

Determination of some pesticides in the Republic of Macedonia's lakes by high performance liquid chromatography

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Abstract

An HPLC-UV-DAD method was applied to the determination in surface waters of pesticides widely used in Macedonia for agriculture purposes, such as dimethoate, 2,4-D, linuron and MCP. The water samples were extracted by off-line methods, either by solid phase extraction (SPE) or by liquid-liquid extraction. After concentrating the samples, the compounds under study were separated by HPLC, using a mobile phase composed of acetonitrile-water-acetic acid. Ultraviolet detection was carried out for dimethoate, 2,4-D, MCP at 229 nm, and for linuron at 249 nm. Recoveries performed on 500-mL volumes of drinking water using the SPE method were found to range between 64 to 92 %, according to the pesticide. Detection limits going from 0.01 µg/L to 0.31 µ/L were obtained for the compounds under study. Our HPLC-UV-DAD method was applied to water samples from the three largest natural lakes in Macedonia, Ohrid, Prespa and Dojran, as well as from three man-made accumulations, Tikveš, Mladost and Paljurci, that receive runoffs from agriculture lands. It was found that the measured pesticide levels depended on the extraction method, the compound, the sampling period, and the sampling site.

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

Introduction

Pesticides have been widely used in agriculture throughout recent decades to improve the efficiency of food production. But adverse effects of this intensive use have resulted in the ubiquitous presence of pesticides residues in surface waters [1].

Official monitoring of pesticides in surface waters samples requires limits of detection (LOD) close to 0.1 µg/L [2]. Pichon and Hennion suggested that sensitive analytical methods providing limits of detection lower than 0.05 µg/L are needed [3]. Since many pesticides present in environmental samples, such as waters and sediments, are present at low concentration levels it is necessary to carry out an enrichment step before their determination [4]. Butz et al. obtained LODs ranging between 0.001 µg/L and 0.01 µg/L, for acidic herbicides in water, by applying solid phase extraction (SPE) [5]. 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 and LC-MS [2, 6-10], 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 [4, 15, 16].

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 [17]. 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 in France by pesticides from a regional priority list [18].

The main objective of our work was to develop and validate a fast and precise method for detection and quantification of dimethoate, 2,4-dichlorophenoxyacetic acid (2,4-D), linuron and mecoprop (MCP) in Macedonian lakes (Ohrid, Prespa, Dojran, Tikveš, Mladost and Paljurci). Determination of these pesticides is based on HPLC coupled with an UV DAD detector after an off line preconcentration step.

Experimental

Chemicals

The pesticide standards (analytical purity) were provided by Riedel-de-Haën (Germany). Acetonitrile (HPLC grade) was obtained from Sigma-Aldrich. Methanol was purchased from Fluka, and acetic acid was supplied from Alkaloid, Skopje (R. Macedonia). HPLC high quality water was used.

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 °C before analysis.

Solid Phase Extraction

Individual water samples (500 mL) were filtered on Schleicher & Schuell 5841 black ribbon ashless filter paper, and pre-concentrated on Bond Elut PPL cartridges containing 250 mg of functionalized divinyl benzene polymer supplied from Varian 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 [2, 7, 15, 19]. 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). 50 µL of mixture MeOH-NH₃ 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-H₂O 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.

HPLC analytical method

The pesticides were separated at room temperature on a Varian HPLC apparatus equipped with a ternary gradient pump and an UV-DAD detector. A Stability RP Pesticides chromatographic column, (dimensions and particle size: 250 mm x 3 mm and 5 µm) was used. 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. All pesticides were separated in isocratic manner within 20 min. For the elution of the pesticides under study, a mobile phase constituted of a mixture acetonitrile/water/acetic acid 39:59:2 (v/v/v) was selected.

Results and discussion

Method validation and analytical performances

The chemical structures of the insecticide dimethoate and herbicides 2,4-D, MCPP and linuron which were investigated in this work are presented in Figure 1.





