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normalization but simultaneously also a background correction. This resulted in underestimation of DE genes as our data had a previous automatic step of background correction.

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DETERMINATION OF TWO HERBICIDES IN DRAINAGE WATER

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ABSTRACT

Pesticides used in agricultural production for weeds and pests control can migrate to surface and ground water after application. Their presence in water used for irrigation can cause yield reduction and decrease product quality. This is very important considering importance of water quality for agricultural production, especially organic. Some of commonly detected pesticides in surface water are chloracetanilide herbicides, acetochlor and alachlor. They are herbicides widely used for control of broadleaf weeds and annual grasses in row crops. This study was carried out to evaluate the content of acetochlor and alachlor in drainage water which is widely used in agricultural production for irrigation. Water samples were collected from drainage canals in agricultural fields in the region of Vojvodina Province, Serbia. This part of Serbia is well-known as region with intensive agricultural production. The sampling was performed during June 2012, on twelve potential risk sites. Solid-phase extraction on C₁₈ ENVI™ SP disc (47 mm) was used for isolation of the investigated pesticides, acetochlor and alachlor, from water samples. Prior to extraction disc was conditioned with 5 ml of methanol and 5 ml of deionized water. Afterward, water sample was filtered through the disc. After drying the disc, acetochlor and alachlor were eluted with mixture of dichloromethane and n-hexane (40/60, v/v) and evaporated to dryness. Finally, the extract was diluted in 1 ml methanol and analyzed. Analysis was performed with a Hewlett-Packard (HP) model 5890 Series II gas chromatograph with EC Ni⁶³ detector (GC/ECD). Most of the analyzed water samples were found to be contaminated. Content of acetochlor and alachlor were ranged from 0.02-0.41 µg/l and 0.05-0.78 µg/l, respectively. This could be due to the frequent usage of the above-mentioned herbicides in these localities.

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Key words: acetochlor, alachlor, drainage water, residues

1 INTRODUCTION

Contamination of surface water with pesticides may be due to drift or runoff from areas where they were applied, while the result of contamination of groundwater leaching into deeper soil layers under the influence of precipitation. This is particularly obvious in areas with sandy soil and intensive pesticide application. The European Union by Framework Directive 2000/60/EC defined the guidelines in protecting and improving the quality of all water resources - rivers, lakes, groundwater, coastal water, etc. Directive 2008/105/EC is updated the Directive 2000/60/EC and by Annex X defines the List of priority substances in the field of water policy. List includes 33 pollutant - 9 are pesticides, including alachlor.

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⁴ BSc, ibid

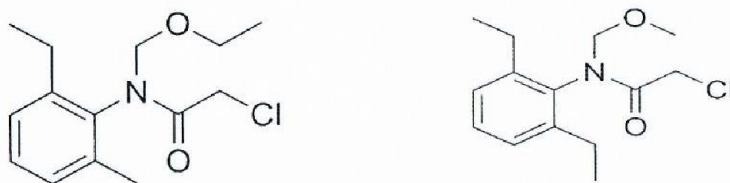


Figure 1: Structure of acetochlor and alachlor

Alachlor [2-chloro-2,6-diethyl-N-(methoxymethyl)acetanilide] (Fig. 1) has been registered since 1969 as a preemergence, early postemergence, or preplant incorporated herbicide for control of most annual grasses or certain broadleaf species. Alachlor is most heavily used on corn, soybeans, and grain sorghum (Schwab *et al.*, 2005).

The intensive use of alachlor herbicides in contemporary agricultural production during previous decades, has led to the accumulation of residues of alachlor and its metabolites in the environment, which endangers surface and ground water.

In order to replace the more widely used corn herbicides alachlor, atrazine, etc., acetochlor was registered for use, first in the USA. Acetochlor is a selective preemergent herbicide used to control broadleaf weeds and annual grasses in corn (Fig. 1). During the first season it was used, acetochlor was detected in rain and surface water in Minnesota at concentration of 0.01-0.25 µg/l. It was predictable, considering that acetochlor has a water solubility of 223 mg/l and it is moderately to very mobile in soil.

