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Advanced Identification and Quantification of Pesticide Residues in Vegetables by GC-ToFMS Technique

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Abstract. *The identification and quantification of a large range of pesticide residues (e.g. fungicides, insecticides, acaricides) in vegetables from Romania markets, in Maramures, Alba Iulia and Botosani region, was performed by gas-chromatography coupled with time-of-flight mass spectrometry (GC-ToFMS) to better assess the impact of agricultural practices that may result from "accidental" application of higher doses of treatment solutions. The ion chromatograms obtained by GC-ToFMS enabled us to easily quantify at ppb level and to record the entire spectrum at high speeds (up to 500 spectra/second) without losing of the data quality. Although, most of the pesticide residues detected were below the limits set by the law, the results highlighted concentrations that exceeded the maximum allowed for toclofos-methyl and malathion from recent treatments and still high levels of alpha- and beta-hexachlorocyclohexane (mostly in green lettuce) from the historical treatments. It was also evidenced that green lettuce accumulated more than 77% higher amount of pesticides than cucumber, green beans and tomatoes.*

Keywords: *pesticides residues, vegetables, soil, water, GC-ToFMS.*

1. Introduction

The need to meet increasing international food consumption has led to the development and enhancement of biocidal research with unfavorable long-term effects to the human body. The use of pesticides in agriculture has important benefits and really combats pests, but their accumulation in vegetables and fruits is not desirable [1,2]. The use of those pesticides with rapid effects, but with decomposition to less toxic components under the action of microorganisms or environmental factors, is currently being promoted. Their permitted maximum concentrations are governed by international and European norms [3]. However, the use of pesticides on a large scale in agriculture leads to their accumulation in both soil and vegetables, fruits or other foods, so that the setting of the administered concentrations is particularly important [4,5]. Classification of pesticides can be achieved depending on their chemical nature (e.g. organic and inorganic), target organism, action timing of their application, the mode of action and degree of their toxicity [6-9].Organic pesticides, of which the most commonly used are carbamates, triazines, neonicotinoids, pyrethrins, organochlorines, guanophosphates, phenylureas and others, contain carbon in their molecular structure [10]. Inorganic pesticides have mineral derivatives in their composition such as lime, sulfur, antimony, cadmium or lead, which lead to their accumulation of toxic deposits in many cases. A very important aspect is the identification and quantification of pesticide residues and other toxic components in foodstuffs as a crucial part of controlling food safety and public health [6,11-13]. Numerous investigations have been made on various processed food, dairy products, meat, plant products, seeds, vegetables, fruits, as well as in the soil in rural areas, monitoring a wide range of pesticides such as DDT, lindane isomers, endosulfan, cyclodiene and methoxychlor. A recent study performed on agricultural topsoil samples from 11 EU countries highlighted that the number of pesticide residues and their concentration in soil varied among the

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countries and cropping systems, and occasionally exceeded their predicted environmental concentrations in soil (≥ 1 mg kg^{-1}), but below the respective toxic endpoints for standard in-soil organisms [14]. However, has been shown that even pesticide (e.g. DDT) contamination in soil was high, in raw food (e.g. vegetable, meat, milk) was low, meaning that was a minimal transfer of pesticides residues from soil to the food segment [15]. Another study performed in the soils of Campagnia Plain (Sothern Italy), evaluated the potential environmental and human risks in terms of the presence of hexachlorobenzene (HCB) and chlorinated pesticides, with registered levels presenting differentiated values depending on the use of soil. To investigate the spatial distribution of the pesticide residues and explore their relationship with the soils properties, a systematic grid and geostatistical sampling method was applied. It was found that CHLs (heptachlor, heptachlor epoxide, *trans*-chlordane, and *cis*-chlordane) showed the most significant concentrations, followed by DRINs (aldrin, dieldrin, and endrin), and SULPHs (α-endosulfan, β- endosulfan, and endosulfan sulphate), while the lowest concentrations were for HCB. Soils sampled from the agricultural area of the region showed high concentrations for most cyclodienes compared to those of corresponding counterparts in the other land uses; however, the registered values do not lead to potential carcinogenic illnesses, but can affect other segments of terrestrial living creatures [16]. Therefore, to ensure food safety, monitoring the level of pesticide residues in various agricultural products, as vegetables, fruits, soil, water and other components of agro-systems, at a lower concentration level than the established maximum residue levels imposed by EU directives [17], remains an important tool in the evaluation of risks for environment and human health. The choice of an appropriate sample preparation method and an accurate analytical method influences considerably the reliability of the analysis of pesticide residues in foodstuffs. It was demonstrated that the PUF-azo-Tan adsorbent behaves as an efficient sorbent for the extraction and preconcentration of pesticides in food, cucumber, guava and potatoes [18]. Currently, for analyzing and monitoring of residue levels of pesticides and other toxic components in food products, complex sample processing methods were used, such as the QuEChERS technique [19-24], followed by the analysis of extracted compounds with gas-chromatography [25-28, 29] or liquid-chromatography, coupled with mass spectrometry. In this work, the gas-chromatography coupled with time-of-flight mass spectrometry (GC-ToFMS) is used to identify and quantify a range of 20 pesticide residues (ex. fungicides, insecticides, acaricides) from vegetables collected in various regions of Romania, considering both different batches and production periods, randomly chosen from several agricultural producers. Validation of the chromatographic method (linearity, limits of detection (LODs) and quantification (LOQs)) was also carried out.

