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Monitoring of the Pesticide Levels in Natural Waters of Greece

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The occurrence of pesticides and their conversion products in aquatic systems is of major concern world-wide. Therefore, monitoring of surface and ground waters, in order to verify whether inadmissible levels of pesticides are present, is highly important. In European Union countries the directive concerning the quality of water intended for human consumption sets a maximum permissible level of 0.1 µg/L in tap water and 1-3 µg/L in surface water for each compound (E.C. Council Directive, 1980). From the analytical point of view the analyst's task is complex, due to the great variety of different chemical structures that pesticides represent and to the very low concentrations of these compounds that must be determined. Screening for these low levels requires high performance from analytical instruments and sample preparation techniques. Sample preparation of water samples is performed either by liquid-liquid or by solid phase extraction (SPE). Of the solid supports available for SPE, octadecyl (C-18) bonded porous silica has become the most popular due to its applicability on non-polar and weakly polar analytes, as most pesticides are (Hennion MC and Scribe P, 1993). Capillary gas chromatography provides high resolving power, and by the use of the various highly selective detectors, e.g. nitrogen-phosphorus detector (NPD) or electron capture detector (ECD), has become a powerful tool for practical residue determinations for a wide range of pesticides at µg/L (ppb) levels and below.

In this study, solid phase extraction was used to monitor natural waters of Greece for pesticide residues. Organochlorine pesticides were determined with ECD according to a previously described method, while a procedure for the quantitative determination of 24 organophosphorus and triazine pesticides was employed by capillary gas chromatography with cold on column injection and NPD. Capillary gas chromatography has been a well established technique for more than 30 years. Due to its higher separation efficiency, gas chromatography on capillary columns is far better suited for the detection of pesticides' residues than the technique using packed columns. The application of cold on column injection improves selectivity and sensitivity of the analysis, thereby permitting the determination of ultratrace levels of pesticide residues. 80 water samples originating from various regions of Greece were analysed according to the methods.

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MATERIALS AND METHODS

Water samples were collected in 2.5-L glass bottles, between March and December 1996 from the lakes: Marathon (12 samples), Iliki (8), Mornos (12), Pamvotida (1), Orestiada (2), Plastira (1), Lissimahia (1), Trihonida (1) and Amvrakia (1) and from the rivers: Mornos (7 samples), Belesitsas (3), Evinos (5), Kokinos (4), Aliakmon (1), Volvozis (1), Pinios (1), Louros (1), Aheloos (1), Axios (1), Gallikos (2), Evros (1) and Lailia (1). The first three lakes and the first four rivers are the major sources of drinking water for the city of Athens and therefore more samples were taken from them. Most of the remaining rivers and lakes provide the other big cities of Greece with drinking water. Samples were taken also from drillings that provide with drinking water the cities of Volos (10 samples), Larissa (1) and Alexandroupoli (1). Samples were stored at 4°C prior to extraction that was conducted within 24h of sampling.

Stock solutions of the compounds (1000 µg/mL) were prepared by proper dilution of analytical standards, purchased from commercial sources, with "pesticide residue" grade acetone. Working solutions (10, 1, 0.1 and 0.01 µg/mL) of the pesticides, as well as solutions of mixtures of them were prepared in acetone from the stock solutions according to the standard practices. The 24 organophosphorus and triazine pesticides were classified in 5 groups A,B,C,D and E. Each group contained compounds of similar sensitivity to NPD and of the same concentration. The group in which each compound was classified is shown in Table 1.

