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DETERMINATION OF DIMETHOATE, 2,4-DICHLOROPHENOXY ACETIC ACID, MECOPROP AND LINURON PESTICIDES IN ENVIRONMENTAL WATERS IN REPUBLIC OF MACEDONIA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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An HPLC-UVDAD method for determination of dimethoate, 2,4-dichlorophenoxy acetic acid (2,4-D), mecoprop (MCPP) and linuron in environmental waters was developed. The water samples were concentrated and extracted by a solid phase extraction (SPE) method on Bond Elut PPL cartridges. After extraction the investigated compounds were separated on Stability RP Pesticides chromatographic column using mobile phase composed of acetonitrile-water-acetic acid in volume fractions of 39:59:2 and flow rate of 0.7 mL/min. Ultraviolet absorption detection was carried out for dimethoate, 2,4-D and MCPP at 229 nm, and for linuron at 249 nm. Recoveries made from 500 mL of drinking waters using solid phase extraction ranged between 64.3–92.1 %.

The method was applied to environmental waters in Macedonia that receive runoffs from agriculture lands. The levels of pesticides under study ranged between 0.31 μg/L and 7.05 μg/L, depending on the compound and sampling period.

Key words: dimethoate; 2,4-dichlorophenoxy acetic acid; linuron; mecoprop; HPLC-UV-DAD; SPE; environmental waters

ОПРЕДЕЛУВАЊЕ НА ДИМЕТОАТ, 2,4-D, МСРР И ЛИНУРОН ВО ПОВРШИНСКИ ВОДИ ВО РЕПУБЛИКА МАКЕДОНИЈА СО ВИСОКО ЕФИКАСНА ТЕЧНА ХРОМАТОГРАФИЈА

Со примена на методот HPLC-UV-DAD извршено е определување на диметоат, 2,4-дихлорофеноксиоцетна киселина (2,4-D), мекопроп (MCPP) и линурон во површински води во Република Македонија. Примероците на вода се концентрирани со примена на цврстофазна екстракција (SPE). Испитуваните пестициди се раздвоени со хроматографска колона Stability RP Pesticides 5 μm; 250 mm × 3 mm мобилна фаза составена од ацетонитрил-вода-оцетна киселина со волуменски однос 39:59:2 и проток од 0,7 mL/min. Детектирањето на испитуваните пестициди е извршено со УВ детектор со диоди во низа на различни бранови должини, и тоа: за диметоат, 2,4-D и MCPP највисоки апсорпциони максимуми се добиени на бранова должина од 229 nm, а за линурон на 249 nm. Аналитичките приноси добиени од екстракција на пестицидите од примероци на дестилирана вода со волумен од 500 mL се движат од 64,3 % до 92,1 %.

Методот е применет на повеќе примероци од површински води околу кои се наоѓаат земјоделски површини. Утврдено е дека концентрациите на испитуваните пестициди се движат од 0,31 μg/L до 7,05 μg/L зависно од компонентата и периодот во кој е извршено земањето на примероците.

Клучни зборови: диметоат; 2,4-дихлорофеноксиоцетна киселина; линурон; мекопроп; HPLC-UV-DAD; SPE; површински води

INTRODUCTION

Natural waters are contaminated with various pesticides or their transformation products. Pesticides, among which mostly herbicides and nematicides, are potential contaminants of natural waters because they are applied to soil and are transported to ground waters or leached directly to the environmental waters.

Pesticides concentrations in environmental samples are usually low, with tolerance limits around 0.1 μg/L in drinking waters. Regarding environmental waters official monitoring of surface waters samples requires LODs close to 0.1 μg/L [1]. Henion and Pichon suggested that sensitive analytical methods providing limits of detection lower than 0.05 μg/L are needed [2]. Butz et al. obtained detection limits of acidic herbicides in water between 0.001 μg/L and 0.01 μg/L, by applying solid phase extraction [3]. Several authors have developed and validated multi residue methods based on liquid-liquid extraction or solid phase extraction coupled with gas-chromatography–mass chromatography (GC-MS), high performance liquid chromatography (HPLC) with UV DAD, LC-MS and LC-ESI-MS [1, 4–10] leading to the determination of pesticides priority lists. The presence of pesticides at trace levels in raw waters, has been confirmed in numerous SPE-LC-MS applications [11–14]. As a consequence, SPE-LC-UV DAD-MS has become a widely used analytical method for the quantitative environmental analysis of pesticides [15–17].