2. Material and methods

2.1. Sample Collection

Fresh vegetable (cucumber, green beans, green lettuce and tomatoes) samples were taken from small producers located in North-West, Center and North-East Romania, namely from Maramureș, Alba and Botosani counties. The samples were collected in 2017, during harvesting of vegetables (June). Samples, in the amount of 1000 g, were stored in original containers and kept in the dark until the date of analysis.

2.2. Reagents and Organic Solvents

Standards for the 20 pesticides under investigation, such as cyprodinil, fludioxonil, boscalid, malathion, iprodione, tolclofos-methyl, tebuconazole, buprofezin, spirodiclofen, endosulfan, diclorvos, endrin, ethion, α-HCH (alpha-hexachlorocyclohexane), β-HCH (beta-hexachlorocyclohexane), chlorpyrifos-ethyl, atrazine, pyrimethanil, chlorothalonil and pendimethalin were obtained from Dr. Ehrenstorfer-Schafers (Augsburg, Germany) and most were certified of purity > 99%. Concentrations of standard solutions were corrected by standards of certified purity, whenever their purity were below 99%. Proper amount of stock solutions of all these standards were prepared in chromatography-grade *n*-hexane. Solutions of varying concentrations from 0.01 μg/mL to 0.80 μg/mL were prepared from each stock solution of 10 μg/mL. All individual standard solutions and dilutions were stored in glass flasks

and kept at -20 °C. Mixed calibration solutions in n-hexane were prepared from the stock solutions and used for controlled contamination of the selected matrix. Acetone, methylene chloride, isooctane, toluene, acetonitrile of chromatography grade and active black carbon were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich. An uncontaminated cucumber sample (for control) was taken from a bio culture to be used in the optimization and validation experiments.

2.3. Extraction

To analyze a large number of pesticides from different chemical classes, a simple method was first developed to extend the applicability of a multi-residue method previously tested for pesticide analysis in vegetable samples. Vegetables with sample units > 250g are cut into four or six parts and from each of them are taken quarters diagonally and homogenized. Vegetables with units between 25 and 250g are homogenized as such (1kg/10 units), at room temperature, using a Stephan UMC5 homogenizer (Sympak Inc., USA).

For each plant matrix we processed only the part of the product for which the maximum admissible limits are set. At an aliquote of 15g of sample, weighed in a centrifuge bottle, a 30 mL of acetone was added and homogenized for 30 s at a rate of 15,000 rpm with a T25 Ultra-Turrax disperser/ homogenizer (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Then, 30 mL of petroleum ether and 30 mL of methylene chloride were added and mix for 60 s at 15,000 rpm followed by centrifugation for 4 minutes at a minimum of 4,000 rpm. Using a volumetric glass pipette, 15 mL from the sample extract were transferred to a round bottomed flask. Sample extract evaporation was performed with a rotavapor apparatus to nearly dryness, at a water bath temperature of up to 40 ºC. We allowed the flask to cool to room temperature and waiting until the last solvent drops evaporated. The obtained residue was dissolved with 3 mL of isooctane containing 0.2 mg/L of internal standard and sonicated for 3 minutes at the ultrasonic bath.

For fortifying, the untreated cucumber sample was subjected to the same treatment as the other vegetables, adding an appropriate volume of the standard working solution (30 µL, 60 µL, 150 µL, and 300 µL) to achieve the concentrations on the calibration curve and obviously following the recovery yield of these concentrations.

