CARBAMATE INSECTICIDES EXTRACTION DEPENDING ON THE SOIL PROPERTIES

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Abstract
The influence of physic-chemical properties of soils on retention of insecticides belonging to carbamate pesticides was studied. The recoveries determination was done in three soils for all pesticides applying QuEChERS method. Identification and quantification were done by LC-MS/MS. Except methiocarb, recovery values for multiple analysis of different soil samples spiked at 1.0 and 10.0 mg/kg of each of the pesticides ranged from 70.2 to 109.1%. The statistical analyses emphasized high statistical differences among pesticides and obtained recoveries.

Introduction
Carbamate consists of a wide spectrum of biologically active pesticides used worldwide to control insects and nematodes [1]. Carbamate insecticides are derivatives of carbamic acids and the first carbamate insecticide, carbaryl, was introduced in 1956 [2]. They inhibit the AChE enzyme and cause overstimulation of nervous system. Carbaryl (1-naphthyl *N*-methylcarbamate), broad spectrum carbamate insecticide is extensively used worldwide in more than 120 different crops and ornamental plants [3]. Because of its very low mammalian toxicity together with the short half-life carbaryl is the most popular insecticide and it effectively acts against 160 harmful insects. Carbaryl is the second most widely detected insecticide in surface waters in the United States [4].
Carbamate pesticides usage in agriculture is increasing significantly compared with other organohalogen pesticides, due to the fact that carbamate compounds have been considered stable in the environment in term of their application for preventing disease attack in case of plants' leaves and fruits [5]. Soil acts as one type of a "filter", providing sufficient time for biological or chemical degradation of pesticides before they reach groundwater. Carbamate pesticides have a low persistence in soil. When they are applied to crops or directly to the soil as systemic insecticides, carbamates generally persist from only a few hours to several months. However, they have been fatal to large numbers of birds on turf and in agriculture; the negative effect is seen on decreased breeding of the birds who have been consumed the treated seeds or plants [6].
In general, measurement of trace compounds such as pesticide residues is highly difficult due to time consumption, while the long procedure causes losses of the analytes [7]. The aim of this study was to determine the recoveries of investigated carbamates depending on the physic-chemical properties of three different soils. For the extraction of the aldicarb sulfone, aldicarb sulfoxide, carbaryl, methiocarb, methomyl, fenoxycarb, propoxur and thiodicarb the QuEChERS method was used, followed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Material and method

Chemicals and apparatus - The analytical standards of aldicarb sulfone, aldicarb sulfoxide, carbaryl, methiocarb, methomyl, fenoxycarb, propoxur and thiodicarb were parched from dr. Ehrenstorfer. The stock (≈ 1.0 mg/mL) and working solutions (10 µg/mL) were prepared in acetonitrile (HPLC purity, J.T. Baker). As an internal standards (10 µg/mL) carbofuran-D3, atrazine-D5 and isoproturon-D6 were used. Three soil types with different physical-chemical characteristics (Table 1) were used.

Table 1 Soil characteristics

Soil	pH (H2O)	$CaCO_3$ %	Organic matter %	Send 2-0.2 mm, %	Send 0.2-0.02 mm, %	Powder 0.02-0.002 mm, %	Clay <0.002 mm, %
1.	8.71	30.66	0.11	1.58	91.7	3.4	3.32
2.	8.16	7.45	3.76	10.25	22.45	25.03	42.27
3.	7. 65	1. 02	0. 88	0. 53	21. 39	29.04	49.04

LC-MS/MS analysis. LC-MS/MS with electrospray ionization. 6410 Agilent Technologies. The separation was performed using a Zorbax Eclipse XDBC18 column (50 mmx4.6mm id 1.8 µm.) at 25 ºC. The mobile phase (0.4 mL/min): methanol with 0.1% formic acid and 0.1% formic acid in water in the gradient mode. Total run was 30 min. The injection volume was 5 µL. The target ion transition with highest intensity (primary ion transition) was used for quantitation, where as the second target ion transition was used for confirmation. The instrument uses MassHunter software version B.06.00 for the quantitation and confirmation.

Method validation - recovery was determined according to SANTE/11813/2017. Recovery was obtained by spiking soil samples in the concentrations 1.0 and 10.0 mg/kg. Limit of detection (LOD) was estimated in the MRM mode analysis as the lowest concentration level that yielded S/N ratio of five.

Pesticides extraction from spiked soil samples was carried out using a modified QuEChERS method [8].

Statistical analyses. In order to determine the statistical differences among obtained recovery values as the dependent variables and the pesticides and soil types as independent variables the factorial and one-way factor analysis of variance (ANOVA) were applied using Statistica 13.2 (TIBCO Software Inc. University license). The calculated differences were tested by Fisher's LSD post-hoc test.

Results and Discussion

Before accessing qualitative analysis or quantification of pesticides it is necessary to set the acquisition parameters of the mass spectrometer - to set the multiple reaction monitoring mode (MRM). MRM-MS sensitivity is dependent upon the appropriate tuning of instrument parameters such as collision energy (CE) and energy of fragmentation (Frag) in order to generate maximal transmission of the pesticide product ions (Table 1).