Baraud et al. developed an improved analytical method for sampling characterization and quantification of pesticides in atmosphere among which were the pesticides investigated in our work [18]. Irace-Guigand et al. have used multi residue HPLC method coupled in tandem with UV DAD and mass spectrometry to investigate the contamination of Vannetin environmental waters by pesticides from regional priority list [19].

Therefore, in this work we have used a fast and precise method for determination of dimethoate, 2,4-D, MCPP and linuron, which are used widely in the agriculture in Macedonia. The method consists of application of SPE-HPLC-UV DAD to investigate contamination at low μg/L levels of environmental waters.

The validation of the developed method was evaluated with the limit of detection, the fit type between the mass and peak areas, intra day precision of retention time and peak area and recoveries from drinking water samples.

This optimized and validated chromatographic method can be used in future routine determinations of the investigated pesticides in environmental waters in the Republic of Macedonia.

EXPERIMENTAL

Materials

All pesticide standards were of analytical purity supplied from Riedel-de-Haën (Germany). Acetonitrile, HPLC grade, was from Sigma-Aldrich. Methanol was obtained from Fluka, and the acetic acid was supplied from Alkaloid Skopje. HPLC high quality water was used.

Solid phase extraction (SPE) was carried out with Bond Elut PPL cartridges containing 250 mg of styrene divinylbenzene copolymer supplied from Varian.

Instrumentation

The pesticides were separated on a Varian high performance liquid chromatograph equipped with ternary gradient pump (model 9012), 50 μL sample loop (Rheondyne model 7125) and with UV-DAD detector (model UV 9065 Polychrom). The analytical column was a Stability RP Pesticides chromatographic column (CIL Cluzeau, France), with the following dimensions and particle size: 250 mm x 3 mm; 5 μm. The optimum eluent flow rate was 0.7 mL/min, and the UV detector wavelength was set at 229 nm for dimethoate, 2,4-D, and MCPP, and at 249 nm for linuron. In order to separate the pesticides, a mobile phase constituting a mixture of acetonitrile/water/acetic acid 39:59:2 (*v/v/v*) was used. The chromatographic system was controlled by software package Varian Version 4.5 and the UV spectra were produced by the program Polyveiw Version 4.5. All analyses were performed at room temperature.

Sampling

Samples were taken from the waterfront of six lakes in the Republic of Macedonia. 500 mL of water were collected in glass bottles during the period from October 2006 to July 2007. Before sampling the bottles were decontaminated from organic pollutants by rinsing them with methanol, followed by combustion at 450 °C in an oven for 24 h. The samples were filtered and kept at 4 ºC before analysis.

Solid phase extraction

Water samples (500 mL) (See Ref. 18) were filtered on Schleicher & Schuell 5841 black ribbon ashless filter paper, and pre concentrated on Bond Elut PPL cartridges optimized for the extraction of highly polar species from large volume water samples. The off-line SPE procedure involves several steps of conditioning, pre concentration, drying and elution [1, 5, 15, 20]. The conditioning was performed with 10 mL of MeOH, followed by 10 mL of water at a flow rate of 2 mL/min. After the conditioning, 500 mL of sample were passed through the cartridge at a flow rate of 10 mL/min. Following this concentration step, the cartridge was dried for 30 s by means of a gentle vacuum. During the elution step, the pesticides were desorbed from the cartridge with 4 mL of a mixture acetonitrile-methanol 1:1 (*v*/*v*). To the eluent 50 μl of a mixture MeOH-NH_3 4:1 was added. The extract was then evaporated using rotary evaporator to a volume of about 40 – 80 μL. After completion, a mixture of MeOH-H_2O 1:4 was added to this extract to obtain a total of 200 μL from which 50 μL were injected into the HPLC column. The whole extraction and concentration procedure took around 2 hours**.**

RESULTS AND DISCUSSION

The chemical structures of dimethoate, 2,4-dichlorophenoxy acetic acid (2,4-D), mecoprop (MCPP) and linuron investigated in this study are given in Figure 1.

