Determination of Organochlorine Pesticides in Some Vegetable Samples Using GC-MS

Cemile Ozcan

Department of Chemistry, Science and Art Faculty, Kirklareli University,
Kirklareli, Turkey

Abstract

This study investigated the level of pesticide pollution using marrow squash (*Cucurbita pepo* L.), cherry tomato (*Solanum lycopersicum var. cerasiforme*), banana pepper (*Capsicum annuum*), lettuce (*Lactuca sativa* L.), purslane (*Portulaca oleracea* L.), green beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), and onion (*Allium flavum* L.) in Kirklareli, Turkey. For this purpose, the concentrations of organochlorine pesticides were determined by gas chromatography-mass spectrometry (GC-MS). Vegetables were prepared for analysis by QuEChERS extraction method. Concentrations of Hexachlorocyclohexane (∑HCH), aldrin, heptachlor, dieldrin, endosulfan, methoxychlor and dichlorodiphenyltrichloroethane (∑DDT) were determined in vegetables collected from different points in Kirklareli. The LOD and LOQ were between 0.02-0.26 and 0.06-0.87 µg/L, respectively. The RSDs of the migration time ranged from 2.4% to 7.7% for the 18 analytes, indicating the good repeatability of the method. Recoveries of the spiked analytes in vegetables ranged from 83% to 104%, respectively. The obtained concentrations for pesticides in all vegetables studied were in the range ND-73.6 µg/kg.

Introduction

Kirklareli, one of the Black Sea provinces in northwestern Turkey, has a catchment area of 6,550 km^2. The use of pesticides in agricultural areas can increase toxicity and pollution in the environment. Pesticides are potential chemical pollutants extensively used for agricultural purposes due to their low cost and high effectiveness. They may accumulate in living creatures as decomposition. Counted among the most important sources of pesticides, such as agricultural applications and industrial pollutants of soil, are inorganic and organic fertilizers, fungicides, liming, sewage sludge, and irrigation waters [1, 2].

The use of pesticides in agricultural areas can increase the levels of toxicity and pollution in an ecological environment. Pesticides are potential chemical pollutants extensively used for agricultural purposes due to their low cost and high effectiveness. They may, for instance, accumulate in living creatures during decomposition, which can cause degradation to the environment. The most prevalent sources of pesticide come from agricultural and industrial applications, thus polluting the soil. Among the culprits are inorganic and organic fertilizers, fungicides, lime, sewage sludge, and irrigation water [3]. About half of all chemicals (whether natural or synthetic) chronically

*e-mail: cemilebal23@hotmail.com

tested in animal cancer tests at the maximum tolerated dose (MTD), were established as carcinogens [4].

Organochlorine pesticides in these vegetables may have accumulated in the environment in various ways such as through industry, fossil fuels, agriculture, and other human activities. The increasing agricultural and anthropogenic activities of humans has intensified the emissions of various pollutants into the environment, including toxic metals and pesticides [5]. Pollution by pesticides is a problem of increasing significance for ecological, evolutionary, nutritional, and human health [6-10]. Organochlorine pesticide compounds are known to create certain health problems such as cancer, immune system malfunction, and disruption of hormonal functions [11].

The extensively used and highly sensitive analytical techniques for determination and quantification of these pesticides at low concentrations in food samples are GC-MS, HPLC-MS, HPLC-DAD, and GC-ECD [12-15]. They can provide compound confirmation and detailed information on the structure of the compound analyzed, such as seperation and detection. The function of GC-MS is identification, quantification, and analysis of a compound [16]. The most widely used extraction techniques for pesticides in plants are microwave extraction (MAE) [17], ultrasound extraction (UAE) [18-21], Soxhlet [22], supercritical fluid extraction [23], and accelerated solvent extraction [24].

The aim of this study is to determine the concentrations of organochlorine pesticides in marrow squash (*Cucurbita pepo* L.), cherry tomato (*Solanum lycopersicum var. cerasiforme*), banana pepper (*Capsicum annuum*), lettuce (*Lactuca sativa* L.), purslane (*Portulaca oleracea* L.), green beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), and onion (*Allium flavum* L.) in the province of Kirklareli (Turkey) by GC-MS (pesticides including ∑BHC (α-BHC, β-BHC, γ-BHC, δ-BHC), ∑DDT (4,4'-DDD, 4,4'-DDE, 4,4'-DDT), α-Endosulfan, β-Endosulfan, Endosulfan sulfate, Heptachlor, Heptachlor-endo-epoxide, Aldrin, Dieldrin, Endrin aldehyde, Endrin ketone, Endrin, and Methoxychlor). Kirklareli is a location with intense industry and agriculture.