Figure 1. Structural formula of dimethoate (I), 2,4-D (II), MCPP (III) and linuron (IV)

The UV absorption spectra of dimethoate, 2,4-D, MCPP and linuron (Figure 2) in a mobile phase of acetonitrile-water-acetic acid 39:59:2 (v/v) show that dimethoate, 2,4-D and MCPP have maximums at 229 nm and linuron at 249 nm.

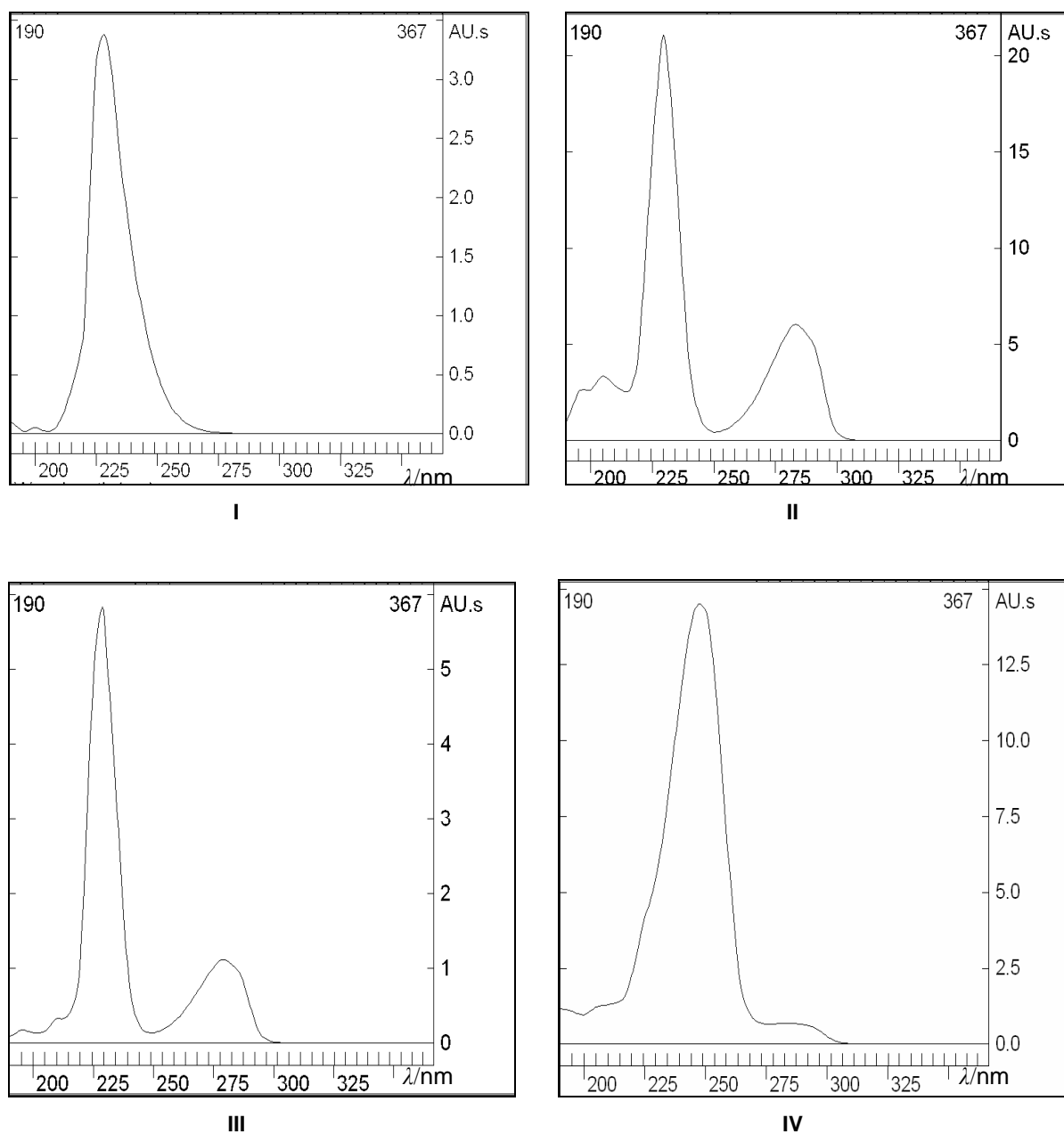


Figure 2. UV spectra of dimethoate (I), 2,4-D (II), MCPP (III) and linuron (IV)