For the green lettuce matrix, beside the extraction method, purification with black activated charcoal (GBC) was also carried out in order to eliminate the very high chlorophyll loading.

2.4. Analytical Conditions of Quantitative Method for Pesticide Residues by GC-ToFMS

Confirmation and quantification analysis was performed by Multiresidual Analysis Method (MRM) with a LECO Pegasus III 4D GCxGC-ToFMS system. The LECO Pegasus III 4D GCxGC-ToFMS system (LECO Corp., St. Joseph, MI, USA) is equipped with a modified AGILENT 6890 series gas chromatograph equiped with a LECO Quad-Jet cryostatic modulator and a LECO secondary GC oven, an AGILENT 7683 Autosampler and a split/splitless injector (the instrument was initially configured as a GCxGC-ToFMS). Practically, for this application the system was used in GC-ToFMS configuration (monodimensional Gas Chromatograph coupled with Time of Flight Mass Spectrometer).

Two columns of different polarities were combined to achieve the apropriate chromatographic separation, using helium as a carrier gas at a flow rate of 1.2 mL/min. Column 1: RXi-5MS, with a length of 30 m, 0.25 mm internal diameter, film thickness of 0.25 µm (Restek, USA); Column 2: BPX-50, length 1.6 m, 0.1 mm internal diameter, 0.1 µm film thickness (SGE Analytical Science, Australia). The injector temperature was set at 250°C, and the splitless injection was performed by opening the split valve after 1 min and providing a 100:1 division ratio.

The temperature program for main GC oven provided an analytical time of about 29 min and worked under the following conditions: the initial temperature of 70°C was maintained for 1 min, increased by 20°C/min to 180°C, then increased until at 250 °C with 5°C/min and finally reaches up to 305°C with 25°C/min and being held for 6 min. The cryostatic modulator was turned off, and the

secondary GC oven follows the shape of temperature program of the main GC oven, but increased by $\Delta T = 25°C$.

The sample injection volume was 1 μL. The transfer line was maintained at 280°C and the ion source temperature was set at 220 °C. The mass spectrometer ion source was operated in electronic ionization mode (EI +) with ionization energy of 70eV. The mass analyzer operated in full scan mode on a m/z range of 40 to 450 amu and a 15 spectra/second acquisition rate.

The MS acquisition method specifies a 350 s delay time for data acquisition used by solvent removal and a minimum 70% superpozability (matching) of the analyzed pesticide spectra compared with the spectra of the same compounds in any of the 10 available mass spectra libraries. At the time of sample analysis, the detector voltage was set to 1700V, while it can be raised up to 2000V depending on the duration of use and the wear of the detector.

The detection limits (LODs) of GC-ToFMS were determined for each pesticide by successive dilution of the mixed solution, followed by injection of the column 10 times for each dilution. Repeatability of the dilution provided the necessary information to calculate the detection limits of each pesticide.

3. Results and discussions

Cucumber, green beans, lettuce and tomatoes matrices were studied using GC-ToFMS method to monitor the following pesticides: cyprodinil, fludioxonil, boscalid, malathion, iprodione, tolclofos-methyl, tebuconazole, buprofezin, spirodiclofen, endosulfan, diclorvos, endrin, ethion, α-HCH (alpha-hexachlorocyclohexane), β-HCH (beta-hexachlorocyclohexane), chlorpyrifos-ethyl, atrazine, pyrimethanil, chlorothalonil and pendimethalin.

The Multiresidual GC-ToFMS Method

Analytes were quantified with calibration curves prepared in isooctane:toluene (9:1 v/v) mixture and for each analyzed pesticide the equation of the calibration curve was presented. Samples whose content exceeded the maximum value in the calibration curve were diluted with the final dilution solvent, this being specified for each pesticide, and a correction factor was applied for the reporting of the results in mg/kg.

Calibration curves were constructed from 7 calibration levels, namely: 0.01 - 0.03 - 0.05 - 0.1 - 0.3 - 0.5 - 0.8 μg/mL. At each calibration solution, 3 mL of internal standard solution was added. Sample integration was achieved by constructing calibration curves for each active substance with 7 concentration levels and 5 injections for each level.