Materials and Methods

Instrumentation

The organochlorine pesticides were analyzed by GC-MS, using an Agilent 5975C MSD system and Agilent 7890A model in Kirklareli University. An HP-5 MS IU column (30 m x 250 µm x 0.25 µm) was used with Helium as the carrier gas. Injector temperature, split flow, and injection volume were 250ºC, 1 mL/min and 1 µL. The GC oven temperature was kept constant at 110ºC for 5 min to 320ºC at a rate of 8°C/min. MS fragmentation voltage was taken at 70 eV. Component identification was carried out using spectrometric electronic libraries (NIST) [25].

Standard and Reagents

The standard stock solutions of organochlorine pesticide mix 2, 2,000 mg/L in toluene, (including ∑BHC (α-BHC, β-BHC, γ-BHC, δ-BHC), ∑DDT (4,4'-DDD, 4,4'-DDE, 4,4'-DDT), α-Endosulfan, β-Endosulfan, Endosulfan sulfate, Heptachlor, Heptachlor-endo-epoxide, Aldrin, Dieldrin, Endrin aldehyde, Endrin ketone, Endrin and Methoxychlor) were purchased from Dr. Ehrenstorfer GmbH (a standard mixture solution containing 2,000 ng/µL, all 18). All solvents were HPLC-grade and other chemicals were analytical reagent grade. Double-distilled water (HPLC-grade, 18.2 ELGA) was used for all preparations.

Using 2,000 mg/L, stock was prepared using a mixture of 1 mg/kg (1000 µg/L) intermediate stocks. The standard solutions were prepared from a dilution of their intermediate stock standard solutions at concentrations of 100, 50, 25, 10, 5, and 1 µg/L in toluene. All solutions were stored in the dark at 4ºC. Working solutions were prepared by dilution of standard stock solution with toluene. Standard obtained through a purification system identification, quantification, and analysis of the compounds was done by GC-MS.

Sample Preparation for Determination of Organochlorine Pesticides

Marrow squash (*Cucurbita pepo* L.), cherry tomato (*Solanum lycopersicum var. cerasiforme*), banana pepper (*Capsicum annuum*), lettuce (*Lactuca sativa* L.), purslane (*Portulaca oleracea* L.), green beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.) and onion (*Allium flavum* L.) were utilized as samples in this study. About 1,000 g of plants were collected from a public bazaar in Kirklareli. All samples were homogeneously mixed and put into dry plastic bags (washed with distilled water).

After homogenization of plant samples, approximately 5 g portions were added to 10 ml dichloromethane in a 50 mL PTFE centrifuge tube. The sample was centrifuged in a 50 mL polypropylene centrifuge tube. Sodium acetate 1.5 g and anhydrous magnesium sulfate 6.0 g in supernatant were added to remove moisture and the compound was stirred for 3 min using the vortex. The sample was then centrifuged again at 5ºC and 10 min at 7,000 rpm. The supernatant extraction was followed by a clean-up step using a solid-phase extraction with 400 mg PSA, 1,200 mg MgSO$_4$ (extracted using an SPE cartridge) [26-28]. Pesticides in the sample extract were concentrated for dryness at 35ºC using a gentle nitrogen stream. This was dissolved in 1 mL of hexane and filtered through a syringe filter of 0.45 µm. Afterward, the extracts (1 µL injection volume) were quantified by gas chromatograph with mass detector (GC-MS).

Quantitation

Linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and recovery

Table 1. The linear regression (y = ax + b), correlation coefficients (R²), LOD (μg L⁻¹), LOQ (μg L⁻¹), MRL (μg kg⁻¹) and %RSD in pesticide standard solution range different levels and spiked recoveries and RSD of the method.