A standard mixture of the pesticides under study was separated within 20 min by applying the SPE-HPLC-UV DAD method. For validation purposes linearity, intra-day precision, LOD, LOQ and recovery values were calculated. The obtained results of the method validation are listed in Table 1.

Table 1. Statistical data of SPE-HPLC UV DAD for the pesticides under study

Compound	r^2	RSD / %	LOD / $\mu\text{g/L}$	LOQ / $\mu\text{g/L}$	Recovery /%	RSD / %
Dimethoate	0.997	7.17	0.31	0.95	88.81	2.63
2,4-D	0.995	9.41	0.06	0.18	71.46	4.54
MCCP	0.998	6.04	0.05	0.14	92.13	8.31
Linuron	0.999	6.13	0.01	0.04	64.31	5.03

The method showed wide linear dynamic ranges (LDR), with correlation coefficients $r^2 > 0.995$ for all pesticides under study, indicating a good linearity. LDR values were, respectively, 0.51 - 10.20 $\mu\text{g/L}$ for dimethoate, 0.21 - 4.32 $\mu\text{g/L}$ for 2,4-D, 0.22 $\mu\text{g/L}$ - 4.48 $\mu\text{g/L}$ for MCCP, and 0.04 $\mu\text{g/L}$ - 4.40 $\mu\text{g/L}$ for linuron. The limit of detection (LOD) and limit of quantification (LOQ) values were determined for all pesticides under study using HPLC-UV DAD. The LODs were found to be comprised between 0.01 and 0.31 $\mu\text{g/L}$, according to the compound. The LODs were satisfactory for 2,4-D, MCCP and linuron, since the monitoring of surface waters generally requires LOD values close to 0.1 $\mu\text{g/L}$ [2]. Regarding dimethoate, the method was suitable for detection of this pesticide at concentration levels larger than 0.3 $\mu\text{g/L}$. Under these chromatographic conditions, linuron showed the best sensitivity.

The extraction efficiency of the pesticide standards from 500 mL distilled water by SPE, using Bond Elut PPL cartridges was investigated. The mean percentage recovery values ranged from 64.3 to 92.1 %, according to the compound. For dimethoate, 2,4-D and MCCP, the recoveries were quite acceptable, whereas in the case of linuron, the recovery value was too low (64.3 %), and further improvement was needed. Intra-day precision of the off-line system 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 MCCP and 13.75 ng of linuron. Under the given chromatographic conditions, the investigated pesticides were very well separated, with t_R values ranging from approximately 3-18 min. Figure 3 shows a typical chromatogram recorded for a mixture of standards. The repeatability of the retention times for all pesticides was lower than 1.08 %. Therefore, all validation data demonstrated the reliability of the proposed method.