The working sequence consists of an injection at each calibration level followed by the samples of cucumbers, green beans and salad analyzed, following by a control sample enriched at 0.05 μg/mL (50 ng/mL) for each extraction, and ends with a new series of injections from the calibration curves. Each pesticide was identified and quantified with at least 3 m/z ratios, with a similarity of at least 70%, comparing with similar compounds spectra from those 10 spectra libraries. The specific characteristics for the analysed pesticides are shown in Table 1.

Table 1. Characteristics of analyzed compounds

Pesticide		Pesticide class/type	Molecular mass [g/mol]	m/z for specific ions	LOD of the method [mg/kg]	LOQ of the method [mg/kg]	RSD [%]
1	Cyprodinil	Fungicide	225.295	224, 225, 210	0.003	0.01	0.998
2	Fludioxonil	Fungicide	248.185	248, 127, 182, 128	0.003	0.01	0.998
3	Boscalid	Fungicide	343.207	342, 142, 140, 167	0.003	0.01	0.996
4	Iprodione	Fungicide	330.165	314, 187, 124, 245	0.003	0.01	0.998
5	Tolclofos-methyl	Fungicide	301.118	265, 125, 250	0.0015	0.005	0.997
6	Tebuconazole	Fungicide	307.822	235, 165, 239, 246	0.003	0.01	0.999
7	Pyrimethanil	Fungicide	199.257	198, 199, 200	0.003	0.01	0.997
8	Chlorothalonil	Fungicide	350.575	97, 197, 199, 314	0.01	0.05	0.998

Pesticide		Pesticide class/type	Molecular mass [g/mol]	m/z for specific ions	LOD of the method [mg/kg]	LOQ of the method [mg/kg]	RSD [%]
9	Malathion	Insecticide	330.358	125, 173, 93,127	0.003	0.01	0.999
10	Buprofezin	Insecticide	305.44	105, 106, 172, 104	0.006	0.02	0.999
11	Spirodiclofen	Insecticide	411.319	71, 157, 312, 159	0.003	0.01	0.998
12	Endosulfan	Insecticide	406.904	170, 241, 195, 207	0.003	0.01	0.999
13	Dichlorvos	Insecticide	220.98	109, 185, 145	0.003	0.01	0.996
14	Endrin	Insecticide	380.907	263, 281, 265, 279	0.0015	0.005	0.997
15	Ethion	Insecticide	384.459	231, 153, 125, 121	0.0015	0.005	0.998
16	alpha-HCH	Insecticide	290.814	183, 181, 219, 217, 109	0.0015	0.005	0.999
17	beta-HCH	Insecticide	290.814	219, 181, 109, 183	0.0015	0.005	0.999
18	Chlorpyrifos-ethyl	Insecticide	350.575	97, 197, 199, 314	0.003	0.01	0.999
19	Atrazine	Herbicide	215.685	200, 215, 173	0.006	0.02	0.998
20	Pendimethalin	Herbicide	281.312	263, 281, 265, 279	0.01	0.05	0.999

Distribution of pesticide residues accumulation in vegetables

The vegetable samples, namely cucumber, green beans, green lettuce and tomatoes, were analyzed for twenty individual pesticides and the results are summarized in Table 2. Most of the compounds, even present in all the samples - like fludioxonil, boscalid, and iprodione, were present in concentrations below the limits set by the EU regulation.

Table 2. Experimental results on investigated compounds – min, max, average [mg/kg];

