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HUMANS IN THE ENVIRONMENT

COMPARATIVE ANALYSIS AND ASSESSMENT OF PESTICIDE RESIDUES FROM FIELD-GROWN TOMATOES

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Abstract

This paper aims to provide a perspective on the possibility that consumers could be exposed to pesticides, by estimating quantities of pesticide residues in tomatoes from field crops. Twelve pesticides, 7 fungicides and 5 insecticides respectively have been analyzed in field-grown tomatoes samples within the Mureş Fitosanitary Unit (Romania) by gas chromatography coupled with mass spectrometer with flight time, using the multi-residual method. This study highlights the necessity of pesticides monitoring in field-grown tomatoes, to warrant that their levels do not pose risks to human health. A number of three treatments were applied at the recommended normal doses to field-grown tomatoes samples at an interval of 20 days during 2012. The final residual amounts of pesticides in tomatoes were below the MRLs, except for chlorothalonil and bifenthrin. The analysis of health risk estimates based on consumption data in Europe and Romania revealed that the target pesticides do not pose a risk to human health.

Keywords: degradation, pesticides, food consumption, human health risk assessment

1. Introduction

1.1. Short history of pesticides use

Agriculture began to be practiced about 10,000 years ago in the Fertile Crescent of Mesopotamia as the population became more established and farming turn out to be to be the way of life. Likewise, rice and millet were

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cultivated in China, even as about 7,500 years ago rice and sorghum were farmed in a region of Africa, the Sahel [1]. During time, the farmed crops began to endure from pests and diseases causing high production breakdown, so that people started to find ways of overcome these problems. About 4500 years ago Sumerians who used sulphur compounds as insecticides to control insects and mites, whilst about 3200 years ago the Chinese were using mercury and arsenical compounds for controlling body lice [2]. Persians used the powder of pyrethrum (derived from the dried flowers of Chrysanthemum cinerariaefolium 'Pyrethrum daisies') as an insecticide for over 2000 years to protect stored grain. In the 1600s, ants were controlled with mixtures of honey and arsenic [1].

Table 1. Main milestones in the modern era of pesticides development [4].

Period	Example	Source	Characteristics
1800s-1920s	Early organics, nitrophenols, chlorophenols, creosote, naphthalene, petroleum oils	Organic chemistry, by-products of coal gas production etc.	Often lacked specificity and were toxic to users or nontarget organisms
1954-1955	Chlorinated organics, DDT, hexachlorocyclohexane (HCCH), chlorinated cyclodienes	Organic synthesis	Persistent, good selectivity, good agricultural properties, good public health performance, resistance, harmful ecological effects
1945-1970	Cholinesterase inhibitors, organophosporous compounds, carbamates	Organic synthesis, good use of structure-activity relationships	Lower persistence, some user toxicity, some environmental problems
1970-1985	Synthetic pyrethroids, juvenile hormones mimics, biological pesticides	Refinement of structure-activity relationship, new target systems	Some lack of selectivity, resistance, costs, and variable persistence
1985-	Genetically engineered organisms	Transfer of genes from biological pesticides to other organisms and into beneficial plants and animals Genetic alteration of plats to resist non-target effects of pesticides	Possible problems with mutations and escapes, disruption of microbiological ecology, monopoly

The start of the 19th century found scientists working for a better understanding of fungi behaviour, and sulphur compounds were developed as fungicides [3, 4]. In 1807, copper sulphate solution was used to control bunt disease in wheat. In the late 19th century, arsenic compounds were introduced to control insect attack on fruit and vegetable crops. Paris green, developed in 1867 from a copper & arsenic mixture, was applied broadly to control the potato beetle and protect grapes from insect damage [2]. Inorganic compounds, such as sodium chlorate and sulphuric acid, or organic chemicals derived from natural sources were still extensively used in pest control up to 1940s. Chemical research was directed toward cheap chemicals with persistence in sunlight and low toxicity to man, but with the ability to kill insect pests rapidly. In 1938 Muller demonstrated that DDT would certainly meet these requirements. Its accessibility for the period of World War II led to early use as a 10% dust on humans, for example in Naples, to repress a typhus outbreak [5].

After the World War II, the production of synthetic pesticides accelerated, with the discovery of the effects of DDT, BHC, aldrin, dieldrin, endrin, chlordane, parathion, captan and 2,4-D [K.S. Delaplane, *Pesticide Usage in the United States: History, Benefits, Risks, and Trends*, 1996, online at http://ipm.ncsu.edu/safety/factsheets/pestuse.pdf]. A new chemical age began, and farmers were the main reason for the new age. By 1952, there were almost 10,000 separate new pesticide products registered with the USDA [B. Ganzel, *Farming in the 1940s. The Chemical Age Dawns in Agriculture*, online at http://www.livinghistoryfarm.org/farminginthe40s/pests_01.html].