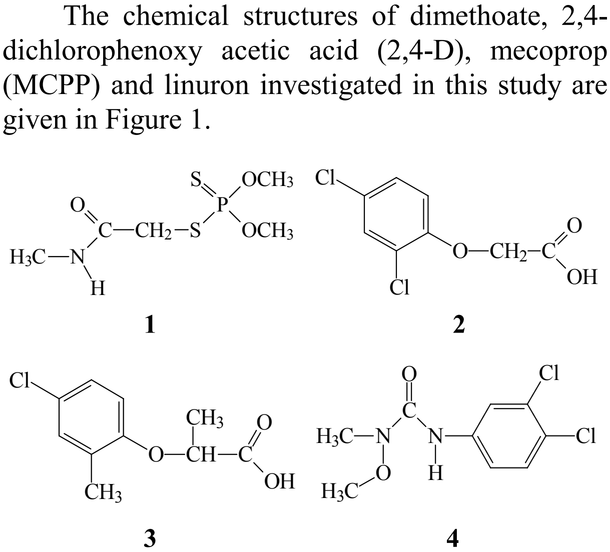

Fig. 1. Structures of dimethoate – **1**: 2,4-D – **2**; MCPP – **3,** and linuron – **4**

The pesticides under study were separated within 20 min by a mobile phase of acetonitrile-water-acetic acid 39:59:2 (*v/v*) at a flow rate of 0.7 mL/min. A series of preliminary experiments were designed to establish the effect of the flow rate over the range 0.2–1.2 ml/min, while the other factors were kept constant. The experiments showed that flow rate of 0.7 mL/min is optimum for performance of short and sensitive analysis. As can be seen from the following figures, under the given chromatographic conditions the investigated pesticides are completely separated from each other. Figures 2 and 3 show typical chromatograms of mixture of standards. Figure 2 shows the absorption maximum for dimethoate, 2,4-D and MCPP is at 229 nm, and Figure 3 gives the absorption maximum at 249 nm for linuron. The linearity of this method was determined for all compounds separately.

The data for the retention factors, separation factors and resolution are summarized in Table 1. Values calculated for separation factors (α) are above 1 and for the resolution *(R)* is above 1,5 for all pesticides under study. The retention factors (*k*) for dimethoate and 2,4-D are below 10 which is highest optimal value for this parameter. Nevertheless it can be seen that under these chromatographic conditions high separation of the investigated pesticides is obtained.

Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) were evaluated for all the pesticides under this study. The limit of detection was calculated as the lowest concentration level at which in three parallel analyses the target compound could be definitely detected with a signal-to-noise ratio of 3:1, and the limit of quantification as the lowest concentration level at which in three parallel analyses the target compound could be quantified with a signal-to-noise ratio of 10:1.

The limits of detection for each compound are reported in Table 2. The limits of detection for the individual compounds were found to be between 0.01 μg/L to 0.31 μg/L. It should be emphasized that the detection limits for 2,4-D, MCCP and linuron are satisfactory, taking into account that the monitoring of surface waters requires LODs close to 0.1 μg/L [1]. Regarding dimethoate, the method is suitable for detection of this pesticides on 0.3 μg/L concentration level.