Sample preparation was performed by solid phase extraction with pre-packed reversed-phase octadecyl (C-18) bonded silica contained in cartridges. Isolute cartridges (International Sorbent Technology), containing 500 mg of packing material were solvated prior to loading the sample, by passing successively 1 mL/100mg sorbent (5 mL) ethyl acetate, 5 mL methanol and 10 mL of organic free water, with a glass syringe. 500 mL of water sample were cleaned by removal of any floating or insoluble material, and were passed through the cartridge. For extraction of the samples a SPE large volume sampler was connected to a vacuum source equipped with a gauge and bleed valve to adjust the vacuum, in order to obtain a sampling rate of 15 mL water/min. Teflon tubes were inserted into the sample containers and the other ends were connected by adapters to the cartridges. In this way, up to four water samples could pass through cartridges simultaneously. After passing the sample, suction continued for 1h to dry out the packing material. The cartridges were disconnected and the adsorbed pesticides were eluted with ethyl acetate into 1-mL volumetric cylinders. The extract was then stored in Teflon-sealed vials until chromatographic analysis.

Organochlorine pesticides were determined by a method previously described (Miliadis GE, 1994 a), while analysis of organophosphate and triazine pesticides was performed with a Fisons HRGC Mega 2 Series gas chromatograph, equipped with a cold on column injector and with a nitrogen-phosphorus detector. A 25 m X 0.25 mm i.d. capillary column, containing DB-5, 0.25 µm film thickness was used. For the protection of the column 0.8 m of empty pre-column was used. Helium was used as carrier gas, at 1 mL/min. Operating temperatures were: ambient for the injection port, preserved with secondary cooling and 280° C for the detector. The temperature program for the

Table 1. Relative retention time (RRT), as to parathion ethyl, of the 24 pesticides for the two capillary columns. Actual retention time for parathion ethyl was ~11.3 min with the DB-5 and ~8.0 min with the DB-1701.

Compound	Group*	Column	
		DB-5	DB-1701
mevinphos-cis	C	0.38	0.27
mevinphos-trans	C	0.39	0.43
cycloate	E	0.44	0.58
ethoprop	A	0.48	0.57
demethon-S-methyl	D	0.51	0.56
phorate	B	0.53	0.63
diazinon	A	0.60	0.73
atrazine	D	0.64	0.69
dimethoate	C	0.73	0.65
prometryn	E	0.80	0.90
pirimphos methyl	B	0.80	0.93
chlorpyrifos	B	0.85	1.00
parathion methyl	B	0.86	0.84
malathion	B	0.93	0.96
fenitrothion	B	0.93	0.92
parathion ethyl	B	1.00	1.00
methidathion	C	1.22	1.22
cyanazine	E	1.29	1.01
fenamiphos	C	1.36	1.37
phosmet	B	1.85	2.22
phosalone	B	1.90	2.38
azinphos methyl	D	1.90	2.37
pyrazophos	B	1.92	2.48
azinphos ethyl	B	1.95	2.47

* The group in which the compounds are classified according to their sensitivity to NPD.

chromatographic separation was: 80 to 180°C at a rate of 30°C/min, then to 210°C at 2°C/min and then to 270°C at 30°C/min, where it remained for 15 min. The injection volume was 1 µl.

Quantification was carried out by use of a computer integrator. Identification of the unknown peaks in the samples' chromatograms was managed by comparing the relative retention time (RRT) of the unknown peaks to the RRTs of the reference standards. In order to confirm the identity of the pesticides found, the relative retention times obtained from another capillary column containing DB-1701 15m X 0.25mm i.d., with 0.25 µm film thickness were used, while further confirmation, whenever necessary, was achieved by the use of GC-MS. Duplicate analysis was performed for the samples with pesticide residues detected.

