UNIUNEA EUROPEANĂ
GUVERNUL ROMÂNIEI MINISTERUL MUNCII, FAMILIEI, PROTECȚIEI SOCIALE ȘI PERSOANELOR VÂRSTNICE AMPOSDRU
Fondul Social European POSDRU 2007-2013
Instrumente Structurale 2007-2013
MINISTERUL EDUCAȚIEI NAȚIONALE OIPOSDRU
UNIVERSITATEA TEHNICA "GHEORGHE ASACHI" DIN IAȘI

"BABEŞ-BOLYAI" UNIVERSITY OF CLUJ-NAPOCA

Faculty of Environmental Science & Engineering

Assessing the incidence of triazine pesticides in environmental samples and foodstuffs

- Summary -

Scientific coordinator:
Prof. univ. dr. Dumitru Ristoiu

PhD student:
Andreea-Bianca Drăguș

CLUJ-NAPOCA - 2015

UNIUNEA EUROPEANĂ

GUVERNUL ROMÂNIEI
MINISTERUL MUNCII, FAMILIEI,
PROTECȚIEI SOCIALE ȘI
PERSOANELOR VÂRSTNICE
AMPOSDRU

Fondul Social European
POSDRU 2007-2013

Instrumente Structurale
2007-2013

MINISTERUL
EDUCAȚIEI
NAȚIONALE
OIPOSDRU

UNIVERSITATEA TEHNICA
"GHEORGHE ASACHI" DIN
IASI

The PhD thesis was realized with the financial support of the project: **"Doctoral Studies for European Performances in Research and Innovation (CUANTUMDOC)"** POSDRU/107/1.5/S/79407.

The project **"Doctoral Studies for European Performances in Research and Innovation (CUANTUMDOC)"** POSDRU/107/1.5/S/79407, *is a strategic project with the general aim of "Applying management, research and didactic strategies designed for the initial improvement of future researchers through the doctoral program, according to the Bologna process, through developing specific competencies for scientific research, as well as general competencies: research management, linguistic and communication competencies, documentation, writing, publishing and scientific communication skills, employing modern tools offered by TIC, entrepreneurial spirit of transferring the results of a research. Developing the human capital for research and innovation will contribute on the long term to forming doctoral students at a European level with interdisciplinary interests. The financial support offered to doctoral students will ensure the involvement in national doctoral programs and in internships to foreign research centers or universities in the EU. The mission of the project is to form a young researcher adapted to the market economy and new technologies, having theoretical, practical, economical and management knowledge at an international level, which will promote the principles of sustainable durability and protection of the environment.*

Project financed during: 2010-2013
Funding project: 16.810.100,00 RON
Beneficiary: Technical University "Gheorghe Asachi" of Iasi
Partner: "Babes-Bolyai" University of Cluj-Napoca
Project manager: Prof. univ. dr. ing. Mihai BUDESCU
Responsible project partner: Prof. univ. dr. ing. Alexandru OZUNU

Summary

Keywords: pesticides, triazines, high performance liquid-chromatography, soil, water, milk, honey

I. Introduction

I.1 Introduction on the topic of pesticides

In the last years the concerns related to an increasing population and food security represent topics which pose interest for both the society at large, as well as the scientific community. The development of modern agriculture would be inferior to the current level without the application of pesticides. Pesticides represent the vast category of certain chemical substances with the role of controlling different types of pests. Fighting pests through chemical methods similar with what we have in the present began to take shape after The World War II. In 1946 the first known herbicide pertaining to the phenoxy class, namely 2,4-D was present on the market (Burnside, 1996). Long before the development of chemical synthesis the control of pests was realized with products of inorganic nature or plant-based, for example, nicotine was known towards the end of 1600s as a potent herbicide, as well as pyrethroids which were extracted from chrysantemum (Gilbert, 2012).

With the intensive herbicide use certain issues can occur, such as the contamination of environmental compartments and the contamination of the food chain (Ackerman, 2007; Drăguș & Beldean-Galea, 2015a; Wang, D.-Q. et al., 2012).