A chronology of the modern era of pesticide development is shown in Table 1 [4].

Pesticides have certainly facilitated to increase agricultural production and control vectors of disease over the past decades. Farmers consider pesticides as an indispensable means to guarantee that they can preserve production of crops of quality and quantity to satisfy an increasing human population. Latest approximations of crop losses due to insect pests, diseases generated by a variety of pathogens and rivalry from weeds, in spite of present control practices, range from 26 to 40% for major crops, with weeds causing the highest potential loss [5, 6]. Unfortunately, some pests became genetically resistant to pesticides under constant chemical pressure, while non-target plants and animals were harmed, and pesticide residues appeared in unexpected places [5]. In 1962, Rachel Carson, in her well-known book, ‘Silent spring’, due to which public confidence in pesticide use was shaken, said that “*…. man is a part of nature, and his war against nature is inevitably a war against himself*” [7].

Nowadays, pesticides are among the most extensively used chemicals in the world, being also among the most hazardous compounds to the environment and humans. The pathways of humans’ exposure to pesticides are numerous, since pesticide residues can be found virtually everywhere. Pesticides have also posed a number of problems for agriculture, since they kill beneficial insects and assist in developing pesticide-resistant pests [3]. In this context, a regulatory framework was developed during time, but, despite of this, the adverse impacts of agricultural pesticide use continue to be serious concern. Simultaneously, incomplete knowledge of existing and potential effects of pesticides continues to influence people perception on the dangers and benefits of pesticide use [8, 9].

1.2. Pesticides in environment and foods

Pesticides are chemical compounds with a structure and mode of action which have rendered them serious pollutants of the environment in general. Some pesticides (in particular organochlorine compounds) are classified as persistent organic pollutants (POPs), with a good ability to volatilize and travel long distances through atmosphere, and then deposited in remote regions. They also have the ability to accumulate and biomagnify, and bioconcentrate up to

70,000 times their original concentration [4]. They are not biodegradable or very little biodegradable. The pesticide degradation needs particular reaction conditions] [10-14].

The U.S. Environmental Protection Agency (USEPA) defines pesticides as [2, http://www.epa.gov/kidshometour/pest.htm]: "*A pesticide is a chemical used to prevent, destroy, or repel pests. Pests can be insects, mice and other animals, weeds, fungi, or microorganisms such as bacteria and viruses. Some examples of pests are termites causing damage to our homes, dandelions in the lawn, and fleas on our dogs and cats. Pesticides also are used to kill organisms that can cause diseases*". USEPA [http://www.epa.gov/kidshometour/pest.htm] made a classification of pesticides, according to their types, purposes and uses, as presented in Table 2.

The Food and Agriculture Organization (FAO) has defined pesticide as [http://www.fao.org/docrep/018/a0220e/a0220e00.pdf]: "*Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport*".

Pesticides are designed to be toxic to living bodies, so that they inevitably pose risks and need to be used safely and disposed properly [15-20]. During years, the intensive use of pesticides led to serious environmental problems such as perturbation of the natural balance, widespread pest resistance, environmental pollution, hazards to non-target organisms and wildlife, and hazards to humans. Unfortunately, due to their impacts, the use of pesticide contributed to biodiversity loss, along with habitat loss and climate change, since they affect wildlife directly and indirectly by means of food sources [16, 17, 21-23].

The use of pesticide in agriculture is the subject to permanent monitoring due to possible risk for human health [24-27]. Intake of active ingredients through food ingestion has been shown to be up to five orders of magnitude higher than other exposure routes like air inhalation and ingestion of drinking water. Furthermore, because fruits and vegetables are consumed raw or semi-processed, it is expected to contain higher pesticide residue levels than other food groups like milk or meat [28, 29]. The consumption of pesticides contaminated food can seriously deplete some essential nutrients in the body that are further responsible for serious damage associated with health risks [9, 22, 23, 27, 30-34]. Fresh vegetables and fruits are representing some of the most important commodities in human diet due to the presence of meaningfully amounts of nutrients and minerals. However, at the same time, due to agricultural practices they can also turn out to be a source of toxic substances such as pesticides [35-38].

Table 2. Main classes of pesticides and areas of application [http://www.epa.gov/kidshometour/pest.htm].