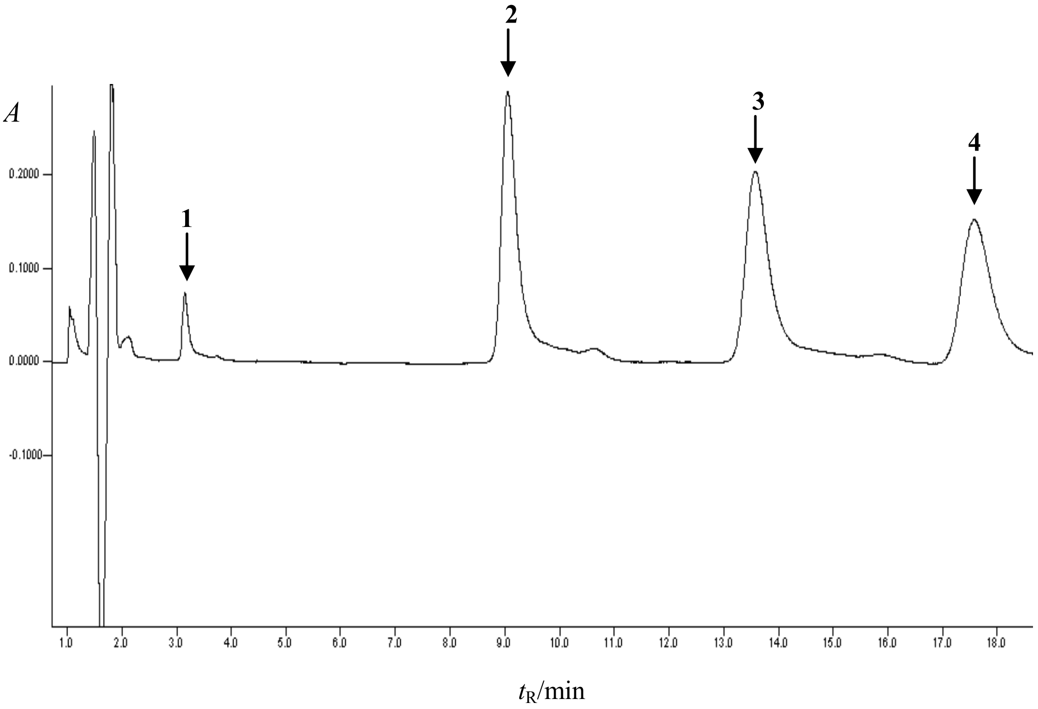

Fig. 2. HPLC chromatogram of a standard mixture of pesticides eluted at 25 °C with acetonitrile-water-acetic acid 39:59:2 at 229 nm. **1** – dimethoate; **2** – 2,4-D; **3** – MCPP; **4** – linuron

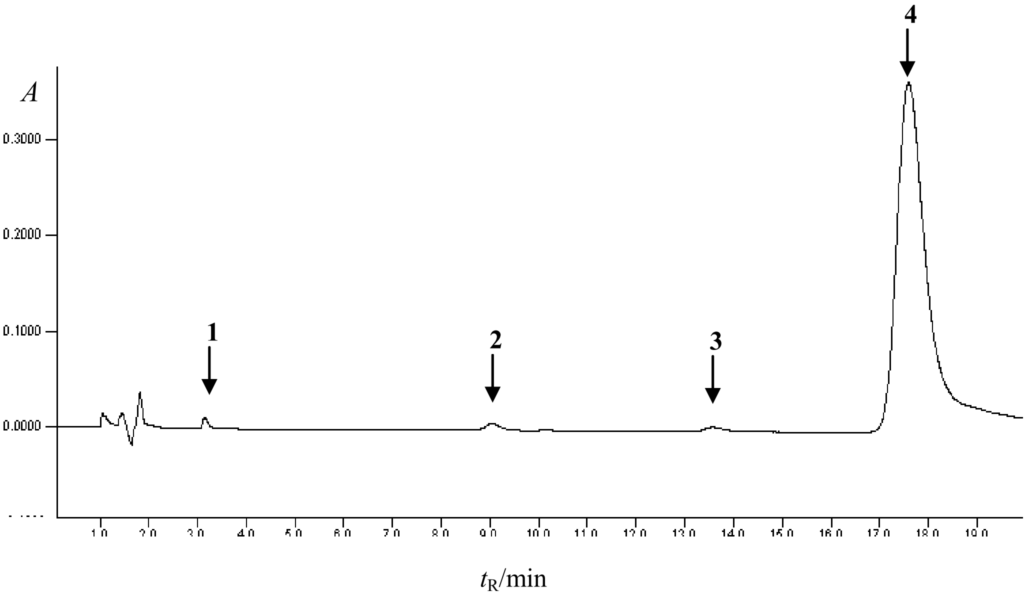

Fig. 3. HPLC chromatogram of a standard mixture of pesticides eluted at 25 °C with acetonitrile-water-acetic acid 39:59:2 at 249 nm. **1** – dimethoate; **2** – 2,4-D; **3** – MCPP; **4** – linuron