Besides the occurrence of pesticide residues in drinking water, control of their presence in drainage water used in agricultural production is very important. This primarily refers to the river water and groundwater, considering that the water and land quality in conventional production, but especially in organic agriculture, are extremely important. The presence of pesticide residues in these matrices may cause yield reduction and decrease product quality, due to its uptake.

This research was carried out to evaluate the content of chloroacetanilide pesticides, acetochlor and alachlor, in drainage water in the region of Vojvodina.

2 MATERIALS AND METHODS

2.1 Sampling area

Water samples were collected from drainage canals in agricultural fields in the region of Vojvodina Province, Serbia. This part of Serbia is well-known as region with intensive agricultural production. The sampling was performed during June 2012, on twelve potential risk sites (Fig. 2).

2.2 Analytical procedure

The standard solution of acetochlor and alachlor were prepared in mixture of ACN/methanol (50/50, v/v) in concentration interval of 0.01 – 2 µg/ml. For the recovery test model solution (500 ml of tap water enriched with 1 ml of acetochlor and alachlor standard solution in concentration 0.01 µg/ml, 0.1 µg/ml and 1 µg/ml) was used.

2.3 SP extraction

The extraction of pesticide from water was performed using ENVITM-C18 DSK 47 mm

extraction fpr disc of 47 mm. Prior to extraction disc was preconditioned with 5 methanol, followed by 5 ml of ultrapure water, at a rate of 2 ml/min. Then model solution filtered through the disc under vacuum at rate of 10 ml/min. After the disc was pesticides eluted from disk with 6 ml of dichloromethane/n-hexane (40/60, v/v) evaporated to dryness. The extract was dissolved in 1 ml of methanol, ultrasor homogenized and analyzed by GC-ECD. Injecting volume was 3 µl. Quantification performed using external standard method. The calibration solutions were analyzed t and after each water samples.

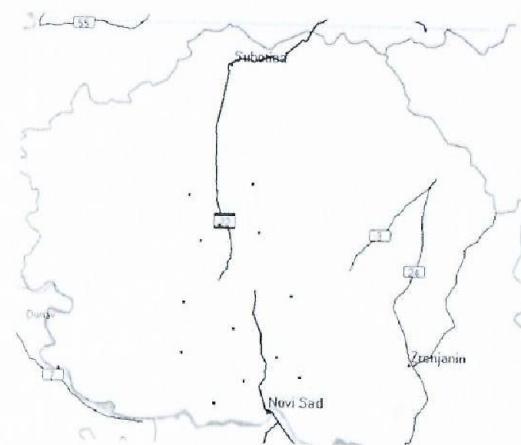


Figure 2: The geographic position of drainage water samples in the area of Vojvodina

2.4 GC-ECD analysis

Content of acetochlor and alachlor was determined using a Hewlett-Packard model 5 Series II gas chromatograph equipped with Supelco column 24048 (SPBTM-5, 30 m × mm id, film thickness 0.25 mil) and an electron capture detector Ni⁶³ (ECD) (Table 1). He was used as the carrier gas at a flow rate of 1 ml/min. The splitless injection was applied.

Table 1: GC/ECD conditions for acetochlor and alachlor determination

	Temperature
Inlet column temperature	100 °C
Rate	9 °C/min
Final column temperature	250 °C
Injector	250 °C
EC Detector	300 °C

Definition of chromatographic conditions was performed by determining the follow chromatographic parameters – linearity, repeatability of the peak area and limit quantification (LOQ). Linearity of detector response was determined by injecting standard acetochlor and alachlor at concentrations of 0.01 to 2 µg/ml. Standard solution for calibr curve was defined as the dependence of the peak area and concentration and expressed the corelation coefficient (R^2). Repeatability of detector response was determined injectiong "chlorpyrifos standard solution (0.1 µg/ml) five times nad tested by calculat the variati coefficient. LOQ was calculated using the formula $10 \times S_{a/a}$, where the S_a standard deviation and a is the slope of the calibration curve.