Triazine herbicides are a class of selective herbicides which can be applied in a pre-emergent and post-emergent mode, aspect that increases their efficiency. Since these herbicides, as well as their degradation products are toxic and present as characteristics a high solubility in water and the capacity of being adsorbed onto soil particles, the aforementioned agrochemicals pose an environmental and health problem (Drăguș & Ristoiu, 2012a; Prado et al., 2014). In Europe, triazine concentrations of up to 5.6 µg/L were found in surface waters (Quednow & Puttmann, 2007). Considering the mobility of these herbicides, their presence was detected also in underground water and rainwater (Boyd, 2000; Mast et al., 2007). With regard to the deleterious effects on human health the exposure to triazines has been connected with the development of ovarian cancer and breast cancer (Donna et al., 1989; Kettles et al., 1997).

Determination of triazine herbicides in complex matrices represents a difficult task due to the numerous interferences present in various types of samples. Therefore, the proper extraction of the target compounds holds major importance in the analytical process. Traditional extraction methods, such as the well-known Soxhlet or liquid-liquid extraction require significant volumes of solvent, take a considerable amount of time and generate waste. For this reason, analytical chemistry has shifted its attention toward developing modern, miniaturized extraction methods that require a significantly decreased volume of solvents, take less time, and minimize waste.

I.2 Aim of the study and original contributions

The aim of the present paper focused on optimizing a multicomponent analysis method in order to determine in a single analysis as many as possible triazine herbicides, and at the same time renders a low limit of detection in accordance with concentrations of these compounds in the environment. For these reasons, in this study we have focused on high performance liquid chromatography, considering that, according to the body of literature this method offers the possibility of accomplishing all the proposed objectives. The optimized method has undergone all the steps of validation in order to assess its performance, errors and to establish its environmental application.

The thesis is focused on the analysis of triazine herbicides in various environmental and food matrices with the scope of evaluating their transfer mechanism in environmental factors, as well as evaluating the human exposure to this pesticide class. The paper comprises of studies that regard:

- the incidence of triazine herbicides in well water samples taken from two areas of Transylvania, Cluj area (Turda) and Mureș area (Sighișoara), respectively;
- the incidence of triazine herbicides in surface water samples collected from Someșul Mic hydrographic basin and the upper Tisza basin;
- the incidence of triazine herbicides in soil samples sampled from Transylvania and Moldavia;
- the incidence of triazine herbicides in honey samples taken from Transylvania, Moldavia and Dobrogea;
- the incidence of triazine herbicides in raw milk samples collected from Transylvania.

It is important to mention that for each type of matrix (soil, water, honey and milk) were developed and validated specific extraction and purification protocols for the extracts, taking into account recent extraction methods, such as solid phase extraction, liquid-phase microextraction and QuEChERS.

I.3 Outline of the thesis

The thesis is divided in two major parts, the theoretical part and the experimental part. In the beginning of the present paper a general introduction into the pesticides topic and a brief outline about the issues that come with the intensive usage of pesticides is presented. The theoretical part contains three chapters the role of each chapter being briefly described in the following.

Chapter I will comprise of information pertaining to various classes of pesticides, their toxicity, and their role in society. The next two chapters, **Chapter II** and **Chapter III**, respectively, will contain a detailed presentation of the organic compounds that make the focus of the present paper, namely triazine pesticides, as well as treating a wide array of analysis and extraction methods from different matrices. The experimental part starts with **Chapter IV** and represents the main chapter of the thesis. It will focus on the assessment of triazine herbicides in various environmental and food matrices (soil, surface waters, underground waters, honey and raw milk), as well as the optimization of a method in order to assay the aforementioned agrochemicals. The last chapter, **Chapter V** will draw a series of conclusions that will complete the present paper and identify future perspectives.

The references will be listed in the **References** list. The thesis includes 21 tables and 42 figures.