Table 1

Retention factors (k), separation factors (α) and resolution (R) for the pesticides under study

Compound	t_R/min	k	α	R
Dimethoate	3.1	2.0	3.8	14.4
2,4-D	9.0	7.6	1.6	6.0
MCPP	13.5	12.0	1.3	3.8
Linuron	17.6	15.8	–	–
Acetonitrile-water-acetic acid 39:59:2	1.0	–	–	-

Table 2

Data for limit of detection (LOD) and limit of quantification (LOQ) for the investigated pesticides

Compound	Dimethoate	2,4-D	MCPP	Linuron
LOD/$\mu g \cdot L^{-1}$	0.31	0.06	0.05	0.01
LOQ/$\mu g \cdot L^{-1}$	0.95	0.18	0.14	0.04

As can be seen in Table 3, the method shows wide linear concentration ranges with correlation coefficient values greater than $R^2 > 0.995$ for all studied pesticides. The linear concentration range was described by the lowest and upper concentration values corresponding to the limits of the linear portion of the calibration curve. The lowest concentration was equal to or slightly higher than the LOQ value of the studied pesticide. The calibration curves were drawn for all pesticides. The results of the regression equations are given in Table 3.

Table 3

Data for statistical assessment on calibration curves for dimethoate, 2,4-D, MCPP and linuron

Compound	Linear concentration range / µg/L	Regression equation[a]	RSD[b] / %	R^2 [c]
Dimethoate	0.95 – 10.2	$y = 14.249x + 10.882$	7.2	0.9966
2,4-D	0.2 – 4.3	$y = 167.51x + 4046.7$	9.4	0.9951
MCPP	0.2 – 4.8	$y = 103.14x – 1780.6$	6.0	0.9978
Linuron	0.04 – 4.4	$y = 227.8x + 670.9$	6.1	0.9988

[a] y = peak area, x = injected mass of pesticide (in ng); [b] RSD = relative standard deviation; [c] R = correlation coefficient.

The intraday precision was determined by measuring six different samples, with the following amount of pesticides: 637.5 ng of dimethoate; 135.0 ng of 2,4-D; 140.0 ng of MCPP and 13.75 ng of linuron. In Table 4. the results obtained for intraday precision of retention time, and the peak area for all investigated compounds are presented. The repeatability of the retention time for all compounds is lower than 1.08 %. The repeatability for peak areas is lower but still acceptable. As can be seen, the RSD values ranged between 2.29 % to 4.29 % for dimethoate. These data indicate a considerable degree of precision of the developed method.

Table 4

Intraday precision of the retention time and peak area for all pesticides under study

Compound	SD (area)	RSD (area) %	SD (t_R)	RSD % (t_R)
Dimethoate	0.02	0.72	276.4	4.29
2,4-D	0.02	0.17	545.4	3.65
MCPP	0.15	1.07	382.8	2.29
Linuron	0.01	0.08	327.5	3.81

The efficiency of the extraction of the pesticides standards from 500 mL distilled water by SPE using Bond Elut PPL cartridges are presented in Table 5. The mean percentage recovery values ranged between 64 to 92 %. The recovery values for dimethoate and MCPP were close to 100 % and quite acceptable for these compounds. However, for linuron and 2,4-D, these recovery values were lower, and further improvement is needed.

Table 5

Recovery values[a] of distilled water spiked with pesticides

Compound	Recovery %	RSD %
Dimethoate	89	3
2,4-D	71	4
MCPP	92	8
Linuron	64	5

[a] The recovery values correspond to the mean of triplicate analysis

The developed HPLC method was used for the analysis of environmental water samples. The exact locations determined by global positioning satellites (GPS) and time of sampling are listed in Table 6. It can be seen that samples taken from three natural lakes and three man-made accumulations in R. Macedonia were included in the study.

The sampling locations are shown on the map of the Republic of Macedonia (Fig. 4).