Compound			Vegetables			
			Cucumber	Green beans	Green lettuce	Tomatoes
1	cyprodinil	Range	<LOQ-0.114	0.0321-0.061	0.657-2.483	0.0357-0.0980
		Average	0.0455	0.0430	2.2647	0.0662
	EU maximum limit [mg/kg]		*0.5*	*2*	*15*	*1.5*
2	fludioxonil	Range	0.0133-0.136	0.0200-0.2587	1.3612-2.0325	0.035-1.2527
		Average	0.0933	0.0996	1.7536	0.4676
	EU maximum limit [mg/kg]		*0.4*	*1*	*40*	*3*
3	boscalid	Range	0.0118-1.3697	<LOQ-1.0258	0.4111 - 1.3410	0.0567-0.6870
		Average	0.5612	0.3503	0.9367	0.3703
	EU maximum limit [mg/kg]		*4*	*5*	*50*	*3*
4	iprodione	Range	0.0369-1.2527	0.3650-1.0050	<LOQ-1.2561	0.0570-0.2580
		Average	0.7481	0.6757	0.7700	0.1674
	EU maximum limit [mg/kg]		*4*	*2*	*25*	*5*
5	tolclofos-methyl	Range	<LOQ-0.1680	<LOQ-0.0136	0.0112-1.3639	<LOQ -0.058
		Average	0.0632	0.0085	0.5234	0.0019
	EU maximum limit [mg/kg]		*0.01*	*0.01*	*2*	*2*
6	tebuconazol	Range	0.0354-0.1250	<LOQ-1.0225	<LOQ-0.3605	n.t.*
		Average	0.0690	0.4610	0.1202	n.t.*
	EU maximum limit [mg/kg]		*0.6*	*2*	*0.5*	-
7	pyrimethanil	Range	n.t.*	n.t.*	0.0690-1.0540	0.0055-0.0390
		Average	n.t.*	n.t.*	0.5790	0.0200
	EU maximum limit [mg/kg]		-	-	*20*	*1*
8	chlorothalonil	Range	n.t.*	n.t.*	n.t.*	0.0740-0.3644
		Average	n.t.*	n.t.*	n.t.*	0.2665
	EU maximum limit [mg/kg]		-	-	-	6
9	malathion	Range	<LOQ-0.1214	<LOQ-0.0120	n.t.*	n.t.*
		Average	0.0442	0.0040	n.t.*	n.t.*
	EU maximum limit [mg/kg]		*0.02*	*0.02*	-	-
10	buprofezin	Range	0.0410-0.0654	0.0141-0.0871	< LOQ	< LOQ
		Average	0.0505	0.0407	< LOQ	< LOQ
	EU maximum limit [mg/kg]		*1*	*1*	*0.5*	*0.5*
11	spirodiclofen	Range	<LOQ-0.036	<LOQ-0.0125	<LOQ-0.0125	<LOQ-0.0018
		Average	0.0139	0.0042	0.0042	0.0006
	EU maximum limit [mg/kg]		*0.1*	*0.02*	*0.02*	*0.02*
12	endosulfan	Range	< LOQ	<LOQ-0.0105	<LOQ-0.0124	< LOQ
		Average	< LOQ	0.0035	0.0041	< LOQ
	EU maximum limit [mg/kg]		*0.05*	*0.05*	*0.05*	*0.05*

	Compound		Vegetables			
			Cucumber	Green beans	Green lettuce	Tomatoes
13	dichlorvos	Range	< LOQ	<LOQ-0.0075	< LOQ	< LOQ
		Average	< LOQ	0.0025	< LOQ	< LOQ
	EU maximum limit [mg/kg]		*0.01*	*0.01*	*0.01*	*0.01*
14	endrin	Range	< LOQ	< LOQ	n.t.*	n.t.*
		Average	< LOQ	< LOQ	n.t.*	n.t.*
	EU maximum limit [mg/kg]		*0.01*	*0.01*	-	-
15	ethion	Range	<LOQ-0.0088	<LOQ-0.0054	< LOQ	< LOQ
		Average	0.0029	0.0018	< LOQ	< LOQ
	EU maximum limit [mg/kg]		*0.01*	*0.01*	*0.01*	*0.01*
16	α-HCH	Range	<LOQ-0.046	<LOQ-0.0071	<LOQ-0.0157	<LOQ-0.0044
		Average	0.0153	0.0046	0.0052	0.0015
	EU maximum limit [mg/kg]		*0.01*	*0.01*	*0.01*	*0.01*
17	β-HCH	Range	<LOQ-0.0080	<LOQ-0.0051	<LOQ-0.0172	<LOQ-0.0047
		Average	0.0050	0.0017	0.0057	0.0028
	EU maximum limit [mg/kg]		*0.01*	*0.01*	*0.01*	*0.01*
18	chlorpyrifos-ethyl	Range	0.0150-0.0358	0.0102-0.0214	<LOQ-0.0122	< LOQ
		Average	0.0222	0.0152	0.0041	< LOQ
	EU maximum limit [mg/kg]		*0.05*	*0.05*	*0.05*	*0.05*
19	atrazine	Range	<LOQ-0.0214	<LOQ-0.0109	< LOQ	< LOQ
		Average	0.0105	0.0036	< LOQ	< LOQ
	EU maximum limit [mg/kg]		*0.05*	*0.05*	*0.05*	*0.05*
20	pendimethalin	Range	n.t.*	n.t.*	<LOQ-0.068	< LOQ
		Average	n.t.*	n.t.*	0.041667	< LOQ
	EU maximum limit [mg/kg]		-	-	*0.1*	*0.1*