Class of pesticides	Main use
Algicides	Control algae in swimming pools and water tanks
Antimicrobials	Kill microorganisms (such as bacteria and viruses)
Attractants	Attractants are traps containing a pesticide and food to lure insects or rodents inside. However, food is not a pesticide even though it certainly attracts pests
Disinfectants and sanitizers	Kill disease-producing microorganisms in the kitchen and bathroom
Fumigants	Produce gas or vapor intended to destroy pests in the house or in the ground
Fungicides	Kill fungi (including blights, mildews, molds, and rusts)
Herbicides	Kill weeds
Insecticides	Kill insects and other arthropods
Miticides	Kill mites that feed on plants and animals
Microbial pesticides	Microorganisms that kill or inhibit pests, including insects or other microorganisms. Sometimes microorganisms get rid of pests simply by growing larger in numbers, using up the pests' food supply, and invading the pests' environment
Molluscicides	Kill snails and slugs
Nematicides	Kill nematodes (microscopic, worm-like organisms that feed on plant roots)
Pheromones	Biochemicals used to disrupt the mating behavior of insects
Repellents	Repel pests, including insects (such as mosquitoes) and birds
Rodenticides	Control mice and other rodents

Maximum Residue Limits (MRLs) encourage food safety by restricting the concentration of a residue allowed on a commodity, and by limiting the type of commodity on which it is allowed. The establishment of MRLs is based on good agricultural practices (GAP) data on food derived from commodities. MRLs are not toxicological limits, but they must be toxicologically acceptable. Exceeded MRLs are strong indicators of violations of GAP [39-41].

Tomatoes represent one of the most appreciated and consumed vegetables. However, the intensive crops could suffer by high infestation of the tomatoes cultures by pests and diseases, causing major losses and depreciations of the quality of the vegetables [42, 43]: this is why the pesticides are used on a large scale to control the infestation [44-46]. The chemical protection used in the case of the tomatoes is usually done by using two or three treatments with different types of pesticides.

Table 3. Target pesticides commercial name and use.

Products commercial name	**Active substance**	**Chemical Group**	**Recommended dose (%)**	**Use***	**MRLs** (mg/kg)**
Merpan 80 WDG (grains dispersible in water)	80% captan	Phthalimide	0.15	Fungicide	<2
Shavit F 72 WDG (grains dispersible in water)	70% folpet	Phthalimide	0.2	Fungicide	<2
	2% triadimenol	Triazole			<1
Systhane Forte (soluble concentrate)	240 g/L myclobutanil	Triazole	0.02	Fungicide	<0.3
Bravo 500 SC (concentrated suspension)	500 g/L chlorothalonil	Chloronitrile	0.2	Fungicide	<2
Orius 25 EW (emulsion – oil in water)	250 g/L tebuconazole	Triazole	0.05	Fungicide	<1
Ridomil Gold MZ 68 WG (grains dispersible in water)	4% metalaxyl-M	Phenylamide	0.25	Fungicide	<0.2
	64% mancozeb	Ditiocarbamati			
Nurelle D 50/500 EC (emulsifiable concentrate)	500 g/L Chlorpyrifos ethyl	Organophosphate	0.06	Insecticide	<0.5
	50 g/L cypermethrin	Pyrethroids			
Talstar 10 EC (emulsifiable concentrate)	100 g/L bifenthrin	Pyrethroids	0.05	Insecticide	<0.2
Fastac 10 EC (emulsifiable concentrate)	100 g/L alfa-cypermethrin	Pyrethroids	0.02	Insecticide	<0.5
Karate Zeon (concentrated suspension)	50 g/L lambda Cyhalothrin	Pyrethroids	0.02	Insecticide	<0.1
Decis 2.5 EC (emulsifiable concentrate)	25 g/L deltamethrin	Pyrethroids	0.05	Insecticide	<0.3

* [48]
***MRLs* - maximum residue limits set by European Union legislation [https://secure.pesticides.gov.uk/MRLs]

The constant usage of the pesticides increases the possibility of finding multiple residuals of these compounds in the tomatoes that result, beyond the legal prescribed limits, creating a significant risk for the human health [45, 46].

The pesticides residuals from the food products have to be low and under the MRLs established by each country [24, 44, 46, 47]. Table 3 includes the MRLs established by the European Union, for each of the analyzed pesticides.