Table 6

Sample label, sampling time and sampling location of environmental waters collected in various Macedonian lakes

Sample	Date	Lake	Place	GPS	
1	8. 6. 06	Ohrid	Close to Hotel Park	N 41.09805	E 20.80392
2	8. 7. 06	Ohrid	St Erazmo's hospital	N 41.13504	E 20.76030
3	9. 7. 06	Ohrid	Walkway in center of town	N 41.10645	E 20.80732
4	9. 7. 06	Prespa	Fisherman's village towards Oteševo	N 40.99752	E 20.92445
5	11. 10. 06	Ohrid	Close to Hotel Park	N 41.09805	E 20.80392
6	11. 10. 06	Ohrid	St Erazmo's hospital	N 41.13504	E 20.76030
7	11. 10. 06	Ohrid	Walkway in center of town	N 41.10645	E 20.80732
8	7. 10. 06	Mladost	In front of the hotel	N 41.77770	E 21.75623
9	19. 6. 07	Ohrid	Close to Hotel Park	N 41.09805	E 20.80392
10	19. 6. 07	Ohrid	St Erazmo's hospital	N 41.13504	E 20.76030
11	19. 6. 07	Ohrid	Walkway in center of town	N 41.10645	E 20.80732
12	19. 6. 07	Prespa	Fisherman's village towards Oteševo	N 40.99752	E 20.92445
13	25.05.07	Dojran	Star Dojran. near restaurant "Kaldrma"	N 41.21454	E 22.70988
14	25.05.07	Paljurci. Valandovo	Near the dam	N 41.20654	E 22.61986
15	25.06.07	Tikveš	Near the dam	N 41.40356	E 21.93960