*LOQ – limit of quantification, n.t. - not tested

The results reveal that tolclofos-methyl and malathion residues from recent treatments exceeded the maximum residue limits (MRL) allowed by the law. Moreover, detectable amounts of α- and β-hexachlorocyclohexane were present mostly in green lettuce, from the historical treatments. Our results show that green lettuce accumulated more than 77% higher amount of pesticides than cucumber, green beans and tomatoes. This may be due to the high-water content of the lettuce (94.9%) and short path of water and nutrients from the soil to the leaf, compared to the other vegetables in the study with a longer route of water-nutrients to the fruits. The distribution of pesticide residues grouped by their action, fungicide, insecticide and herbicide, in the investigated vegetable samples is shown in Figure 1.

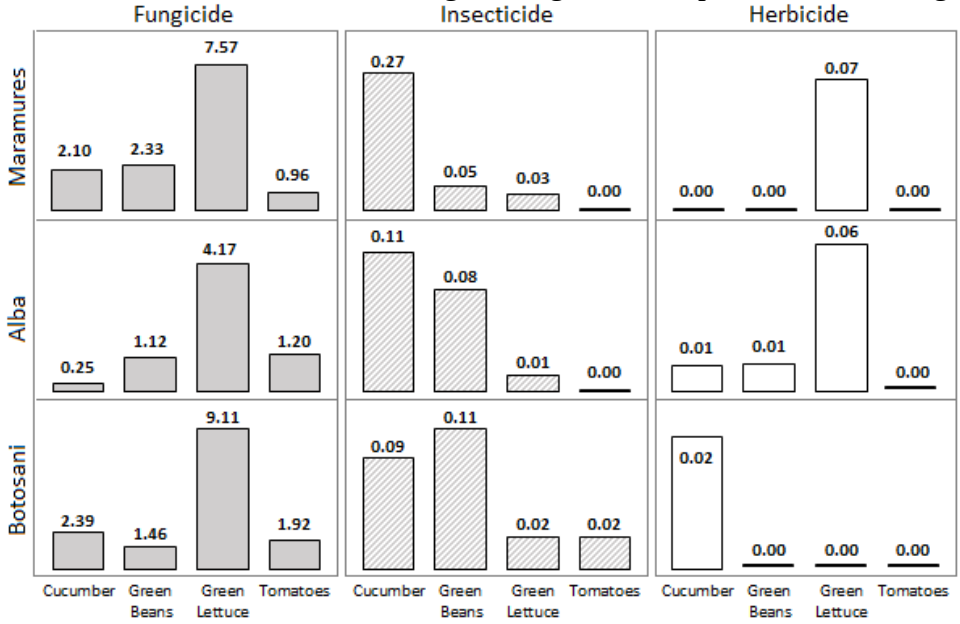

Figure 1. Distribution of measured fungicide, insecticide and herbicide in cucumber, green beans, green lettuce and tomatoes, by sampling provenance (the amount of pesticide residue found in each vegetable, in mg/kg)

No matter is provenance (location of origin), the green lettuce accumulated the highest level of fungicide (9.11 mg/kg in Botosani, 7.57 mg/kg in Maramures, and 4.17 mg/kg in Alba) compared with cucumber (0.25 mg/kg in Alba, to 2.39 mg/kg in Botosani), green beans (1.12 mg/kg in Alba, to 2.33 mg/kg in Maramures) and tomatoes (1.03 mg/kg in Maramures, to 2.28 mg/kg in Botosani).

The variability of the pesticide residues in vegetable samples by individual compounds, vegetable category and sampling location is highlight in Figure 2. The influence of the specific current and past practices in the provenance region of vegetables is evidenced.