Analysis of multiple pesticide residues in fruits and vegetables is often a time-consuming, labour-intensive, and expensive process due to the complexity of the many analytes and matrices involved [49]. A large variety of methods have been used in the determination of different pesticides in these foods. The most frequently used technique for analysis of pesticide residues in fruits and vegetables is gas chromatography with different selective detectors as flame photometric (FPD) (Ueno, et al, 2003) [50, 51], pulsed flame photometric (PFPD) [52], nitrogen–phosphorus (NPD) [53], and electron-capture detectors (ECD) ([54, 55]. Numerous method use gas chromatography coupled with mass spectrometry (GC-MSD) [51, 56, 57], due to the possibility of confirming pesticide identity in these matrices.

In this context, the main objectives of this paper consists in the investigation of the occurrence and distribution of residues of 11 pesticides in tomatoes cultivated within the Mureş Fitosanitary Unit (Romania), during a vegetation period, using a rapid multi-residue method for the analysis based on by gas chromatography with mass-selective detection (GC-MSD). Human health risk estimations due to pesticides presence in tomatoes at harvest has been done based on food consumption rate for vegetables in Europe and in Romania.

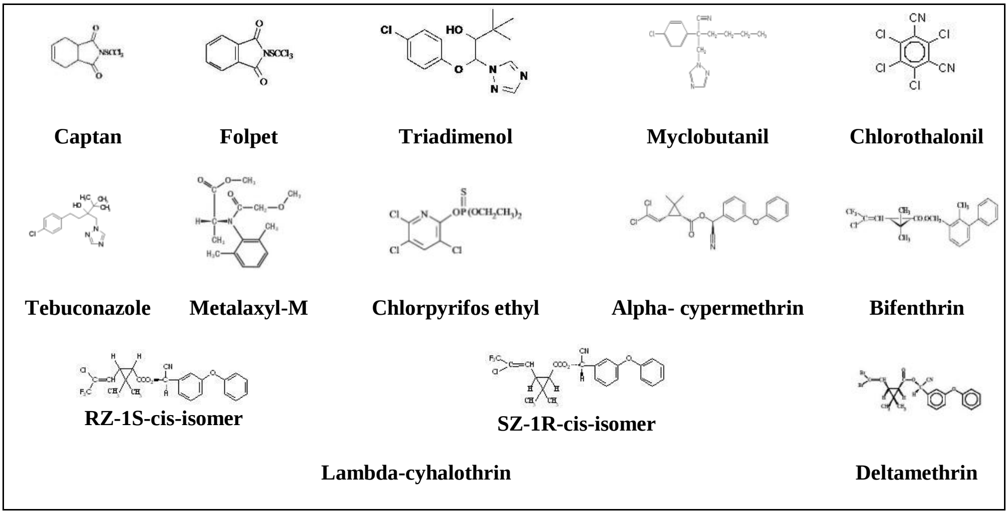


Figure 1. Chemical structure of the selected pesticides for the present study.

2. Materials and methods

2.1. Chemicals and solvents

Reference pesticide analytical standards were purchased from Chem Service (West Chester, SUA) and Sigma Aldrich Laborchemikalien GmbH

(Seelze, Germany) with the purity certified between 95.1% and 99.7%. Acetone, petroleum ether, dichloromethane, toluene and isooctane were Super Purity Solvents supplied by Fluka& Riedel-deHaën (Sigma-Aldrich, UK). The distilled water used was provided by a Thermo Scientific TKA system (Niederelbert Germany). All samples were stored in a refrigerator at 4^0C until further use. The standard solutions were dissolved in toluene and later stored in a refrigerator at 4^0C. The commercial pesticide products used in the study (Figure 1, Table 3) were purchased from Dafcochim SRL (Tg. Mures, Romania) and Chemark Rom SRL (Tg. Mures, Romania).

2.2. Gas chromatography-mass spectrometry analysis

The pesticide residues were analyzed by a gas chromatograph coupled with a mass spectrometer with flight time, CG*GC-TOF-MS Pegasus 4.21 (LECO, SUA). The system consists of an Agilent 7890 gas chromatograph with 2 ovens. The conditions used for the gas chromatography analysis were: capillary column Rxi-Ms (30m*0.25mm*0.25µm) – main oven, and BPX-50 (1.6m*0.1mm*0.1µm) – secondary oven. Helium was used as carrier gas and make-up gas at a flow rate of 1.0 mL/min. The injector temperature was set at 250^0C. The oven temperature was programmed as follows: main oven, 70^0C held for 1 min; ramp at 20^0C/min to 140^0C, held for 1min; ramp at 5^0C /min to 310^0C, held for 4 min; secondary oven, 95^0C held for 1 min; ramp at 20^0C/min to 165^0C, held for 1min; ramp at 5^0C /min to 330^0C, held for 4 min. The injection volume of the GC was 1.0µL. The mass spectrometer operated in the following conditions: ion source temperature, 220^0C; ionization mode EI, 70 eV; detector Voltage 1800; Start mass 40; End start 450; Acquisition Rate *spectre/second, 5; temperature of transfer, 280^0C; time of analysis, 43 min. The high-performance auto sampler software enables the syringe washing with several solvents (at least four different solvents in the same washing phase) to end the contamination.