II. Studies regarding the incidence of triazine pesticides in the environment

Monitoring of the environmental factors represents a part of the sum of operations that form the basis of a complete and consequent environmental protection strategy. Each of these factors, namely the soil, water and air can be contaminated through point and diffuse pollution, such as agriculture (Drăguș et al., 2012b). Contamination of these environmental factors through point sources indicates that the origin of the toxic substances can be traced back to its source, while with diffuse points the contamination source is difficult to trace back. Therefore, agriculture is considered to be a diffuse source of pollution and its synergistic effects reflect upon the quality of the environmental factors. The usage of triazine pesticides in weed control has lead to an increase in the agricultural yield, but at the same time the exposure to these chemicals has also risen. According to the scientific literature, triazine pesticides have a high persistency in soil, water, sediments, suspended soils and thus, can lead to the contamination or pollution of the environmental factors (Bottoni et al., 2013; Du Prez et al., 2005; Dagnac et al., 2005; Gfrerer et al., 2002).

In the following, we will present the results of the studies conducted within the framework of this thesis regarding the incidence of triazine pesticides in environmental matrices, as well as in foodstuffs whose production is closely linked to the environment. The studies were focused on analysing a group of agrochemicals from well waters, surface waters, soil, honey and raw cow milk.

II.1 Studies regarding the incidence of triazine pesticides in well waters

The main beneficiary of pesticide use is agriculture. Triazine compounds are applied especially on corn crops in order to control weeds both in the pre-emergent or post-emergent case. Due to the fact that triazines are mobile in the environment their capacity to negatively impact water bodies in proximity to agricultural fields is high.

The objective of this study was to assess the incidence of triazine pesticides from well waters pertaining to areas with a different agricultural activity, Turda (Cluj County) and Sighisoara (Mures County) in order to evaluate the exposure of the population using the water for domestic purposes. The study was conducted between May-July 2011, a period when these agrochemicals

II.1.1 Experimental section

Chemicals and Reagents

A mixture consisting of four triazine pesticides (simazine, atrazine, propazine and trietazine) was purchased from Supelco, USA. For the ensuing analyses a standard solution of 2 ppm (µg/mL) was prepared. Extraction solvents (methanol, acetone, acetonitrile) of HPLC purity were obtained from Merck, Germany. The compounds were extracted from the liquid matrix by solid phase extraction employing a SPE system (Supelco, USA). The cartridges used in the extraction were Strata C_{18} EC purchased from Phenimenex, USA. The water used for preparing the mobile phase and for conditioning the cartridges was obtained by employing a Milli-Q-Plus ultra-pure water system by Millipone, USA. Determination of the analytes was performed with a HPLC system, ABL&E Jasco 980, equipped with a JASCO PU-980 pump, and UV-VIS detector JASCO UV-980-975. The separation of the target compounds was made by a NUCLEOSIL 100 C18 column (25 cm x 4 mm ID) the mobile phase containing a mixture of acetonitrile: water (55:45, *v/v*). Detection was made at 220 nm. Data acquisition and processing were performed by Soft ChromPASS.

The water samples were collected in polyethylene bottles and kept at 4°C until extraction. Before passing the samples through the cartridge the water was filtered by a filter with a porosity of 0.45 µm (Phenomenex, USA).

Extraction procedure

The C18 EC cartridges were conditioned with 5 mL of water, 5 mL of methanol and again 5 mL of water. After this step a volume of 400 mL of sampled water was passed through the cartridge at a 2 mL/min flow rate. Elution of the retained compounds was made with 3 mL of acetone. The eluate was subjected to evaporation under nitrogen and resumed with 200 µL acetonitrile and further injected into the HPLC system.

II.1.2 Results

Atrazine was the predominant compound found at a concentration ranging between 3.07-8.07 µg/L. Propazine was detected as well, but at a much lower concentration ranging between 0.05-2.87 µg/L. A single water sample was contaminated with trietazine, while simazine was detected only in Turda area water samples with a concentration ranging between 0.05-0.07 µg/L. The chromatogram of tested compounds (sample code P5) is presented in Figure 1 and in table 1 the results for all the water samples can be observed.

