to be satisfactory and much lower than the restrictions given in proposals of EU Directive.

Benzo[a]pyrene is the most studied and measured PAH and serves as an indicator of the total PAHs content.

In the green coffee beans, no Benzo[a]pyrene was found. But Benzo[a]pyrene was found in the roasted, the soluble and coffee without caffeine. Its presence can be explained by the processing of the coffee, due probably to the roasting of the coffee. Some processes as drying of seeds, roasting and processing are potential ways to contaminate food with Benzopyrene. In order to minimize health risk as well as for enforcement activities, monitoring of polycyclic aromatic hydrocarbons and organochlorine pesticide residues is increasingly important and essential.

References

Avallone S., Guyot B., Brillouet J.M., Olguin E., Guiraud J.P., (2001), Microbiological and biochemical study of coffee fermentation, *Current Microbiology*, **42**, 252–256.

Barragan-Huerta B.E., Costa-Perez, C., Peralta-Cruza J., Barrera-Corte J., Esparza-Garcia F., Rodriguez-Vazquez R., (2007), Biodegradation of organochlorine

pesticides by bacteria grown in microniches of the porous structure of green bean coffee, *International Biodeterioration & Biodegradation,* **59**, 239–244.

Badolato E.S.G., Martins M.S., Aued-Pimentel S., Alaburda J., Kumagai E.E., Baptista G.G., Rosenthal A., (2006), Sistematic Study of Benzo[a]Pyrene in Coffee Samples, *Journal of the Brazilian Chemical Society,* **117,** 989-993.

Bishnoi N.R., Mehta U., Sain U., Pandit G.G., (2005), Quantification of Polycyclic Aromatic Hydrocarbons in Tea and Coffee Samples of Mumbai City (India) by High Performance Liquid Chromatography, *Environmental Monitoring an Assessment*, **107,** 399-406.

Carls M.G., Short J.W., Payne J., (2006), Accumulation of polycyclic aromatic hydrocarbons by Neocalanus copepods in Port Valdez, Alaska, *Mar. Poll. Bull.*, **52,** 1480-1489.

Chuang J.C., Hart K., Chang J.S., Boman L.E., Van Emon J.M., Reed AW., (2001) Evaluation of analytical methods for determining pesticides in baby foods and adult duplicate-diet samples. *Anal. Chim. Acta,* **444,** 87.

Colume A, Cardenas S, Gallego M, Valcarcel M., (2001), Semiautomatic multiresidue gas chromatographic method for the screening of vegetables for 25 organochlorine and pyrethroid pesticides, *Anal. Chim. Acta,* **436**, 153.

Commission Regulation (EC) No. 208, (2005), Official Journal of the European Union 2005, L 34/3.

Cooke M., Dennis A.J., (1986) *Polynuclear aromatic hydrocarbons: chemistry, characterisation and carcinogenisis.*, Battle Press, Columbus, Ohio.

Covaci A., Gheorghe A., Hulea O., Schepens P., (2002), Levels of organohalogenated pollutants (PCBs, OCPs, and PBDEs) in biota from the Danube Delta, Romania, *Organohalogen Compounds*, **59**, 9.

Di Bella G., Licata P., Bruzzese A., Naccari C., Trombetta D., Lo Turco V., Dugo G., Richetti A., Naccari F., (2006), Levels and congener pattern of polychlorinated biphenyl and organochlorine pesticide residues in bluefin tuna (Thunnus thynnus) from the Straits of Messina (Sicily, Italy), *Environ. Int.,* **32,** 705–710.

Dobrinas S., Soceanu A., Birghila S., Coatu V., (2008), Polycyclic aromatic hydrocarbons and organochlorine pesticides contents in tap and surface waters, *Environmental Engineering and Management Journal*, 7(1**),** 57-60.

Dobrinas S., Coatu V. and Chitimia C.S., (2002), Utilizarea gaz cromatografiei pentru determinarea pesticidelor din apa de mare, *Rev. Chim. (Bucuresti),* **53(8),** 633.