Table 4. Conditions for pesticide identification.

Pesticides	**$t_{R,}$ (min)**	**MS Selected ions (m/z)**
Captan	16.38	117, 149, 264
Folpet	16.31	104, 260, 262
Triadimenol	16.14	112, 128, 168
Myclobutanil	17.29	179, 181, 245
Chlorothalonil	12.39	109, 264, 268
Tebuconazole	18.24	125, 250, 252
Metalaxyl-M	13.50	132, 160, 206
Chlorpyrifos ethyl	14.51	197, 199, 314
Bifenthrin	19.09	165, 181, 182
Alpha-cypermethrin	21.33	127, 163, 165
Lambda-cyhalothrin	19.47	141, 181, 208
Deltamethrin	23.50	135, 173, 350

The major ions (m/z) and retention time (t_R) were considered for pesticide identification (Table 4). To evaluate the efficiency of the analytical procedures, a recovery assay was accomplished. The method was validated by determining the limits of quantification (LOQ), recovery percentages and coefficient of variation. In all cases, regression coefficients (R^2) resulted were higher than 0.99. Recoveries were found from 80% to 110%.

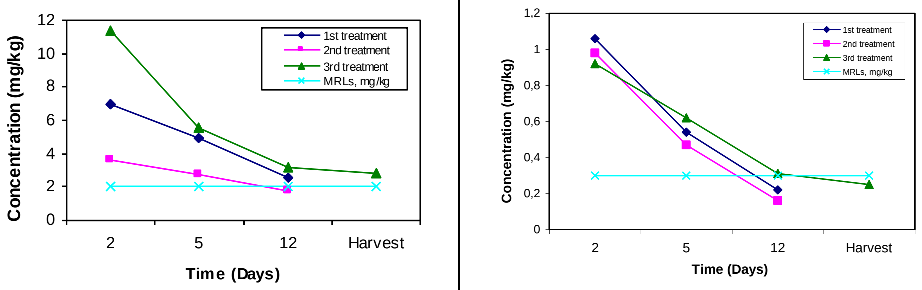
2.3. Field experiments and sample preparation

A field survey was conducted within the Mureş Fitosanitary Unit (Romania) during 2012. Tomatoes plants were transplanted in the open field in mid May 2012, on two rows to 0.6 m wide and 0.3 m distance between tomato plants on the same row, at a density of 45-50 thousand plants/ha. A number of 3 treatments were applied to field-grown tomatoes samples at an interval of 20 days between treatments, in the period 3rd of August 2012 and 29th of September 2012. Buffer areas have been ensured between the tomatoes plants subjected to the experiment. Each applied treatment contains a fungicide and an insecticide. Fungicides were based on *Chlorothalonil, Captan, Folpet, Tebuconazole, Triadimenol, Myclobutanil, Metalaxyl-M* and insecticides were based on *Deltamethrin, Alpha-cypermethrin, Lambda-cyhalothrin, Chlorpyrifos ethyl and Biphenthrin.* Each sample contains tomatoes of approximately the same size, randomly picked, after 2, 5 and 12 days following the application of the treatment. All tomatoes samples were put in sterile bags and stored at 4^0C until further use.

A known quantity of whole tomatoes picked from different areas of the plant were cut in quarters and mixed at a speed of 6,000 rpm. From the mixed sample, 15g were weighed. For the extraction procedure dichloro-methane, acetone and petrol ether were used as solvents. The vial which contains the sample and the solvents was blended in an ultraturax shaker at 15,000 rpm and centrifuged at 4,000 rot/min. In order to ensure an advanced homogenization of the sample, 15 mL were pipetted into a Heidelph balloon of 100 mL attached to a Heidolph rotoevaporator coupled with a vacuum pump at a rotation of the balloon of 120 rpm. After solvent evaporation, the sample was sonicated for 5 minutes, at room temperature and analyzed through the GC-MS (CG*GC-TOF-MS Pegasus 4.21).