Table 1. MRM transitions with retention times of the tested pesticides

Pesticide	**Formula**	**Rt (min)**	**Precursor ion (*m/z*)**	**Product ion (*m/z*)**	**Frag (V)**	**CE (V)**
Aldicarb sulfone	$C_9H_{10}Cl_2N_2O_2$	16.01	249 249	182.3 160.1	100 100	8 20
Aldicarb sulfoxide	$C_{12}H_{12}D_3NO_3$	13.28	225.1 225.1	165 123.1	94 94	10 22
Carbaryl	$C_{12}H_{11}NO_2$	19.38	202.1 202.1	145 127	100 100	20 35
Methiocarb	$C_{11}H_{15}NO_2S$	23.86	226.1 226.1	169 121	62 62	6 18
Methomyl	$C_5H_{10}N_2O_2S$	5.75	163.1 163.1	106 88	80 80	5 5
Fenoxycarb	$C_{17}H_{19}NO_4$	25.93	302.1 302.1	116.1 88	100 100	5 20
Propoxur	$C_{11}H_{15}NO_3$	17.00	210.1 210.1	168.1 111	60 60	5 10
Thiodicarb	$C_{10}H_{18}N_4O_4S_3$	20.87	355.1 355.1	108 88	80 80	10 15

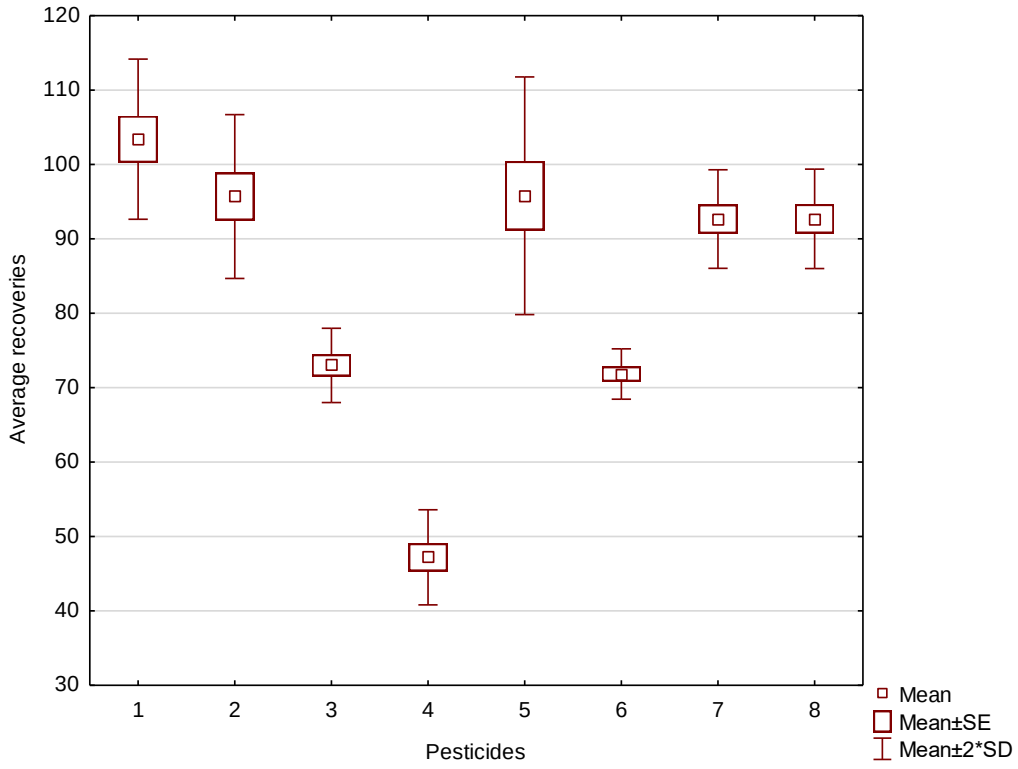
The obtained recoveries with RSD (%) values are given in the table 2. The obtained RSD values represent the precision of the method.

Table 2. Average recoveries of investigated pesticides (%)

Pesticide	**Soil 1**	**Soil 2**	**Soil 3**
Aldicarb sulfone	109.1 (16.33)	102.7 (17.96)	98.4 (12.04)
Aldicarb sulfoxide	101.6 (12.72)	94.8 (19.02)	90.7 (14.25)
Carbaryl	75.0 (9.43)	73.8 (6.23)	70.2 (10.32)
Methiocarb	50.8 (15.1)	46.1 (8.64)	44.7 (10.51)
Methomyl	104.2 (16.65)	94.9 (12.46)	88.3 (10.46)
Fenoxycarb	73.7 (9.57)	70.4 (8.92)	71.4 (5.98)
Propoxur	96.4 (10.16)	91.5 (4.78)	90.1 (7.49)
Thiodicarb	96.4 (9.93)	91.8 (4.55)	89.9 (8.69)

*RSD, % were given in brackets

The factorial ANOVA did not show any statistical significances regarding the influence of the paired values of different pesticides and soil types. The same result was obtained by one-way ANOVA calculated for different soil types and the values of average recoveries (p_s=0.694684 for $p<0.05$). However, the applied statistical analyses emphasized high statistical differences among pesticides and obtained recoveries (p_p=0.000000 for $p<0.01$). Fisher's LSD test distinguished aldicarb sulfone, aldicarb sulfoxide and methomyl as the pesticides with the highest values of average recovery values and high statistical significances compared to the other prospected pesticides (Graph 1).



Graph 1. Statistical analyses

Conclusion

The influence of main physic-chemical properties of three soils on carbamate insecticides recoveries in this matrix were studied applying QuEChERS soil sample preparation followed by LC-MS/MS determination.

The organic matter and clay content affected the recovery of studied pesticides. The obtained dependence indicates that with increasing organic matter and clay content (soil 2 and 3), the recoveries were lower than in soil 1.

The applied statistical analyses emphasized high statistical differences among pesticides and obtained recoveries (p_p=0.000000 for $p<0.01$). Fisher's LSD test distinguished aldicarb sulfone, aldicarb sulfoxide and methomyl as the pesticides with the highest values of average recovery values and high statistical significances compared to the other prospected pesticides.

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