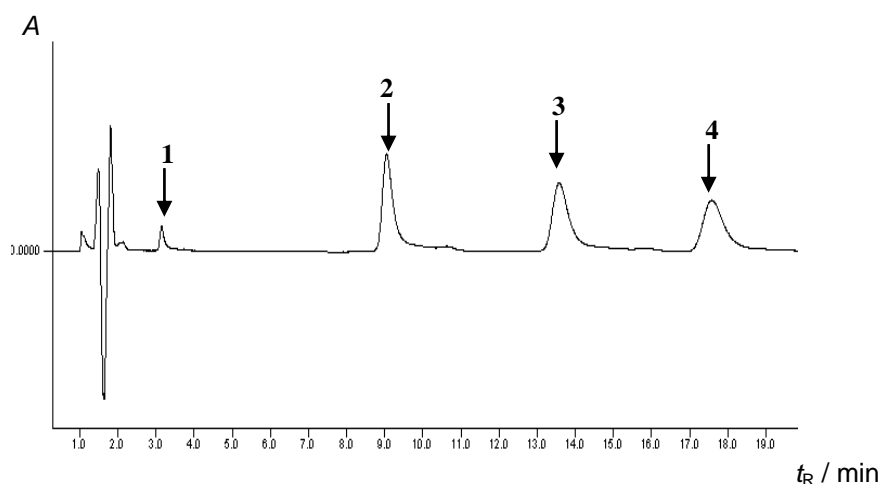


Figure 3. Analysis of a standard mixture of pesticides with UV DAD detector at 229 nm. Peaks of 1-dimethoate (3.11 min), 2-2,4-D(9.04 min), 3-MCCP(13.57 min) and 4-linuron (17.58 min)

Application to multi-residue water analysis

The developed HPLC method was applied to the pesticides determination in water samples from three natural lakes and three man-made accumulations in R. Macedonia, which were selected for this study. The location sites and sampling periods of the environmental samples are symbolized by numbers from 1-15 (Figures 4-7).

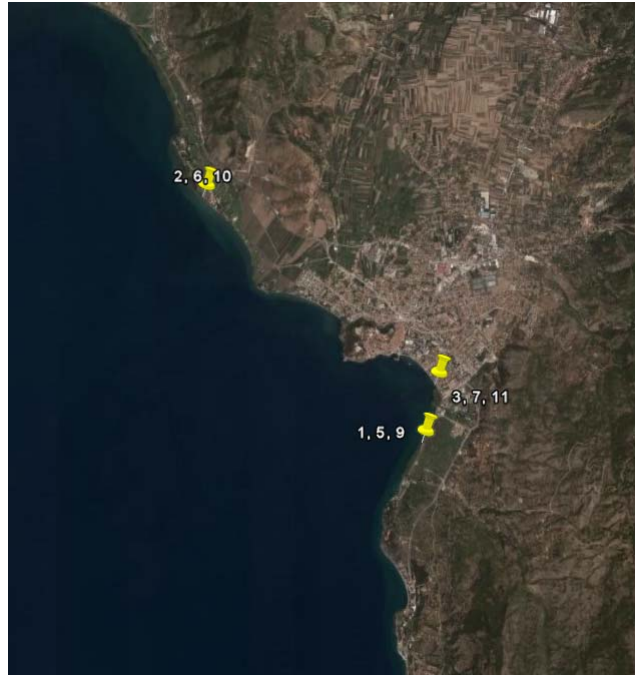


Figure 4. Map of the sampling locations in Lake Ohrid: close to the Hotel Park [1(06/08/2006), 5 (11/10/2006), 9 (19/06/2007)]; St Erazmo's Hospital [2 (08/07/2006), 6 (11/1/2006), 10 (19/06/2007) and walkway in the city center [3 (09/07/2006), 7(11/10/2006), 11 (19/06/2007)]