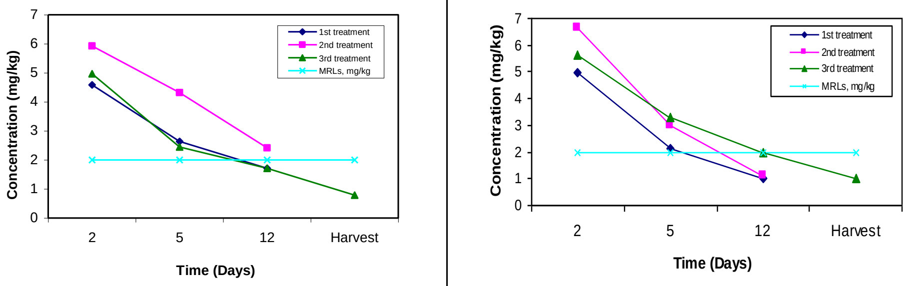
2.4. Human health risk assessment

The human health risk estimation due to pesticides presence in tomatoes at harvest has been done based on food consumption rate for vegetables in Europe, 0.166 kg/person/day [58] and in Romania, 0.284 kg/person/day [59]. The estimated lifetime exposure dose (mg/kg/day) was obtained by multiplying the residual pesticide concentration (mg/kg) in the tomatoes samples times the food consumption rate (kg/day), and dividing the product by the body weight (kg) [58]. The study used the U.S Environmental Protection Agency's guidelines: (a) a hypothetical body weights 70 kg for adults and (b) maximum absorption rate is 100% and bioavailability rate is 100% [60, 61]. The hazard indices for adults were calculated as the ratio between pesticide exposure doses, and the reference doses which are considered to be safe levels of exposure over the lifetime [60].



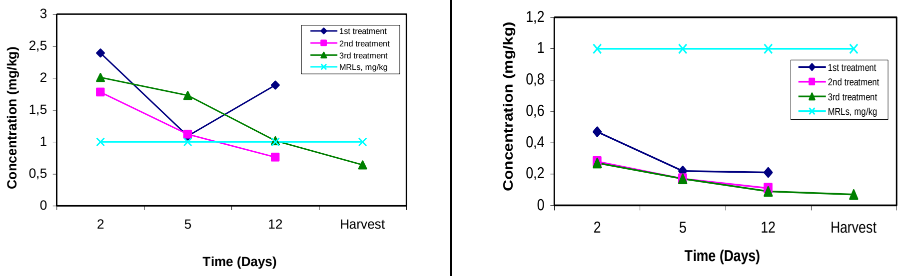
(a) *Chlorothalonil* degradation

(b) *Myclobutanil* degradation



(c) *Captan* degradation

(d) *Folpet* degradation



(e) *Tebuconazole* degradation

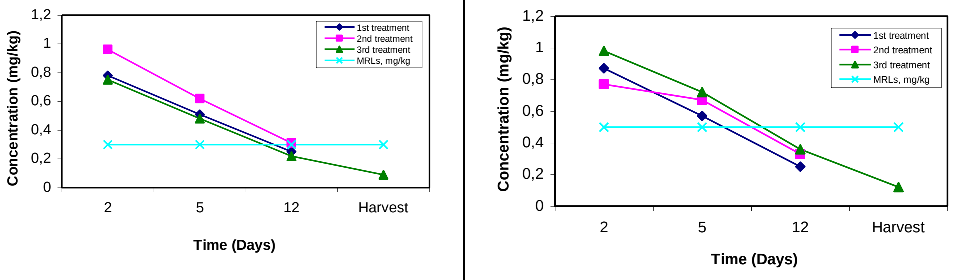
(f) *Triadimenol* degradation

Figure 2. Dynamics of the degradation of *Chlorothalonil*, *Myclobutail*, *Captan*, *Folpet*, *Tebuconazole*, *Triadimenol* in tomatoes