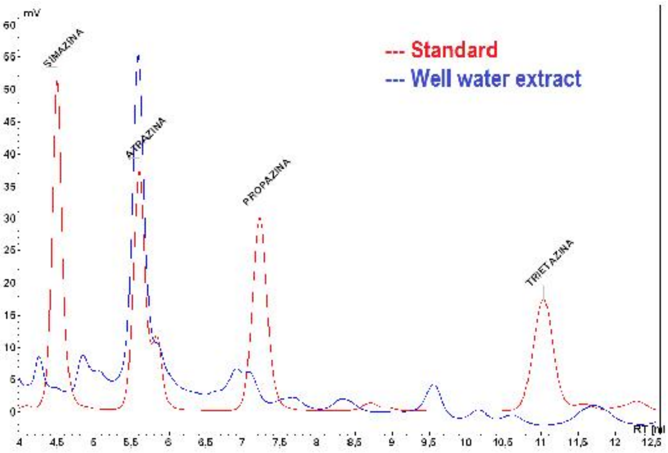

Fig.1 The chromatogram of tested triazine herbicides (Red colour: triazine standard; blue color: well water (sample code P5))

Table 1. Pesticide concentration in the sampled well water

Sampling points	**Simazine (µg/L)**	**Atrazine (µg/L)**	**Propazine (µg/L)**	**Trietazine (µg/L)**	**Sum (µg/L)**
Turda					
P1	0.15	3.07	0.2	nd	3.42
P2	0.1	7.5	2.87	nd	10.47
P3	0.1	6.6	0.22	0.07	6.99
P4	nd	5.8	0.2	nd	6
P5	nd	8.07	nd	nd	8.07
Sighişoara					
P6	nd	5.2	nd	nd	5.2
P7	nd	4.6	nd	nd	4.6
P8	nd	5.47	nd	nd	5.47
M.A.C. US E.P.A.	3	4	-	-	-
M.A.C. UE	0.1	0.1	0.1	0.1	0.5

nd - under the limit of detection

- limit not established

Data in Table 1 presents information that shows the sampled well water was found with pesticide concentrations that according to Directive no. 98/83/EC are above its maximum allowable concentration (M.A.C.). It is to be noted that Romania has obtained a transition period for the quality of water for human consumption until 2015. Atrazine was detected in all the samples, while propazine was detected in 50% of the samples, simazine was detected in 37% of the samples, and trietazine in 12% of the samples. It can be observed in table 1 that in the area with an increased agricultural activity (Turda area) triazine pesticides are found in a higher percentage, while in the area with a decreased agricultural activity (Sighisoara area) the percentage is lower. In Sighisoara, atrazine was the single compound detected, while the rest of the triazines were under the limit of detection. This aspect underlines the fact that the aquifers were contaminated through percolation from the spraying activities, circumstance revealed in the scientific literature as well (Nordmark et al., 2008).

II.1.3 Conclusions

The analysis and extraction method for the triazine class was successfully applied to environmental samples pertaining to underground water from areas with different agricultural activity in Transylvania. The compounds were detected as expected in the area where the agricultural activity is predominant. For the rural communities where the drinking water networks are nonexistent and concentrations are above 0.1 µg/L investigation activities must be accompanied by monitoring activities.

II.2 Studies regarding the incidence of triazine pesticides in surface waters

The present study focused on assessing triazine pesticides in surface waters sampled from Somesul Mic Basin and the upper Tisza Basin. The aim of this study was to assess the differences between areas with various agricultural activity and if the usage of pesticides constitutes a diffuse pollution source for watercourses.

In the case of Somesul Mic Basin 5 water samples were taken:

- after Apahida;
- after Rascruci;
- after Gherla;
- upstream Dej;
- Dej after meeting Somesul Mare.