Pesticide	y = ax + b	R^2	LOD	LOQ	Recovery (%)	MRL	% RSD
α-HCH	1998,9x—9625.8	0.9944	0.13	0.43	85	10	7.2
β-HCH	868,8x—3487	0.9949	0.26	0.87	83	10	6.1
γ-HCH	803.77—3602.9	0.9927	0.26	0.87	92	10	6.6
δ-HCH	1567.2x—9449.6	0.9925	0.02	0.06	85	10	5.1
Heptachlor	983.7x—5414.6	0.9923	0.06	0.19	104	10	5.5
Heptachlor-endo-epoxide	577.48x—2803.9	0.9943	0.18	0.60	96	10	4.4
Aldrin (HHDN)	1541.9x—6458	0.9958	0.03	0.10	83	10	7.7
α-Endosulfan	492.08x—2610.4	0.9937	0.14	0.46	85	50	3.0
4,4'-DDE	4884.1x—24947	0.9932	0.03	0.10	83	50	3.1
4,4'-DDD	6000x—50000	0.9855	0.04	0.13	96	10	3.8
4,4'-DDT	3176.4x—18901	0.9978	0.03	0.10	94	10	2.4
Dieldrin	1922.6x—10689	0.9932	0.12	0.40	88	50	4.0
Endrin	247.79x—856.09	0.9955	0.12	0.40	85	50	6.9
β-Endosulfan	581.31x—2944.3	0.9937	0.03	0.10	88	10	3.8
Endrin-aldheyde	996.05x—7104.4	0.9901	0.06	0.21	92	50	4.9
Endosulfan-sulfate	535.88x—4463.1	0.9885	0.08	0.27	86	10	7.1
Endrin-ketone	815.84x—5530.5	0.9909	0.07	0.23	103	10	5.7
Methoxychlor	3566.4x—21247	0.9964	0.06	0.20	101	5	4.1

parameters were determined for the pesticides. An eight-level 1-100 μg/L calibration series was established with three analyses at each concentration level for determining linearity. The calibration curve was plotted automatically. LOD and LOQ had signal-to-noise ratios of 3 and 10, respectively [29].

Calibration graphs for pesticides were established in a range of 1-100 μg/L with correlation of coefficients from 0.9855 to 0.9978 for all analytes. The maximum residual limits (MRL) in foods of organochlorine pesticides, retention time (RT min), linear regression (y = ax + b), correlation coefficients (R2), LODs, LOQs, and %RSDs are shown in Table 1. Limits of detection ranged from 0.02 to 0.26 μg/L for organochlorine pesticides. Limits of quantification ranged from 0.06 to 0.87 μg/L for pesticides. The RSDs percentage of migration time ranged from 2.4 to 7.7 for the analytes, indicating good repeatability of the method. The procedure was checked for recovery efficiencies by analyzing uncontaminated vegetable samples spiked with organochlorine pesticide standard (n = 5). The spike blanks, solvent blanks, and duplicate samples were analyzed in each of 10 samples. In addition, surrogate standard was added to each of the samples to monitor procedural performance and matrix effects. The recoveries of surrogate spiked in plant samples ranged from 83% to 104% (Table 1).

Results and Conclusion

In the article we studied the content of organochlorine pesticides in marrow squash (*Cucurbita pepo* L.), cherry tomato (*Solanum lycopersicum var. cerasiforme*), banana pepper (*Capsicum annuum*), lettuce (*Lactuca sativa* L.), purslane (*Portulaca oleracea* L.), green beans (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), and onion (*Allium flavum* L.). This could possibly have been the result of industrial pollution, as pesticides could easily have been absorbed into plant leaves from the air or soil. The presence of organochlorine pesticide residues in foods has been a problem for decades. Residues of organochlorine pesticides were found in all food samples analyzed. However, their concentrations were many times lower than the permissible limits in place in other countries. Organochlorine pesticides are resistant to degradation, which allows them to persist in the environment for a long time and become widespread via atmospheric and water transport mechanisms [30-32].

In this study, organochlorine pesticides in vegetables were determined and analyzed by GC-MS. The optimized conditions were found to be 1 ml/min for flow rate in the mobile phase, 1 μl for injection volume, and 70 V for fragmentation potential. The optimized method was applied for the determination of organochlorine pesticide in vegetable samples. The results obtained for vegetables

Table 2. Concentration of organohlorine pesticides detection in vegetables (µg kg⁻¹).