3 RESULTS AND DISCUSSIONS

3.1 Method validation

Using the presented conditions, the obtained value of correlation coefficient (Table 1) and coefficient of variation ($CV=2.62\%$) for acetochlor and alachlor indicating that achieved good linearity and high reproducibility and LOQ was $0.01 \mu\text{g/ml}$. These data are within the accepted range for determination of pesticide residue in matrices such as water.

Table 1: Analytical parameters for GC-ECD determination of acetochlor and alachlor

Parameter	Concentration interval μg/ml	Retention time Min	Correlation coefficient	LOQ μg/ml	Recovery %
Analit					
Acetochlor	0.01-2	10.955	0.993	0.01	97±1.9
Alachlor		10.737	0.975		96±2.5

For the extraction of pesticides from water C_{18} solid phase in the form of columns is mostly used. (Kolpin *et al.*, 1996; Louter *et al.*, 1992). This study used a C_{18} solid phase in the form of disc. Activation and conditioning of the disk is carried out by applying methanol. Otherwise, the hydrophobic C_{18} ligands are entangled and reduce the active surface interaction with the analytes from the water. Average value of the recoveries for acetochlor and alachlor at three fortification levels was 97% and 96%, with associated relative standard deviations (RSDs) of 1.9 and 2.5%, respectively.

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3.2 Monitoring

The European Union (Council Directive 98/83/EC of 3 November 1998) specifies a limit (MRL) of $0.1 \mu\text{g/l}$ for individual pesticides in water intended for human consumption, while the total concentration of pesticides should not exceed $0.5 \mu\text{g/l}$.

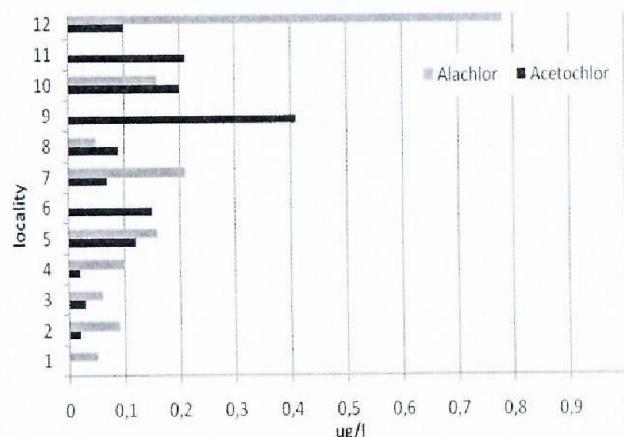


Figure 2: Content of acetochlor and alachlor in drainage water samples in the Vojvodina region

Obtained results show that the extraction and analysis procedures are efficient for determination of acetochlor and alachlor in water samples, considering that the EU MRL for

applied to real water samples. Drainage water samples were taken from several locations in the territory of Vojvodina during 2012. The presence of acetochlor was detected in 92 analyzed drainage water samples, while alachlor founded in 75% samples. The corresponding range of acetochlor concentrations was $0.02-0.41 \mu\text{g/l}$ and $0.05-0.78 \mu\text{g/l}$ for alachlor. Pollution of surface waters by tested pesticides may be the result of drift or runoff from fields that have been applied. In this sampling program content of acetochlor and alachlor and their metabolites were analyzed. Some investigators (Kalkhoff *et al.*, 1998) reported that acetochlor and alachlor were detected much more frequently than their respective parent compounds. This is probably because the metabolites have higher water solubility than the parent compounds.

4 CONCLUSIONS

In this work SPE-GC-ECD method for determination of acetochlor and alachlor in water samples was developed. High values of the recovery suggested the possible use of this method for acetochlor and alachlor extraction from different type of water, especially bearing in mind that the described method does not use large amounts of toxic organic solvents. The optimized method was applied for analysis of drainage water samples from Vojvodina region. The corresponding range of acetochlor and alachlor content in water samples was $0.02-0.41 \mu\text{g/l}$ and $0.05-0.78 \mu\text{g/l}$, respectively.

5 ACKNOWLEDGEMENT

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