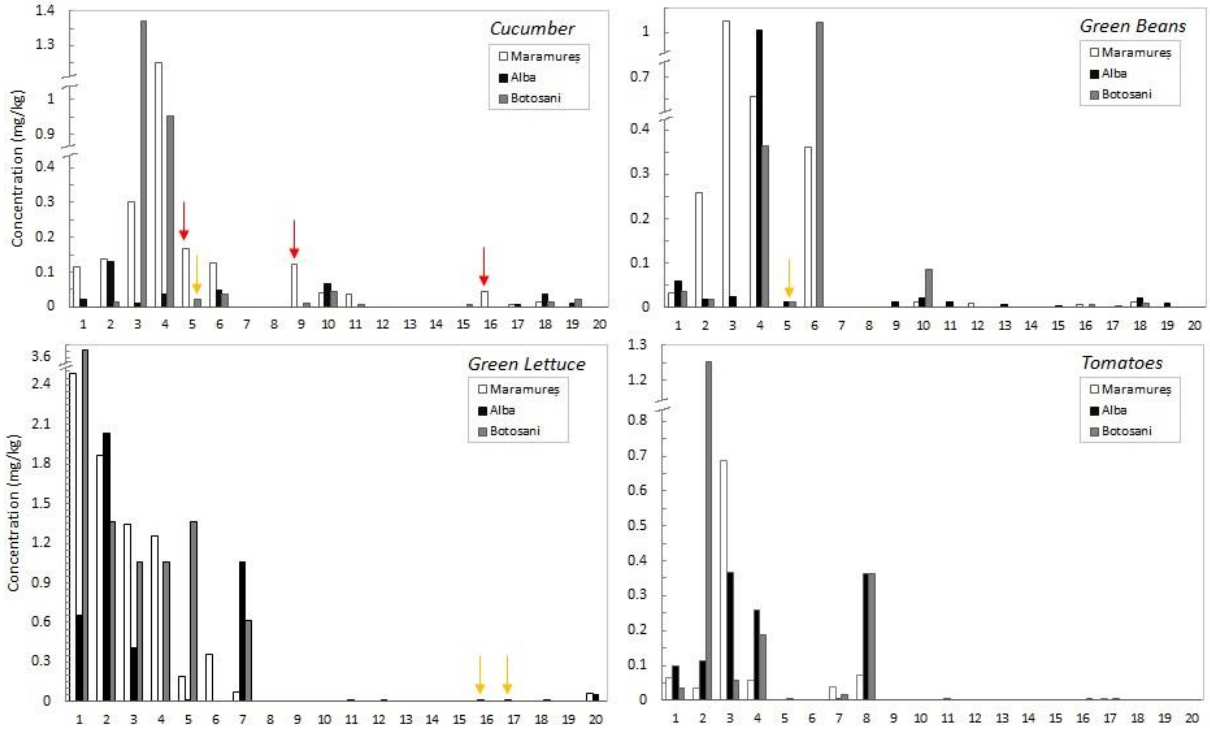

Figure 2. Variability of pesticide residues in vegetable samples by individual compounds (1-8 fungicide, 9-18 insecticide and 19-20 herbicide), vegetable category and sampling location. Compound concentration values exceeding the MRLs marked with red; values near MRLs marked with yellow

Cucumber contained the highest concentrations for iprodion (1.25 mg/kg in Maramureş) and for boscalid (1.37 mk/kg in Botosani), but well below the MRL of these compounds, of 4 mg/kg. Instead, the most concerning values for pesticide residue concentrations was observed for malathion and tolclofos-methyl. For malathion, the measured value of 0.12 mg/kg, in Maramures, was six time higher than the EC Reg. 396/2005 MRL (0.02 mg/kg). The tolclofos-methyl values of 0.17 mg/kg in Maramures, and 0.02 mg/kg in Botosani, significantly exceed the MRL of 0.01 mg/kg. The soil contamination derived from past treatments is reflected by HCH congeners in cucumber (0.046 mg/kg) which is in samples from Maramures, 4.6 times higher than the EU MRL of 0.01 mg/kg for α-HCH. Values near the limit of 0.01 mg/kg were determined also for β-HCH in cucumber samples from Maramures (0.008 mg/kg). Considering the region of provenance, all the compounds measured in cucumber samples from Alba were under the MRL. Our data reveal the occurrence of some organochloride pesticides (e.g. malathion, tolclofos-methy, α-HCH) in cucumber fruits at levels that exceed the MRLs and are in agreement with Manosur et al. (2009) [30] who reported high concentration of organochloride pesticides residues (e.g. HCB, lindane, heptachlor, aldrin, dieldrin, and some DDT metabolites) in cucumber samples grown in different conditions.