RESULTS AND DISCUSSION

The SPE procedure used for the preparation of the samples is a fast and

Table 2. Mean recoveries (%) standard deviation (N=3) for the studied pesticides in fortified water samples at various fortification levels (μg/L)

Group	Compound	Fortification level			
		C_1*	$2C_1$	$4C_1$	$10C_1$
A	diazinon	97± 10.6	99±1.98	94±6.58	87±2.61
	ethoprop	100± 15.0	102±7.14	98±4.90	91±1.82
B	azinphos ethyl	99± 15.8	94±7.52	93±5.58	90±0.90
	chlorpyriphos	110± 8.80	88±9.68	75±4.50	65±1.30
	fenitrothion	110± 8.80	103±4.12	93±6.51	87±0.87
	malathion	86± 17.2	98±7.84	102±5.10	90±0.90
	parathion ethyl	98± 10.8	102±7.14	96±7.76	89±1.64
	parathion methyl	67± 12.7	96±9.60	97±5.82	90±1.80
	phosalone	108± 9.72	92±6.44	89±2.67	86±1.72
	phosmet	93± 8.37	85±8.50	85±5.95	78±1.56
	pirimiphos methyl	75± 12.0	96±0.96	94±7.52	87±2.61
	pyrazophos	100± 15.0	95±3.80	91±3.64	83±4.15
C	dimethoate	26± 0.26	12±0.12	14±0.28	15±0.15
	fenamiphos	23± 0.69	17±0.85	15±0.30	10±0.40
	methidathion	101± 15.1	95±4.75	97±4.85	92±0.92
	mevinphos-cis	68± 3.40	50±0.50	42±1.26	43±2.58
	mevinphos-trans	20± 2.40	11±0.11	10±0.70	20±0.40
D	atrazine	98± 8.82	97±6.79	88±7.92	85±0.85
	azinphos methyl	100± 3.00	87±1.74	99±6.93	87±0.87
	demeton-S-methyl	-	-	18±0.18	11±0.22
E	cyanazine	90± 5.40	87±2.61	88±3.52	85±0.85
	cycloate	87± 4.35	92±0.92	92±4.60	87±1.74
	prometryn	89± 2.67	86±6.88	85±3.40	84±0.84

*C_1=0.05, 0.1, 0.2, 1 and 2 μg/L for the groups A,B,C,D and E respectively.

economical technique, currently gaining acceptance for the determination of pesticide residues. Using this procedure, as we found in other studies, it is possible to isolate also other classes of pesticides, such as carbamates (Aplada-Sarlis P and Miliadis GE, 1995). Thus the final extracts can also be used for the determination of these compounds. Cold on column injection used avoids losses caused by thermal degradation of components and direct injection avoids component discrimination and inaccuracies during the transfer of the sample to the column. The 24 compounds studied are separated by the two capillary columns used, as their relative retention times, that are presented in Table 1, indicate, and as it is seen from figure 1.

The linearity of the 24 compounds was tested over the concentration range 0.05-2 μg/L and a linear relationship was found, with correlation coefficients $r \geq 0.990$ for all analytes except for fenitrothion, malathion and parathion methyl with $r \geq 0.982$ and for azinphos methyl with $r = 0.936$. Quantitation of the compounds in the samples was made by comparing the detector response for the sample to that measured for the calibration standard within the linear range.

For statistically validating the method's efficiency, HPLC-grade water was spiked in the laboratory with the 24 compounds at various concentration levels. A typical chromatogram of spiked water sample is shown in Figure 1. The results