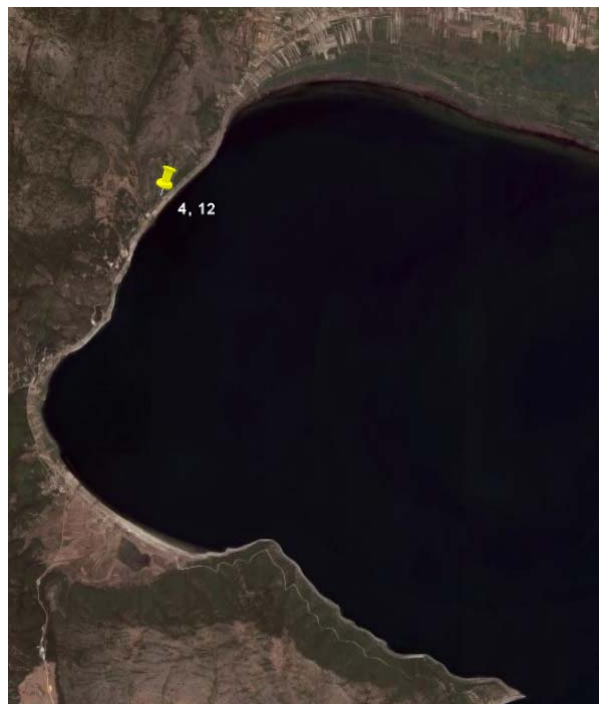


Figure 5. Map of sampling locations in Lake Prespa: close to the Fisherman's village close to Oteševo [4 (09/07/2006) and 12 (19/06/2007)]



Figure 6. Map of sampling locations in Lake Dojran: close to restaurant Kaldrma [13 (25/05/2007)] and in the man-made accumulation Lake Paljurci: close to the dam (14 (25/05/2007))



Figure 7. Maps of sampling locations in man-made accumulation Lake Mladost: near Veles close to the hotel [8 (07/10/2006)] and Lake Tikveš: near Kavadarci close to the dam [15 (25/06/2007)]

The occurrence of the pesticides residues present in water samples was confirmed by comparing the retention time (t_R) values of HPLC peaks measured in the extracted sample and their UV spectra with those of the pesticide standards. Linuron was detected only in sample 13, in Lake Dojran. The results for the other pesticides are presented in Figures 8-10. The water samples were collected from June 2006 to July 2007 mostly in the spring and autumn seasons, when these pesticides are mainly applied. As can be seen from Figures 8-10, dimethoate, MCPP and 2,4-D residues were detected in the three natural lakes and man-made accumulations Lakes Mladost and Tikveš Lake, although none of the pesticides was found in the man-made accumulation Lake Paljurci. Dimethoate was detected in only five of all investigated samples maybe due to the relatively higher LOD value (0.3 $\mu\text{g/L}$), achieved for this pesticide. The concentrations of this pesticide ranged between 0.76-7.05 $\mu\text{g/L}$ which are the highest among the investigated pesticides found in the same samples. 2,4-D concentrations were comprised between 0.39 $\mu\text{g/L}$ at Tikveš Lake in June 2007 and 1.92 $\mu\text{g/L}$ at Dojran Lake in May 2007. This pesticide was found in most of the samples under study, probably resulting from its wide use. The MCPP concentration range was between 0.31 $\mu\text{g/L}$ and 2.85 $\mu\text{g/L}$ in Ohrid in October 2006. The highest concentration were found for all pesticides in May and October probably because of their intensive use during these periods of the year.

All lakes and major rivers in Macedonia are officially classified into class I or II, which means that the maximum allowed concentration for organophosphorous pesticides is 0.1 $\mu\text{g/L}$. All sampling sites in this study correspond to these two classes, except the accumulation lake Paljurci near Valandovo. Of the four pesticides of 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 [20], 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 $\mu\text{g/L}$ which means that concentrations of this pesticide under this figure could not be detected. These results suggest that the investigated pesticides significantly contribute to the surface water contamination.