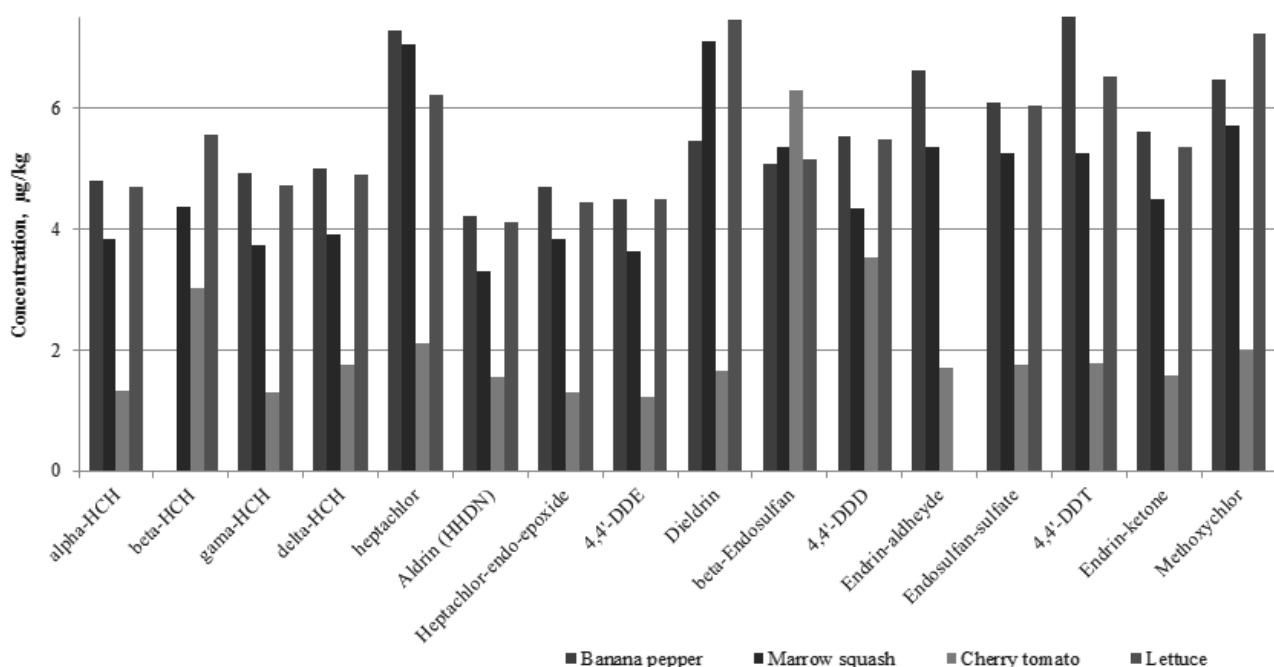| Pesticides	Banana pepper	Marrow squash	Cherry tomato	Lettuce	Onion	Purslane	Green beans	Cucumber
α-HCH	4.80±0.09	3.82±0.03	1.31±0.01	4.70±0.04	3.84±0.01	4.02±0.03	3.11±0.02	4.06±0.01
β-HCH	ND	4.36±0.07	3.02±0.09	5.55±0.05	ND*	4.51±0.03	ND	10.8±0.9
γ-HCH	4.91±0.11	3.73±0.08	1.30±0.01	4.71±0.09	3.84±0.02	3.86±0.02	3.89±0.09	10.9±0.9
δ-HCH	5.00±0.08	3.90±0.07	1.76±0.01	4.89±0.05	3.97±0.02	4.00±0.09	3.98±0.09	4.25±0.02
Heptachlor	7.28±0.11	7.05±0.13	2.12±0.02	6.21±0.08	9.65±0.11	10.2±0.5	7.70±0.11	10.7±0.8
Heptachlor-endo-epoxide	4.21±0.08	3.29±0.09	1.56±0.05	4.12±0.04	3.33±0.08	3.35±0.04	3.33±0.03	21.2±1.1
Aldrin (HHDN)	4.70±0.06	3.84±0.08	1.29±0.02	4.45±0.03	4.18±0.03	4.16±0.06	3.82±0.03	14.2±1.1
α-Endosulfan	ND	ND	ND	ND	ND	ND	ND	9.25±0.11
4,4'-DDE	4.50±0.09	3.63±0.03	1.22±0.09	4.50±0.03	3.83±0.03	3.69±0.04	3.81±0.05	4.09±0.11
4,4'-DDD	32.7±1.3	59.9±3.4	11.8±0.9	18.6±1.1	36.4±2.2	70.2±5.4	32.1±1.9	73.6±5.7
4,4'-DDT	5.45±0.09	7.10±0.17	1.64±0.03	7.45±0.09	5.89±0.08	7.49±0.11	5.14±0.05	5.10±0.05
Dieldrin	5.08±0.11	5.36±0.09	6.29±0.08	5.15±0.08	5.02±0.05	6.76±0.09	4.93±0.09	4.48±0.08
Endrin	5.53±0.15	4.35±0.11	3.52±0.03	5.47±0.06	4.46±0.05	4.56±0.04	4.48±0.03	4.55±0.04
β-Endosulfan	6.63±0.11	5.36±0.09	1.70±0.02	ND	5.51±0.03	6.78±0.04	4.41±0.05	ND
Endrin-aldheyde	6.10±0.18	5.25±0.14	1.74±0.02	6.03±0.09	4.95±0.08	5.49±0.05	5.65±0.05	5.34±0.08
Endosulfan-sulfate	7.58±0.09	5.24±0.07	1.79±0.01	6.53±0.06	5.33±0.05	5.41±0.02	5.67±0.05	5.57±0.02
Endrin-ketone	5.60±0.11	4.49±0.09	1.58±0.02	5.36±0.06	4.78±0.04	4.79±0.05	4.51±0.06	4.62±0.04
Methoxychlor	6.47±0.04	5.72±0.04	1.98±0.01	7.22±0.05	5.87±0.05	5.86±0.01	4.64±0.01	5.91±0.02