For green beans, the highest values were found for boscalid, in amount of 1.03 mg/kg in Maramures but much lower than the MRL of 5 mg/kg, and for iprodione, of 1 mg/kg to Alba, almost 2

times lower than the MRL (2 mg/kg). Significant values have also been observed for other pesticides, such as tebuconazole (1.02 mg/kg in Botosani) and tolclofos-methyl (0.012 mg/kg in Alba and 0.0136 mg/kg in Botosani), but lower or quite near the MRL of 2 mg/kg, and 0.01 mg/kg, respectively. For compounds as cyprodinil and fludioxonil, even found in all the green beans samples, the values were well below the MRL of 2 mg/kg and 1 mg/kg, respectively. From regional point of view, all the compounds measured in green beans samples from Maramures were within the MRL.

Green lettuce samples contained the highest pesticides residues among the vegetables, but most of them were well below the MRLs. For example, cyprodinil had values from 2.5 mg/kg in Maramures, to 3.7 mg/kg to Botosani, compared with the limit set at 15 mg/kg, while fludioxonil concentrations were 2 mg/kg in Alba, 1.9 mg/kg in Maramures and 1.4 mg/kg in Botosani *versus* the MRL of 40 mg/kg. Even found in all the sampling sites, other compounds as the boscalid, tolclofos-methyl and pyrimethanil, had values under the MRL. The historical soil residual contamination with HCH congeners is present in relatively high concentrations of α-HCH (0.016 mg/kg) and β-HCH (0.017 mg/kg) found in green lettuce samples from Maramures that exceed with 57% and respectively 72% the EU MRL (0.01 mg/kg) set for the sum of the isomers.

For tomatoes, low levels of pesticide residues concentrations were found. Compounds such as buprofezin, endusolfan, dichlorvos, ethion, chlorpyrifos-ethyl, antrazin and pendimethalin were not detected. The biggest concentration was determined in Botosani for the fludioxonil (1.25 mg/kg), near the MRL of 3 mg/kg. Cyprodinil, boscalid, iprodione, pyrimethanil and chlorothalonil were found in all the tomatoes samples, but well below the MRLs. The historical soil residual contamination with HCH congeners is reflecting in relatively low concentration of α-HCH in tomatoes samples (less than 50% of MRLs), and β-HCH was not detected.

The literature reports the occurrence of these organochlorine persistent compounds (OPCs) in vegetables grown both in traditionally conditions [31] and organically ones [32]. Has been shown that beside the hydrophobic uptake of the OPCs from soil [33], the atmospheric deposition of these volatile and semi-volatile compounds on the cultivated crops [34, 35] it is a possible way for plant contamination. It was also revealed that vegetables grown in greenhouse conditions have a high level of pesticide contamination compared to those grown in the open field, possibly due to the favorable conditions of spreading insect pests in the confined space, thus leading to excessive use of organic pesticide [30].

4. Conclusions

The use of pesticides in agriculture is a necessity, first of all for controlling the weeds and pests, and secondly for stimulating fructification, ripening and fertilization. Their persistence in the environment due to the slow decomposition rate, long half-life and high stability, may have a negative impact to human health. A way of human exposure to pesticides is through food, including vegetables/fruits.

In this work, a GC-ToFMS (gas-chromatography coupled with time-of-flight mass spectrometry) method was developed for the simultaneous determination of 20 pesticide residues (ex. fungicides, insecticides, acaricides) from vegetables. Compared with method published previously, the method set in this study had many advantages, such as record the entire spectrum at high speeds (up to 500 spectra/ second) without losing of the data quality, possibility to easily quantify at ppb level. Good quality results, including recovery, precision, linearity, LOD and LOQ were achieved.

Finally, vegetable samples, namely cucumber, green beans, green lettuce and tomatoes collected from producers originated from three different geographical areas were analyzed to demonstrate the applicability of the method. In most samples no exceedances of the MRLs specified by the European legislation were found. However, residues in some batches of some vegetable crops, as green lettuce, have been found to exceed the maximum levels permitted by European legislation. This may be due to the specific agricultural practices performed on batches for which the prescribed doses or the application time imposed for these types of pesticide treatments have not been respected. Also, in

some case-specific situations, values of α-HCH and β-HCH concentrations near or slight over the levels accepted by the legislation were measured on some batches, although the crops have not been treated with lindane for decades. Even if the level of soil contamination with lindane and its congeners decreases slowly every year, there are still soils with historical contamination for which decontamination measures should be envisaged.

The method developed is suitable for a complete screening and rapid determination of trace amounts of pesticide residues in routine analysis.

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