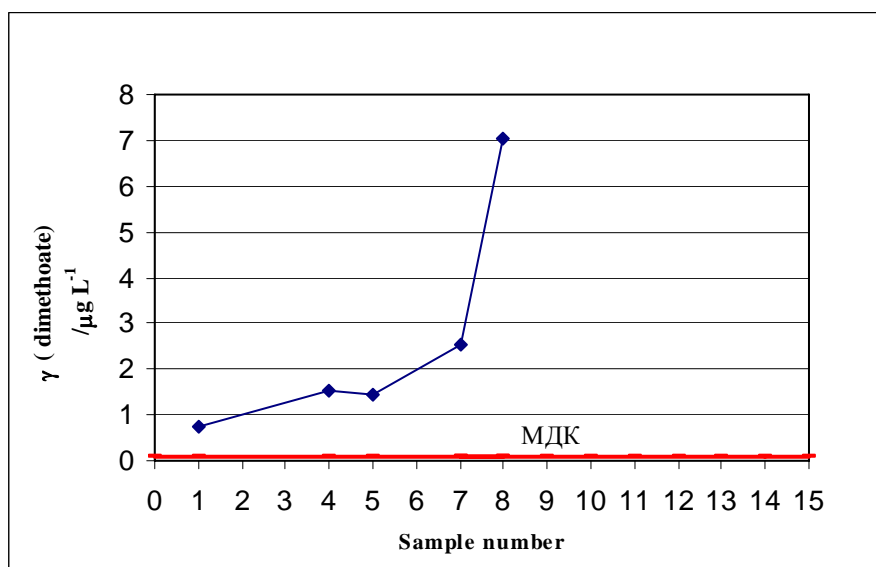


Figure 8. Occurrence and concentration of dimethoate in the analyzed samples (the line drawn in bold indicates the official allowed limit for organophosphorus pesticides)

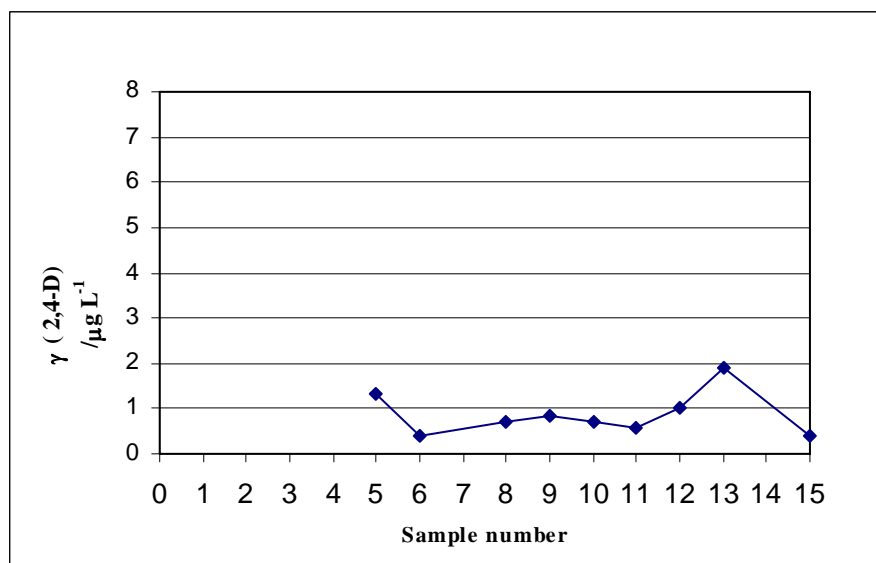


Figure 9. Occurrence and concentration of 2,4-D in the analyzed samples

Conclusion

The aim of this study was to develop a fast, reliable and simple method for investigating several pesticides at the $\mu\text{g/L}$ level in Macedonian environmental waters by means of SPE-HPLC-UV DAD. From the obtained results and the method validation, we can conclude that:

- Good linearity was obtained for all pesticides under study with coefficient of correlation > 0.995 and RSD $< 9.41\%$.
- Intra-day precision for the retention times below 1.08% was reached for all investigated pesticides.
- Detection limits were found to be between 0.01-0.31 $\mu\text{g/L}$. In the case of dimethoate, lower LOD values would be needed.