Garcia-Falcon M.S., Cancho-Grande B., Simal-Gandara J., (2005), Minimal clean-up and rapid determination of polycyclic aromatic hydrocarbons in instant coffee, *Food Chemistry,* **90,** 643-647.

Garrido-Frenich A., Romero-Gonzalez R, Martinez-Vidal J.L., Plaza-Bolanos P., Cuadros-Rodriguez L., Herrera-Aabdo M.A., (2006), Characterization of recovery profiles using gas chromatography-triple quadrupole mass spectrometry for the determination of pesticide residues in meat samples, *J. Chromatogr. A*, **1133,** 315-321.

Gong X., Qi S., Wang Y., Julia E.B., Chunling LV., (2007), Historical contamination and sources of organochlorine pesticides in sediment cores from Quanzhou Bay, Southeast China. *Mar. Poll. Bull.,* **54**, 1434-1440.

Ho W.H., Hsieh S.J., (2001), Solid phase microextraction asociated with microwave assisted extraction of organochlorine pesticides in medicinal plants, *Anal. Chim. Acta,* **428,** 111.

Kayali-Sayadi M.N., Rubio-Barroso S., Cuesta-Jimenez M.P., Polo-Diez L.M., (1999), A new method for the determination of selected PAHs in coffee brew samples by HPLC with fluorimetric detection and solid-phase extraction, *Journal of Liquid Chromatography and Related Technology*, **22,** 615-627.

Kulhanek A., Trapp S., Sismilich M., Jankl J., Zimova M., (2005), Crop-specific human exposure assessment for polycyclic aromatic hydrocarbons in Czech soils, *Sci. Total Environ.* **339,** 71– 80.

Nieva-Cano M.J., Rubio-Barrosso S., Santos-Delgado M.J., (2001), Determination of PAH in food samples by HPLC with fluorimetric detection following sonication extraction without sample clean-up, *Analyst,* **126,** 1326.

Paterson S., Mackay D., Tam D. and Shiu W.Y. (1990), Uptake of organic chemicals by plants: A review of processes, correlations and models, *Chemosphere,* **21,** 297.

Reddy C. M., Quinn J.G., (2001), The North Cape oil spill: hydrocarbons in Rhode Island coastal waters and Point Judith Pond, *Mar. Environ. Res.,* **52,** 445-461.

Report of the twenty-eighth session of the codex committee on food additives and contaminants. Manila, the Philippines, (1996), Appendix VII, Annexes IV and V to the general standard for contaminants and toxins in foods.

Soceanu A., Dobrinas S., Popescu V., Birghilă S., Coatu V., Magearu V., (2006), Determination of some organochlorine pesticides and heavy metals from Capsicum Anuum L., *Environmental Engineering and Management Journal*, **5(**4**),** 597-604.

Stall W. and Einsenbrand G., (1988), *HPLC in Food Analysis*, ed. R. Macrae, Academic press, New York, 377-408.

Vinas P., Campillo N., Aguinaga N., Perez-Canovas E., Hernandez-Cordoba, M. (2007), Use of headspace solid-phase microextraction coupled to liquid chromatography for the analysis of polycyclic aromatic hydrocarbons in tea infusions, *J. Chromatogr. A*, **1164,** 10–17.

Vo-Dinh T, (1989), *Chemical Analysis of Polycyclic Aromatic Compounds,* ed. T. Vo-Dinh, Wiley, New York, 1-30.

Zaghden H., Kallel M., Elleuch B., Oudot J., Saliot A., (2007), Sources and distribution of aliphatic and polyaromatic hydrocarbons in sediments of Sfax, Tunisia, Mediterranean Sea, *Mar. Chem.*, **105,** 70-89.

Zawiyah S., Cheman Y.B, Nazimah S.A.H., Chin C.K., Tsukamoto I., Hamanyza A.H., Norhaizan I. (2007), Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using SAX/PSA clean-up column, *Food Chemistry,* **102,** 98-103.