*ND = not detected

Fig. 1. Concentration graph of organochlorine pesticides in banana pepper, marrow squash, cherry tomato, lettuce.
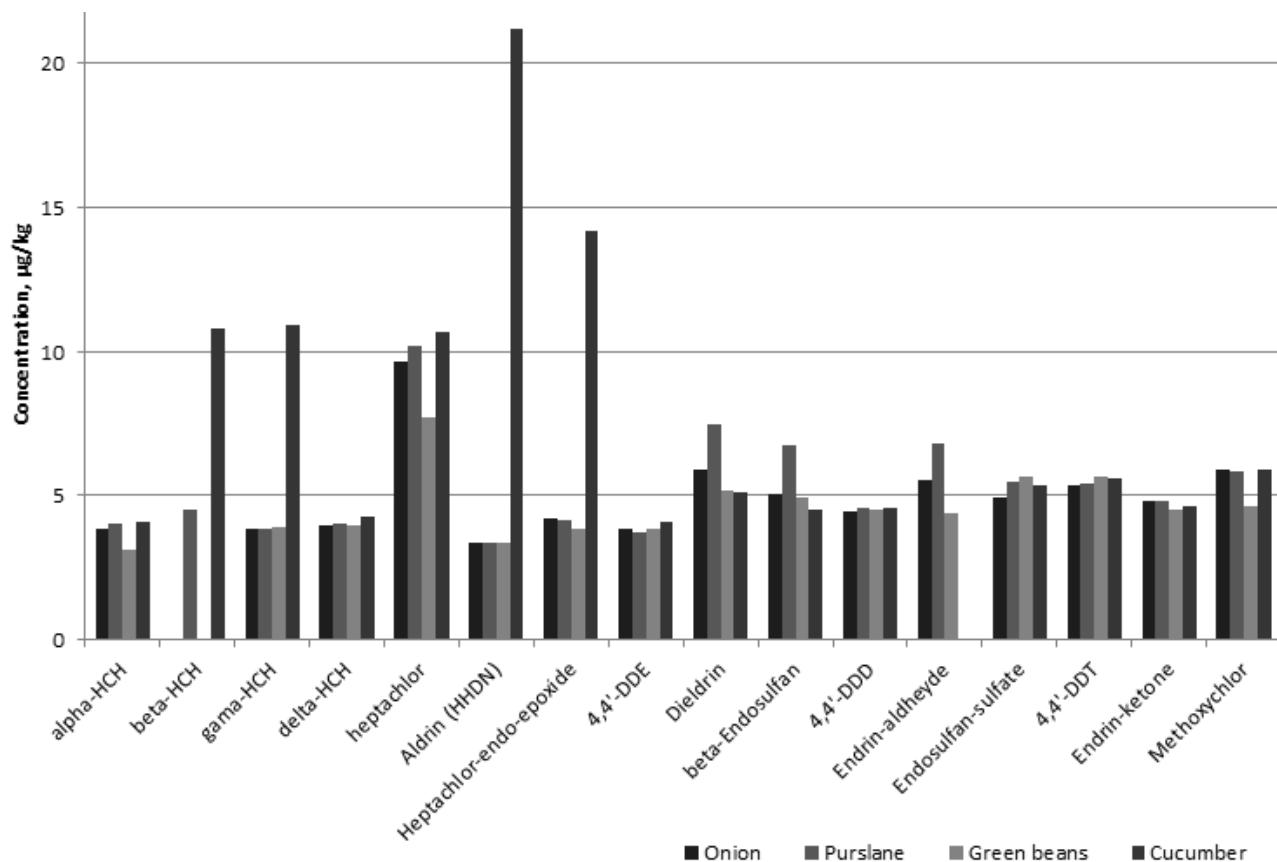
Fig. 2. Concentration graph of organochlorine pesticides in onion, purslane, green beans, cucumber.