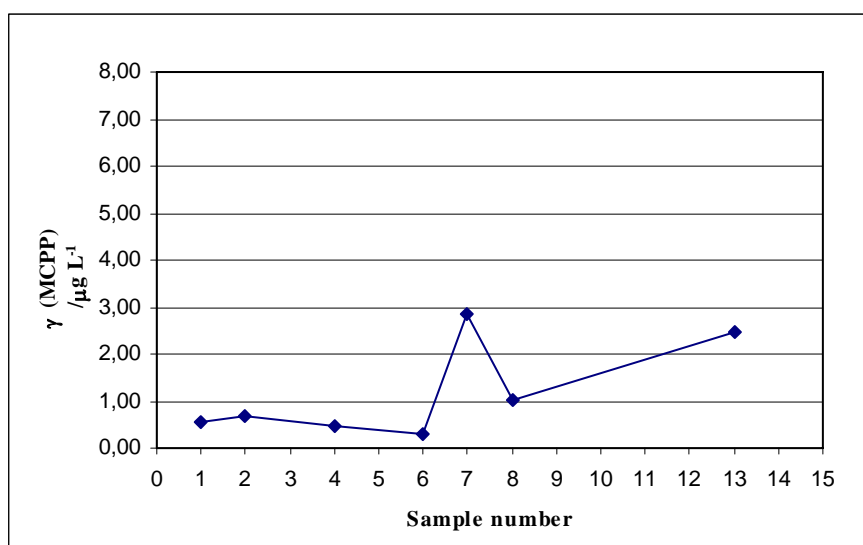


Figure 10. Occurrence and concentration of MCPP in the analyzed samples

- Dimethoate, 2,4-D and MCPP could be extracted with recoveries better than 70 %. The lowest recovery was obtained for linuron probably because of its lower solubility in water compared to the other pesticides under study. Further improvement of the recovery value of linuron is also needed.
- The proposed method required approximately 2 h for the concentration and extraction steps, and around 20 minutes for HPLC analysis of each sample. This justifies the use of this method, to determine the investigated pesticides in Macedonian environmental waters.

From the implementation of the proposed method on the surface water samples, the following, important points can be made:

- The investigated pesticides were found at concentrations ranging from 0.31 to 7.05 µg/L, depending on the compound, the collecting site and the sampling period.
- The generally higher concentrations than 0.1 µg/L that were detected in the samples under study indicate that it would be beneficial to monitor the pesticides levels more frequently and on a longer term basis.
- The limit of detection values below 0.1 µg/L obtained for MCPP, 2,4-D and linuron allow this method to be also applied for the investigation of these pesticides in drinking water.
- The described procedure can be used to determine dimethoate in surface waters in which the concentration of this pesticide is expected to be above 0.3 µg/L.

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References

1. [Irace-Guigand S., Aaron J.J., \(2003\). The role of organic herbicides transfer to rivers: Quantitative study of triazine and phenylurea interactions with colloids, *Analytical and Bioanalytical Chemistry*, vol.376, pp.431-435.](#)
2. [Chiron S., Dupas S., Scribe P., Barceló D., \(1994\). Application of on-line solid-phase extraction followed by liquid chromatography – thermo-spray mass spectrometry to the determination of pesticides in environmental waters. *Journal of Chromatography A*, vol. 665, pp. 295-305.](#)
3. [Pichon V., Hennion M.C., \(1996\). Convention N° 93307. French Ministry of Environment. Report 2 bis. pp. 44.](#)
4. [Pichon V., \(1998\). Multi-residue solid phase extraction for trace analysis of pesticides and their metabolites in environmental waters. *Analisis Magazine*. vol. 26, pp. M91-M98.](#)