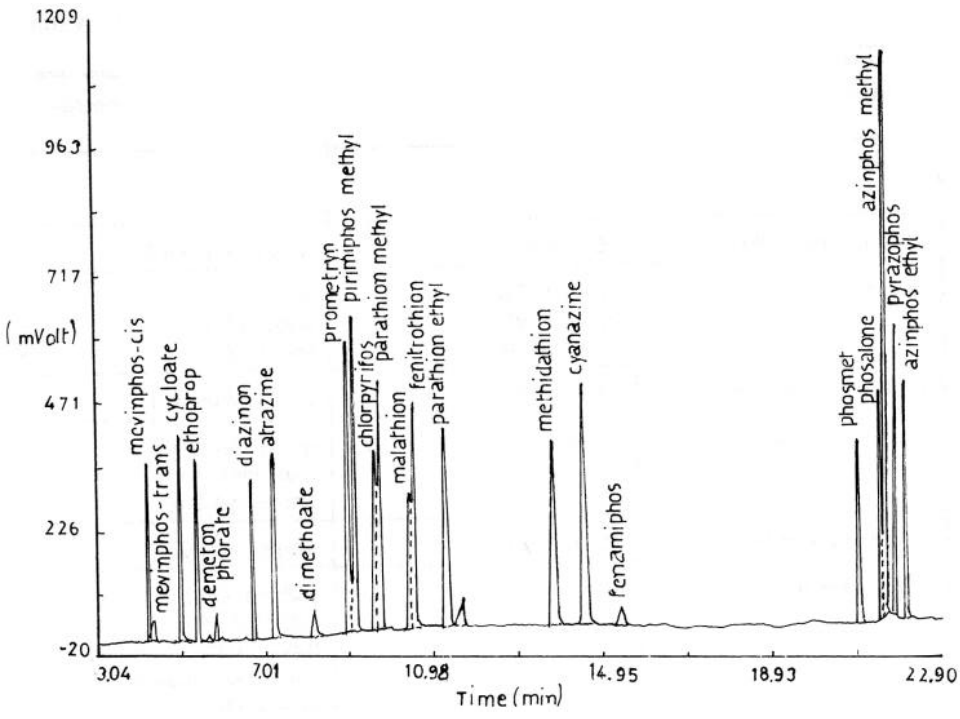

Figure 1. Gas chromatogram of 1 µL of spiked water with the 24 pesticides, at concentration $10C_1$ (see Table 2)

of the recovery study for the 24 compounds, as well as the repeatability of the method are presented in Table 2. Most average recoveries were found between 80 and 110% (except for mevinphos-cis with 42-68%), values acceptable for residue analysis (Greve 1984). For the compounds demeton-S-methyl, dimethoate, fenamiphos and mevinphos - trans the recoveries were lower, between 10 and 26%, making their quantitation unsatisfactory. Such low recovery values have been attributed to breakthrough during the percolation of the water sample through the SPE cartridge (Moreno-Tovar and Santos-Delgado MJ, 1995) especially for polar and moderately polar analytes that are not well retained by the C-18 sorbent. The difference in the recovery values observed in the two isomers of mevinphos is attributed to polarity differences of the two molecules. Mevinphos-cis is a more polar molecule than its trans isomer and a lower breakthrough volume is therefore expected (Lacorte S and Barcelo D, 1996). Finally phorate, that was also included in the study, was found to have recovery values lower than 10%, making its determination not feasible.

The repeatability of the method was checked by measuring the relative standard deviation (RSD) values from the recovery experiments. These values ranged from 1 to 11%, as seen in Table 2, and are satisfactory for residue analysis,

Table 3. Water samples in which pesticide residues were detected. N=total number of samples taken from each sampling site during 1996.

Sample Origin	Date of sampling	Compound/Concentration (μg/L)
Kokinos river* (N=4)	15.5.1996	phosalone:0.1 pyrazophos:0.1
Mornos lake* (N=7)	15.5.1996	azinphos ethyl:0.1
Iliki lake* (N=8)	a) 8.10.1996 b) 6.11.1996 c) 5.12.1996	lindane:0.1 lindane:0.2 lindane:0.2
Drilling of Volos* (N=10)	21.10.1996	pyrazophos: 0.1
Pinios river (N=1)	22.10.1996	quintozene:0.02 malathion:0.1 prometryn:0.8
Trihonida lake (N=1)	29.10.1996	dicofol, p.p':0.03
Axios river (N=1)	6.11.1996	lindane: 0.02 α-BHC:0.45 β-BHC:0.02 demeton-S-methyl:0.6 atrazine:0.6
Evros river (N=1)	6.11.1996	diazinon:0.01

* Water intented for human consumption after treatment

while higher values (up to 20%) were found in some cases at the lowest validation level as expected, since it is known that quantitating at very low concentrations results in high values of RSD (Greve, 1984). A conservative estimate of the method's detection level is known to be the product of the worst case standard deviation at the lowest validation level with the Student t-value (U.S.E.P.A. 1984) which is 6.96 at 99% confidence level for 2 degrees of freedom (3 replicates). The detection limits evaluated by this approach were found to be 0.05, 0.1, 0.2, 0.6 and 0.8 μg/L for the compounds of the groups A to E respectively. However the detection limits were found to be much lower, when the demand for a signal to noise ratio 3:1 was used as the criterion of their estimation.