are summarized in Table 2 and Fig. 1. The target pesticides were analyzed with good reproducibility and low detection limits using gas chromatography-mass spectrometry with electron impact ionization-possitive mode (GC-MS-EI (+)). After elution-cleaning with SPE cartridge for pesticides in vegetables we developed a simultaneous method for analysis by GC-MS. The main advantage of this method is that extraction and clean-up are performed in less time with a low volume of solvent. Additionally, the proposed method is a sensitive, reproducible, and reliable alternative to the normally used methods; moreover, it is inexpensive, easy, and rapid (QuEChERS method). This study demonstrated that the method using QuEChERS and GC-MS is very effective in analyzing the organochlorine pesticides in the vegetable samples.

The concentrations of α-HCH, β-HCH, γ-HCH, δ-HCH, Heptachlor, Aldrin (HHDN), Heptachlor-endo-epoxide, α-Endosulfan, 4,4'-DDE, Endrin, Dieldrin, β-Endosulfan, 4,4'-DDD, Endrin-aldheyde, Endosulfan sulfate, 4,4' DDT, Endrin-ketone, and Methoxychlor in vegetable samples were found among 1.31 to 4.80 μg/kg, ND to 10.8 μg/kg, 1.30 to 10.8 μg/kg, 1.76 to 5.00 μg/kg, 2.12 to 10.7 μg/kg, 1.56 to 21.2 μg/kg, 1.29 to 14.2 μg/kg, ND to 9.25 μg/kg, 1.22 to 4.50 μg/kg, 11.8 to 73.6 μg/kg, 1.64 to 7.49 μg/kg, 4.48 to 6.76 μg/kg, 3.52 to 5.53 μg/kg, ND to 6.78 μg/kg, 1.74 to 6.10 μg/kg, 1.79 to 7.58 μg/kg, 1.58 to 5.60 μg/kg and 1.98 to 7.22 μg/kg, respectively (Table 2 and Figs 1-2).

Concentrations of α-Endosulfan were not determined in vegetable samples except for cucumber (9.25 μg/kg). In addition, the amount of α-HCH, β-BHC, Heptachlor, Aldrin, Heptachlor-endo-epoxide, Endrin, and Methoxychlor in cucumber were determined over the MRL border value. The concentrations of Endrin in all samples were found 1.2 to 7.4 times greater than the MRL value. Therefore, over MRL value of Heptachlor we found 1.02 to 1.07 times greater in purslane and cucumber, respectively. The amount of Endrin-aldheyde was not determined in lettuce and cucumber. The concentrations of organochlorine pesticides in cherry tomato were found below the MRL values, except for endrin. Eventually, this could have been the result of industrial pollution, as pesticides could have been easily absorbed translocated in plant leaves from air or soil. But it may have been also a result of eating the leaves of animals. In addition, the relative abundances of individual percentage graph of organochlorine pesticides in vegetables are shown in Fig. 3.