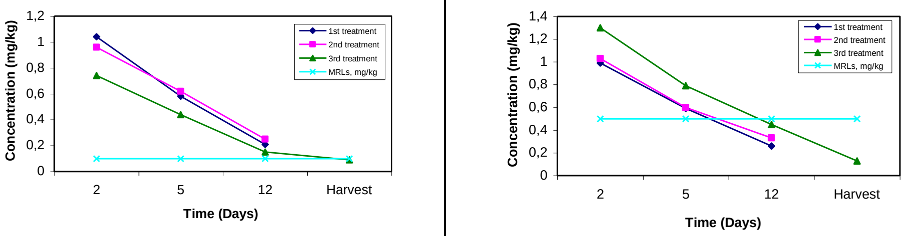
3. Results and discussions

3.1. Pesticides degradation in tomatoes

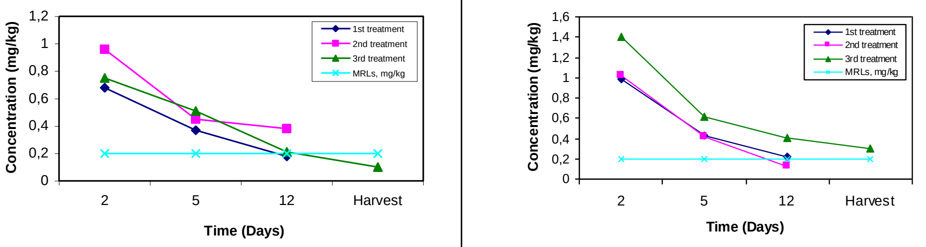
The pathway of pesticides degradation has been monitored for each compound and during various episodes of treatment (Figures 2, 3). In the case of all three treatments carried out with Chlorothalonil (Figure 2a) it was found that the MRLs (2 mg/kg) have been overrun. As it can be seen in Figure 2a, after 12 days, and the third treatment applied the chlorothalonil residues were higher than MRLs, reaching 2.83 mg/kg at harvesting.



(a) *Deltamethrin* degradation (b) *Alpha - cypermethrin* degradation



(c) *Lambda-cyhalothrin* in tomatoes (d) *Chlorpyrifos ethyl* degradation



(e) *Metalaxyl-M* degradation (f) *Bifenthrin* degradation

Figure 3. Dynamics of degradation of *Deltamethrin, Alpha – cypermethrin, Lambda-cyhalothrin, Chlorpyrifos ethyl, Metalaxyl-M, Bifenthrin* in tomatoes

In the case of systemic fungicide *Myclobutanil*, the content in residues at harvest is below 0.3 mg/kg, the maximum limit allowed. As seen in Figure 2b, myclobutanil residues content were below the MRLs, for the first two treatments applied, after 12 days. After the first 5 days of application of the 3 treatments with *Captan*, the content in residues decreases almost by half the maximum residue limit value surpassing 2 mg/kg, but reaching a value of 0.79 mg/kg at harvesting, below the MRLs (Figure 2c).

After 12 days after application of each of the three treatments, *Folpet* residues are below the maximum residue limit of 2 mg/kg (Figure 2d).

Tebuconazole degradation is gradual. After 12 days of application of each treatment, the residue content of the target pesticide is reduced to about half the content of the residue obtained after 2 days of treatment. The final value reached 0.64 mg/kg at harvesting, below the maximum allowed of 1 mg/kg (Figure 2e).

Triadimenol is the second active substance *Folpet* along side commercial product Shavit F 72 WDG. The values obtained in all three treatments applied, after 2 days, 5 days and 12 days respectively is the maximum residue limits. Finally, at harvest, the amount obtained being 0.07 mg/kg far below the MRLs of 1 mg/kg (Figure 2f).

After 5 days in each of the 3 treatments applied, the content of *Deltamethrin* residues were higher than the MRLs (0.3 mg/kg). *Deltamethrin* degradation occurs gradually, reaching a concentration of 0.1 mg/kg at harvesting, below the MRLs (Figure 3a). Salghi et al. [62] compared the contents of *Deltamethrin* residues in tomatoes grown in the field with those of tomatoes grown in greenhouses. Tomatoes cultivated under greenhouse conditions are highly sensitive to pests and need frequent pesticide treatments, leading to higher residues in the final products.

The content of *Alpha-cypermethrin* residues is below the maximum permitted, 0.5 mg/kg, after 12 days for all three treatments applied. Finally the value obtained at harvest reached 0.19 mg/kg much below the MRLs (Figure 3b).

Compared with *Alpha-cypermethrin*, *Lambda-cyhalothrin* was degraded slowly due to its special structure (Figure 1). After each of the three treatments applied, the residues content of *Lambda-cyhalothrin* exceeds the MRLs of 0.1 mg/kg. At harvest the *Lambda-cyhalothrin* concentration reaches a value of 0.09 mg/kg, below the MRLs (Figure 3c).

The degradation of *Chlorpyrifos ethyl* exceeds the MRLs value of 0.5 mg/kg, after 2 days and 5 days of the treatments application. After 12 days the contents in residues were below the MRLs, reaching at harvest a value of 0.13 mg/kg (Figure 3d).