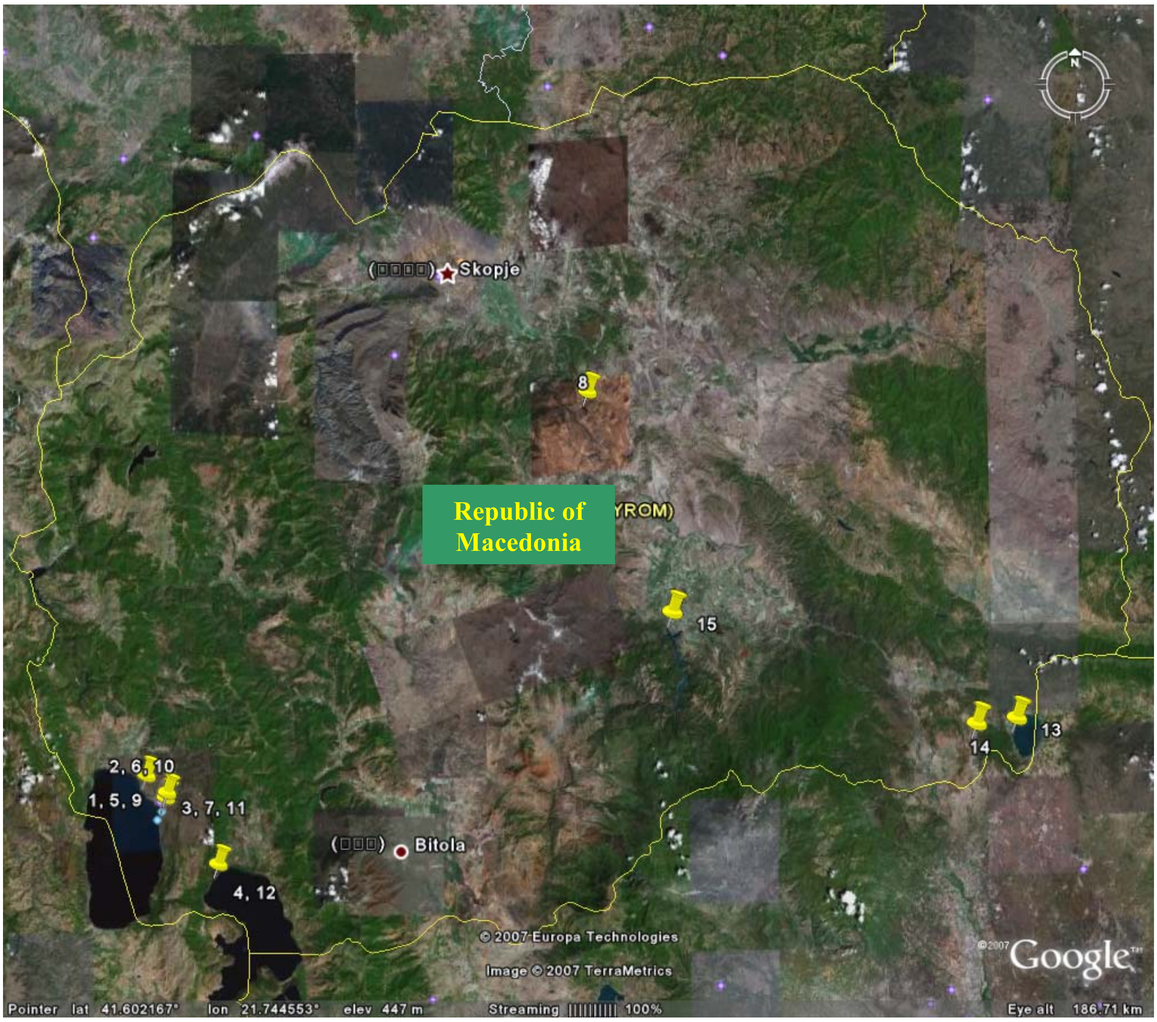

Fig. 4. Map of the Republic of Macedonia with the labeled sampling locations on the lakes

In order to characterize the pesticides residues present in water samples, we compared the retention time (t_R) values of HPLC peaks measured in the extracted sample with those of the pesticide standards. The identification of the investigated pesticides was also confirmed by the comparison of the UV spectra of the pesticides standards and the UV spectra of the peaks of the substances detected in the samples. The concentrations of the pesticides under study, as determined for each sample, are presented in Table 7.

Table 7

Concentrations[a] of the pesticides under study in environmental data samples

Sample	Dimethoate (μg/)	2,4-D (μg/L)	MCPP (μg/L)	Linuron (μg/L)
1	0.76[b]	nd[c]	0.57	nd
2	nd	nd	0.70	nd
3	nd	nd	nd	nd
4	1.53	nd	0.45	nd
5	1.45	1.33	nd	nd
6	nd	0.38	0.31	nd
7	2.55	nd	2.85	nd
8	7.05	0.74	1.04	nd
9	nd	0.86	nd	nd
10	nd	0.71	nd	nd
11	nd	0.56	nd	nd
12	nd	0.99	nd	nd
13	nd	1.92	2.45	2.64
14	nd	nd	nd	nd
15	nd	0.39	nd	nd

[a] The concentration values correspond to the mean of three injections.

[b] Approximate value slightly lower than the LOQ.

[c] nd = not detected.

As can be seen from Table 7, pesticide residues were detected in all natural investigated lakes. However, none of the studied pesticides was identified in the man-made accumulation Paljurci. Typical examples of the chromatograms of samples taken from the Mladost Lake (07/10/2006) and Dojran Lake (25/05/2007) are presented on Figures 5 and 6.

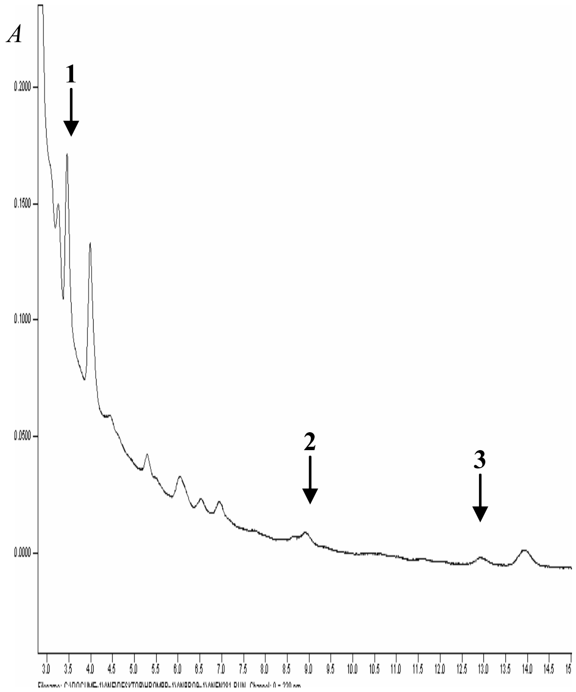

Fig. 5. Chromatogram of the sample 8 obtained with HPLC UV DAD at 229 nm. Peaks: (1) Dimethoate, (2) 2,4-D and (3) MCPP