Eighty water samples, collected from the previously mentioned rivers and lakes of Greece, were analyzed according to the methods mentioned above for the determination of organophosphorus, organochlorine and triazine pesticides' residues. Residues were detected in ten samples, whose origins and concentrations of the compounds found in them are shown in Table 3. The concentrations of the compounds detected in these samples are in some cases lower than the statistically evaluated detection limit of the method described. This was the case, when very small peaks appeared in the samples' chromatograms, which however were quantitated when they exceeded the noise by at least 3 times As seen from Table 3 lindane was detected in the samples taken from the Iliki lake between October and December 1996, while it was not detected in the

previous samples of the same lake taken between March and September. This is in accordance to our previous findings (Miliadis G, 1994b) when, during a two year survey lindane residues in the water of this lake were detected between autumn and spring of each year and were attributed to misuse of lindane in the nearby cultivations. From the same Table it is also seen that residues of 5 pesticides were detected in the sole sample taken from Axios river. This river is located in Northern Greece, and is known from previous studies (Albanis 1991) to be contaminated with pesticides released from the agricultural fields of the nearby areas, during the cultivation seasons. In conclusion pesticide residues were detected in 12.5% of the total samples analyzed. However the concentration levels found in all cases are much lower than the European Union maximum acceptable concentration for surface waters.

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REFERENCES

Albanis TA (1991) Runoff losses of EPTC, molinate, simazine, diuron, propanil and metolachlor in Thermaikos gulf, N. Greece. Chemosphere Vol. 22, No.7, p. 645-653.

Aplada-Sarlis P and Miliadis GE (1995). Determination of N-methy l carbamates in natural waters of Greece by HPLC-post column derivatization. 16th Panhellenic Chemistry Congress, Athens, p. 614-617.

E.C. Council Directive relating to the quality of water intended for human consumption (1980/778/EEC). Off J. Eur. Commun. No L 229/11-29.

Greve PA (1984) Good Laboratory Practice in pesticide residue analysis. In: Ambrus A and Greenhalgh R (ed) Pesticide residue analysis WHO/FAO, Rome, p. 281.

Hennior MC and Scribe P (1993) Sample handling strategies for the analysis of organic compounds from environmental water samples. In: Barcelo D. (ed.) Environmental analysis: techniques, applications and quality assurance. Elsevier, Amsterdam, p. 24.

Lacorte S , Barcelo D (1996) Improvements in the determination of organophosphorus pesticides in ground and wastewater samples from interlaboratory studies by automated on-line liquid solid extraction followed by liquid chromatography diode array detection. J Chromatogr A, 725: 85-92.

Miliadis GE (1994a) Determination of pesticide residues in natural waters of Greece by solid phase extraction and gas chromatography. Bull Environ. Contam Toxicol, 52: 25-30.

Miliadis G.E. (1994b) Lindane Residues in the water of the lake Iliki, Greece. Bull Environ Contam Toxicol, 53: p. 598-602.

Moreno Tovar J and Santos-Delgado MJ (1995) SPE and determination of carbamate pesticides in water samples by reverse phase HPLC. Anal Chem, 91, p. 365.

U.S. Environmental Protection Agency (1984) Guideline establishing test procedures for the analysis of polutants. Fed Register Vol 49, No 209, p. 198. US Government Printing Office, Washington, DC.