In the case of the upper Tisza Basin 5 samples were also taken:

- Viseu River downstream Viseul de Jos;
- Viseu River at Valea Viseului;
- Iza River after meeting Mara River at Vadul Izei;
- Iza River after Sighetul Marmatiei;
- Tisza River at Sarasau.

The areas taken into the study and the sampling points are presented in Fig. 2 and Fig. 3.

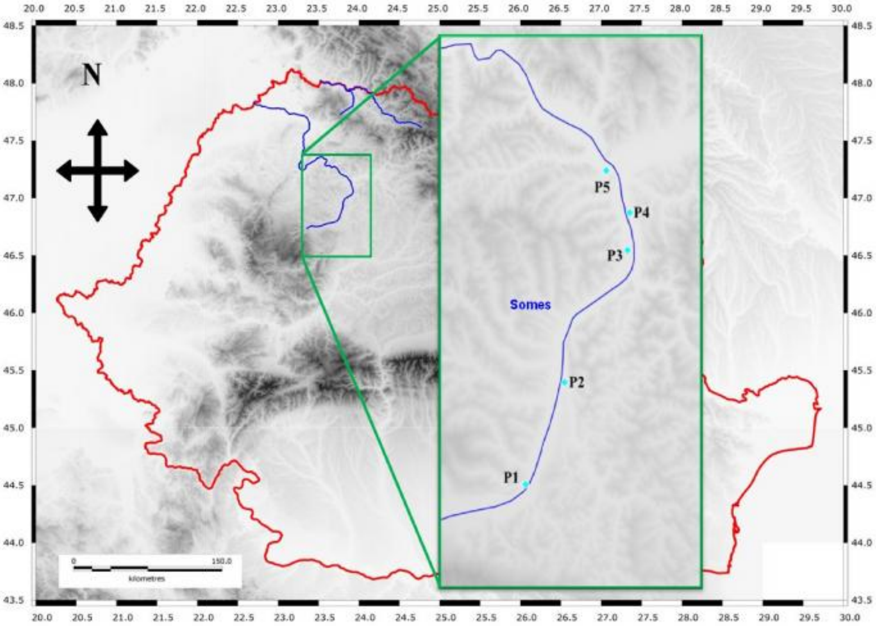

Fig.2 The Somes area and its corresponding sampling points

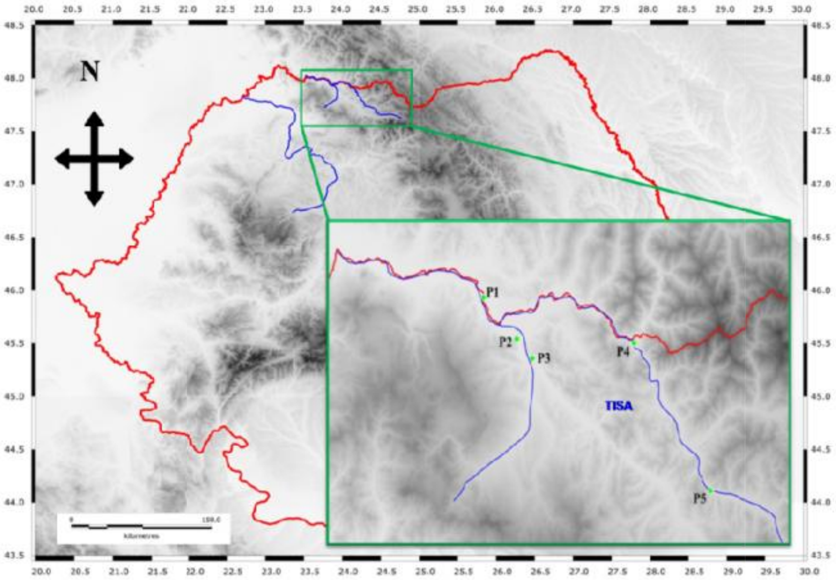

Fig. 3 The Tisza area and its corresponding sampling points

II.2.1 Experimental section and results

The extraction of triazine pesticides from water samples was conducted according to the protocol previously described with the mention that acetonitrile was employed on the extraction cartridges. In order to verify the accuracy of the method a sample of 400 mL water was spiked with 500 ng of pesticide standard and extracted according to the protocol previously mentioned. Extraction recovery of triazine pesticides is situated above 80% for all the compounds. The values for the extraction recovery are given in Table 2.