Organochlorine pesticides are of global concern because of their widespread occurrence, bioaccumulation, persistence, and toxicity. So for the improment of the world and food safety, sources of pollution should be identified and eliminated. Such information is valuable to health care professionals, researchers, and food manufacturers in preparing nutritious products. Levels of organochlorine pesticides in some products may also be
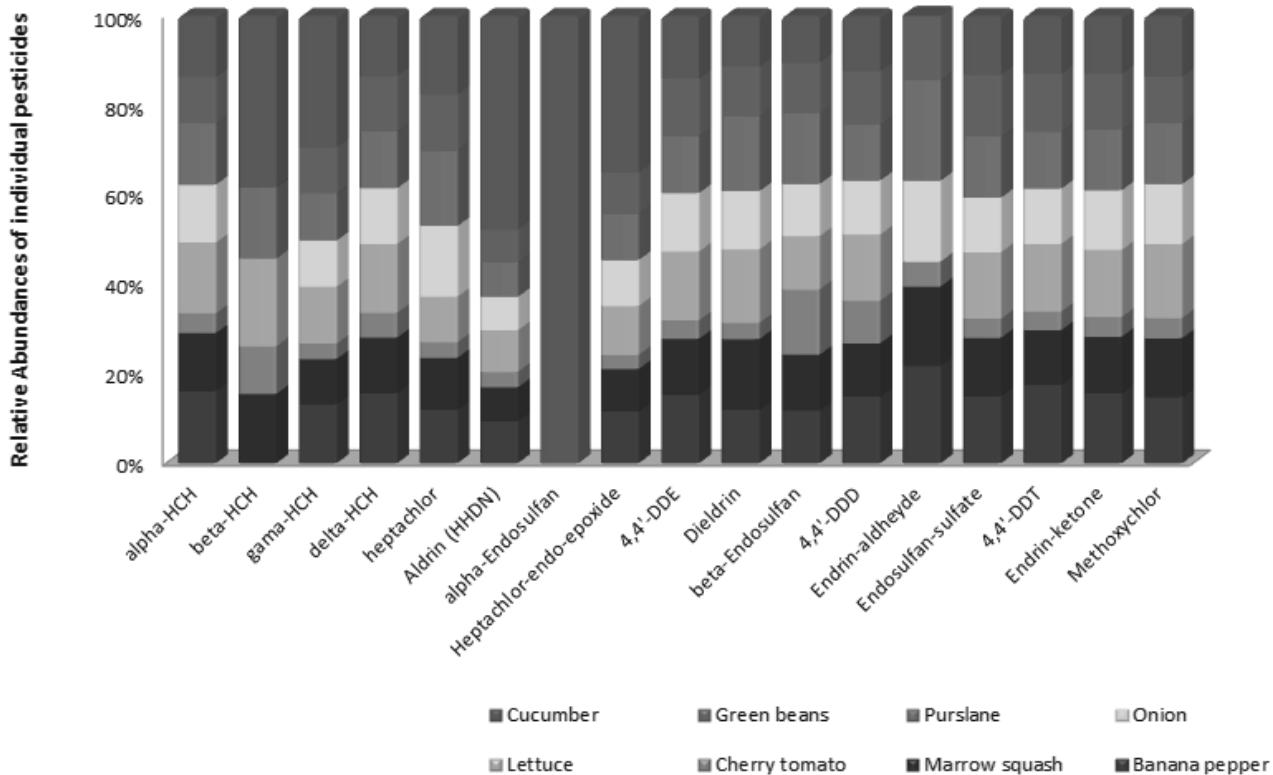
Fig. 3. The relative abundances of individual percentages graph of organochlorine pesticides in vegetables.

unexpected and hence informative and may lead to further analysis and research. Most farmers in Turkey rely on their own experience and on pesticide sellers to help them select the appropriate pesticide. Overall, these findings provide insight into the habits of pesticide use during vegetable cultivation by Kirklareli farmers. Most do not rely on advertisements, extension officers, or government agencies for guidelines on proper pesticide use. While levels have declined in recent years in many countries, a number of these chemicals (called persistent organic pollutants), persist in our environment.

Therefore, a multi-residue method has been developed and successfully validated for the rapid detection of 18 pesticide residues of the same chemical classes in vegetable samples by GC-MS. The high pesticide levels in some of the samples suggest that these pesticides are being used indiscriminately. This could cause considerable health problems – not only to the public but also to farmers. Therefore, training and information on pesticide use and their residues might be established. This research is required to further elucidate the pollution sources of organochlorine pesticides in the region. In addition, more studies on accumulation of organochlorine pesticides are required to accomplish a comprehensive ecological risk assessment.

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