5. [Butz S., Heberer T., Stan H.J., \(1994\). Determination of phenoxyalkanoic acidic herbicides at the low ppt level in water applying solid-phase extraction with RP-C18 material. *Journal of Chromatography A*. vol. 737, pp. 117-126.](#)
6. [Brinkman U.A.T., Slobodnik J., Vreuls J.J., \(1994\). Trace level detection and identification of polar pesticides in surface waters. *Trends of Analytical Chemistry*, vol. 13, pp. 373-381.](#)
7. [Dupas S., Scribe P., Dubernet J.F., \(1994\). On-line and off-line solid-liquid extraction and liquid chromatographic analysis at trace levels. for monitoring of herbicides and their degradation products in river and fluvioestuarine freshwater-seawater interfaces. *Journal of Chromatography A* , vol. 665. pp. 295-305.](#)
8. [Niessen W.M.A., \(1999\). State-of-the-art in the liquid chromatography mass spectrometry. *Journal of Chromatography A*. vol. 856, pp.179-197.](#)
9. [Hogenboom A.C., Hofman M.P., Jolly D.A., Niessen W.M.A., Brinkman U.A.T., \(2000\). On-line dual-precolumn-based trace enrichment for the determination of polar and acidic micro contaminants in river water by liquid chromatography with diode-array UV and tandem mass spectrometric detection. *Journal of Chromatography A* , vol. 885, pp. 377–388.](#)
10. [Geerdink R.B., Niessen W.M.A., Brinkman U.A.T., \(2002\). Trace level determination of pesticides in waters by means of liquid and gas chromatography. *Journal of Chromatography A* , vol. 970. pp. 65-93.](#)
11. [Slobodnik J., Hogenboom A.C., Vreuls J.J., Rontree J.A., Van Baar B.L.M., Niessen W.M.A., Brinkman U.A.T., \(1996\). Trace-level determination of pesticide residues using on-line solid phase extraction-column liquid chromatography with atmospheric pressure ionization mass spectrometric and tandem mass spectrometric detection. *Journal of Chromatography A*, vol. 741, pp. 59-74](#)
12. [Aguilar C., Ferrer I., Borrull F., Marcel R.M., Barcelo D., \(1998\). Comparison of automated on-line solid-phase extraction followed by liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization and particle beam mass spectrometry for the determination of a priority group of pesticides in environmental waters. *Journal of Chromatography A*, vol. 794, pp. 147-163.](#)
13. [Hogenboom A.C., Niessen W.M.A., Brinkman U.A.T., \(1999\). On-line solid-phase extraction short-column liquid chromatography combined with various tandem mass spectrometric scanning strategies for the rapid study of transformation of pesticides in surface water. *Journal of Chromatography A*, vol. 841, pp. 33-44.](#)
14. [Reemtsma T., \(2001\). The use of liquid chromatography atmospheric pressure ionization-mass spectrometry in water analysis. *Trends of Analytical Chemistry* vol. 20, pp. 533-542.](#)
15. [Shreiber. A., Efer J., Engewald W. \(2000\). Application of spectra libraries for high performance liquid chromatography-atmospheric pressure ionization mass spectrometry to the analysis of pesticide and explosive residues in environmental samples, *Journal of Chromatography A*, vol. 869, pp. 411-425.](#)
16. [Bruchet A. \(2001\). Report EUR 19707 EN. European Commission, pp. 110.](#)
17. [Baraud L., Tessier D., Aaron J.J., Quisefit J.P., Pinart J., \(2003\). A multi-residue method for characterization and determination of atmospheric pesticides measured at two French urban and rural sampling sites. *Analytical and Bioanalytical Chemistry*, vol. 377, pp. 1148-1152.](#)
18. [Irace-Guigand S., Aaron J.J., Scribe P., Barcelo D. \(2004\). A comparison of environment impact of pesticide multi residues and their occurrence in river waters surveyed by liquid chromatography coupled in tandem with UV diode array detection and mass spectrometry. *Chemosphere*. vol. 55, pp. 973-981.](#)
19. [Hennion M.C., \(1998\) Automation in multi residue analysis of pesticides using on-line solid phase extraction and liquid chromatography, *Analisis Magazine*, vol. 26, pp. M131-M137.](#)
20. [Degree on water categorization, \(1999\). Table VIII, Limit values/maximum allowed values or concentration of harmful and dangerous substances, *Official Gazette of R. Macedonia* 18.](#)