Table 2 Extraction recovery for the studied compounds

Compounds	Extraction recovery (%)
Simazine	93.67
Prometon	96.62
Atrazine	103.48
Ametryn	93.35
Propazine	97.21
Prometryn	96.70
Terbutryn	99.17

The analysis of triazine pesticides in surface water samples was carried out by high performance liquid chromatography using the apparatus and the methodology described in Chapter IV.2 Method Validation (see the doctoral thesis).

The results show that in the case of Tisza Basin samples the occurence of triazines was not detected. This can be explained by the fact that in the proximity of the sampling points crops were a scarce presence. However, in Somesul Mic Basin, crops, especially corn crops were situated extremely close to the watercourse. The concentrations found range between 0.08-5.29 µg/L (Table 3).

Table 3. Concentrations of triazine pesticides in surface water - Somesul Mic Basin

Sampling point	Simazine (µg/L)	Prometon (µg/L)	Atrazine (µg/L)	Ametryn (µg/L)	Propazine (µg/L)	Prometryn (µg/L)	Terbutryn (µg/L)
Apahida	0.09	nd	nd	nd	nd	nd	nd
Rascruci	0.09	nd	0.15	nd	0.18	nd	nd
Gherla	0.17	nd	0.27	nd	0.41	nd	nd
Dej	0.08	nd	nd	nd	0.12	nd	nd
Downstream Dej	0.52	nd	5.29	1.86	nd	nd	nd

nd - under limit of detection

According to the date from table 3 the predominant compound in all the samples is simazine with concentrations ranging between 0.08-0.52 µg/L. Atrazine and propazine were detected in 60% of the samples, while prometon, prometryn and terbutryn were not detected.

II.2.2 Conclusions

Atrazine, simazine and terbutryn are priority substances according to the Directive 2013/39/EU which modify the Directives 2000/60/EC and 2000/105/EC. The maximum allowable concentration in the case of atrazine is 2 µg/L, while in the case of simazine is 4 µg/L, and for terbutryn the MAC is 0.34 µg/L. According to the data, the results previously mentioned do not exceed the limits with the exception of atrazine at Dej after meeting Somesul Mare. This situation can be explained by the fact that pesticide residues are carried by different affluents and they accumulate at the confluence of Somesul Mare with Somesul Mic.

II.3 Studies regarding the incidence of triazine pesticides in soil samples

Although certain representatives of the triazine class have been banned in the E.U., triazines are the most used class of herbicides used in the U.S. and in Asia. These agrochemicals are applied at a rate of 1.12-2.24 kg/ha which leads at a concentration in soil of up to 3.6 mg/kg (Funari et al., 1998). Their degradation and mobility in soil is influenced by various factors, such as pH, type of soil, temperature, humidity and microbial activity, and through percolation it can be a source of underground water contamination (Koskinen et al., 2008; LeBaron, 1970). Regarding their persistency in soil, studies have been concluding it is ranging between 30 to 245 days (Accinelli et al., 2001; Li et al., 2008).

Although the efficiency of traditional extraction methods, such as SPE and LLE cannot be denied, recently, the trend of extraction methods has a more environmentally friendly approach. Modern extraction methods such as SBSE and MAE are one of the new methods used for the extraction of triazine pesticides from soil (Nakamura & Daishima, 2005; Shah et al., 2011).

The objective of this study was to develop a modern and efficient extraction method of triazines from soil. To obtain an adequate extraction the following parameters were optimized: salt addition, pH and the extraction solvent.