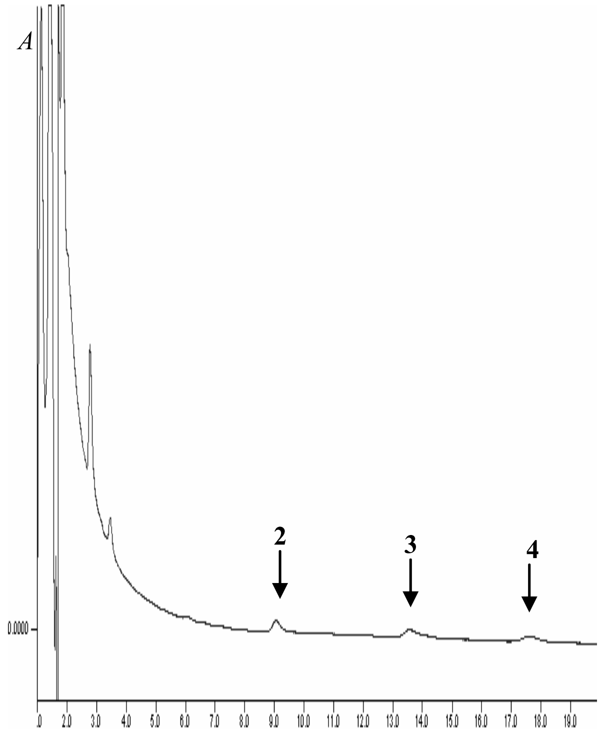

Fig. 6. Chromatogram of the sample 13 obtained with HPLC UV DAD at 229 nm. Peaks: (2) 2,4-D, (3) MCPP and (4) linuron

The analytical results obtained from the investigation on the water samples show that dimethoate, 2,4-D, and MCPP could be identified in many of the samples collected from various lakes. However, linuron could only be quantified in Lake Dojran. The maximum allowed concentration, according to the officially accepted classification of waters for organophosphorous pesticides, is 0.1 μg/L in waters of class I and II [21][1]. Of the four pesticides in this study only dimethoate belongs to this group of organophosphorous pesticides. Since all investigated waters in which dimethoate was detected belong to class I or II, we can conclude that the detected concentration values of this pesticide are above the limit value. Unfortunately, the LOD of dimethoate is higher than 0.1 μg/L which means that pesticides under this figure could not be detected. Regarding 2,4-D and MCPP all detected concentrations are higher than 0.1 μg/L. Higher concentrations of dimethoate and MCPP occurred in October and maximum concentration of 2,4-D was measured in May. These results obtained by indicative measurements suggest that the investigated pesticides contribute to surface water contamination.

CONCLUSION

A fast and simple method has been developed and applied to the investigation of several pesticides at μg/L level in Macedonian environmental waters by means of SPE-HPLC-UV DAD. The investigated pesticides were found at concentration ranges from 0.31 to 7.05 μg/L depending on the compound, the collecting site and the sampling period. The extraction procedure is acceptable for dimethoate, 2,4-D and MCPP. However, regarding linuron further improvement of recovery is needed. For dimethoate lower LOD need to be obtained. HPLC analysis with UV detection seems to be a precise, sensitive and simple method. The proposed method requires approximately 2 h for the concentration and extraction step, and less than half an hour for the HPLC analyses. This justifies the use of this method, for determination of the investigated pesticides in environmental waters.

Due to the relatively high concentrations > 0.1 μg/L that were detected for all pesticides, and the possible adverse effects to the environment, it would be beneficial to monitor the concentrations of these pesticides in the Macedonian lakes at more frequent intervals and on a long term basis.

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[1] All lakes and major rivers in Macedonia are classified into these groups, so in all sampling sites, except the accumulation Paljurci near Valandovo, the allowed concentration limit for organophosphorus pesticides is 0.1 μg/L.

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