The content of *Metalaxyl-M* residues falls below the MRLs of 0.2 mg/kg, after 12 days, considering the application of the first and third treatment. After 5 days from each treatment application the content in residues of *Metalaxyl-M* decreases approximately by half compared with the concentration obtained after 2 days of spraying solution applying, reaching at harvest a residue content of 0.1 mg/kg much below the MRLs (Figure 3e).

As seen in Figure 3f the *Bifenthrin* residues were below the MRLs, 0.2 mg/kg, after 12 days of applying the second treatment. At harvesting, considering the third treatment application, the content in residues of bifenthrin reaches a value of 0.3 mg/kg, above the MRL.

3.2. Health risk estimates

Risk assessment is essential to the process of decision making about pesticide effects on ecological systems and human health, both new and existing. The human health risk estimation entails to assess the nature and probability of adverse health effects in humans potentially exposed to chemicals in contaminated environmental media. The risk assessment process depends on the existing suitable data and testing models.

Table 5. Health risk estimation associated with pesticide residues in tomatoes, at harvesting.

Pesticide	Reference dose (mg/kg/day)	Concentration of pesticides (mg/kg)	Europe estimated dose (mg/kg/day)	Romania estimated dose (mg/kg/day)	Europe Hazard Index	Romania Hazard Index	Health risk
Captan	1.3 x10^{-1}	0.79	0.18 x10^{-2}	0.32x10^{-2}	0.0144	0.0246	No
Folpet	1 x10^{-1}	1.03	0.24 x10^{-2}	0.41 x10^{-2}	0.0244	0.0417	No
Triadimenol	5 x 10^{-2}	0.07	0.01 x10^{-2}	0.02 x10^{-2}	0.0033	0.0056	No
Myclobutanil	3.1 x10^{-1}	0.25	0.05 x10^{-2}	0.10x10^{-2}	0.0019	0.0032	No
Chlorothalonil	1.5 x10^{-2}	2.83	0.67 x10^{-2}	1.14x10^{-2}	0.4474	0.7654	No
Tebuconazole	3 x 10^{-2}	0.64	0.15 x10^{-2}	0.25 x10^{-2}	0.0505	0.0865	No
Metalaxyl-M	6 x10^{-2}	0.10	0.02 x10^{-2}	0.04 x10^{-2}	0.0039	0.0067	No
Chlorpyrifos ethyl	1 x10^{-1}	0.13	0.03 x10^{-2}	0.05 x10^{-2}	0.0030	0.0052	No
Bifenthrin	1.5 x10^{-2}	0.30	0.07 x10^{-2}	0.12 x10^{-2}	0.0474	0.0811	No
Alfa-cypermethrin	1 x10^{-2}	0.19	0.04 x10^{-2}	0.07 x10^{-2}	0.0450	0.0770	No
Lambda-cyhalothrin	5 x10^{-3}	0.09	0.02 x10^{-2}	0.03 x10^{-2}	0.0426	0.0730	No
Deltamethrin	1 x10^{-2}	0.10	0.02 x10^{-2}	0.04 x10^{-2}	0.0237	0.0405	No

Table 5 summarizes the health risk estimates associated with pesticide residues in tomatoes, at harvesting. The Table comprises of reference daily dose, computed average maximum daily intake values and corresponding hazard indices during the study period for adults. The human health risk estimation has been performed based on Romanian and Europe consumption data. The health risk analysis considered hazard indices and showed that the target pesticides residues available in field-grown tomatoes at harvest do not pose a risk to human health, although the doses of *Chlorothalonil* and *Bifenthrin* exceed the MRLs.

It should be noted that during our study, processing factors were ignored, since often tomatoes are peeled, cooked or boiled before consumption, resulting in an overestimation of the actual exposure to pesticide residues. Additionally, the effect of pesticides on other groups such as children or pregnant women should be of high interest in future studies.

4. Conclusions

The present study shows that, despite the high number of treatments with pesticides applied to field-grown tomatoes during 2012, the contamination level could not be considered a public health problem. From a group of 12 target pesticides chosen in our study for tomatoes treatment, only *Chlorothalonil* and *Bifenthrin* concentration exceeded the MRLs allowed by the UE legislation, at the final of the treatment upon harvesting.

Estimating the degradation of pesticides in tomatoes is important for the assessment of the diet risks and to ensure continuous monitoring of residues. Although the study focused on the consumption data both in Europe and Romania for adults, the human health risk estimates indicated that adults are not subjected to any health problems due to consumption of tomatoes treated with pesticides. A future study should aim the health risk assessment from pesticide treated fruit and vegetable consumption by more vulnerable groups such as children and pregnant women.

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