II.3.1 Experimental section

Chemicals and reagents

A standard consisting of seven triazine pesticides (simazine, prometon, atrazine, ametryn, propazine, prometryn, terbutryn) of purity >98 was purchased from Sigma-Aldrich. Extraction solvents 1-undecanol, acetonitrile, methanol and acetone were obtained from Merck (Germany). The water used for preparing the mobile phase and samples was obtained with a Milli-Q-Plus purification system from Millipore (Milford, MA, USA).

Instrumentation and chromatographic conditions

The analysis of the triazine compounds were conducted on a HPLC system, model Shimadzu, equipped with a 10 LC pump, 10 LSD UV-Vis detector according to the protocol described in Chapter IV.1 Method Validation. Detection of the target chemicals was made at a wavelength of 220 nm. Separation of the analytes was carried out on a Nova Pak C 18 reverse phase column (3.9x300 mm, 4µm particle diameter). Elution was made with an isocratic flow consisting of a mix between 25 mM KH_2PO_4 : acetonitrile (60:40, *v/v*) at 1.2 mL per min. Centrifugation of the samples was carried out by an Eppendorf centrifuge, model 5804 R (Austria). Ultrasonication of the samples was conducted with a ultrasonication bath, model Elmasonic S 10 H (Germany).

II.3.2 DLLME-SFO Extraction

A volume of 10 mL of Milli-Q water brought at a pH of 8 with 0.1 M NaOH was placed in a conical tube and spiked with the triazine mix. The working solution of concentration 10 ppm was obtained by dilution of the standard with methanol. 2 g of NaCl were then added in the solution and vigorously shaken for 15 minutes. After the salt dilution, 200 µL of extraction solvent was added which consisted of 150 µL acetonitrile acting as a dispersive solvent and 50 µL 1-undecanol as the extraction solvent. This will lead to the formation of a cloudy solution due to the dispersion of 1-undecanol droplets in the aqueous solution. Separation of the phases was made by centrifugation for 4 minutes at 4500 rpm. After centrifugation the sample was subjected to the ice bath for 10 minutes in order to solidify the extraction solvent. The solidified extraction solvent was then collected and transferred to a conical tube. After melting at room temperature, 5 µL of the extract was injected into the HPLC system.

II.3.3 Sample preparation

For this step, the sampled soil was air dried and finely ground. Afterwards, three grams were weighted and transferred into a screw cap conical vial. Due to the polarity of the triazine pesticides the solvent extraction chosen for the analysis was methanol. Therefore, 3 mL of methanol were added into the conical vial and vigorously shaken for 15 minutes, then subjected to ultrasonication and centrifugation for 4 minutes at 4 500 rpm.

In a clean screw cap conical vial were added 10 mL of Milli-Q water, adjusted at pH 8 with 0.1 M sodium hydroxide solution, adding one milliliter of the clear phase that was collected from the centrifuged soil samples, 2 g of sodium chloride and lastly, 200 µL (150 µL acetonirile - disperser solvent, 50 µL 1-undecanol - extraction solvent) were injected into the sample. The resulting solution was vigorously shaken for 5 minutes and then centrifuged for 5 minutes at 4 500 rpm. After centrifugation the sample was placed in an ice-bath for 10 minutes to solidify the extraction solvent which rises at the surface of the solution. The droplet was collected and further analyzed with the HPLC.

II.3.4 Optimization of DLLME-SFO method

II.3.4.1 Optimization of the pH

For the extraction of the triazine pesticides from water a miniaturized method was adapted from Sanagi et al. (2012). The method was adapted regarding the pH value in order to increase the recovery of the compounds. Therefore, a recovery study of the analytes was conducted at different pH values with the objective of increasing the extraction efficiency. 10 mL of Milli-Q water spiked with 100 ng of each pesticide were brought to various pH values such as: 2, 3, 4, 5, 7 and 8 with sodium hydroxide 0.1 M. Furthermore were added 0.5 g of sodium chloride and 200 µL extraction solvent containing 150 µL acetonitrile and 50 µL 1-undecanol and extracted according to the previously described protocol.

The results showed that extraction efficiency increases with the pH (Fig. 4). Accordingly for subsequent extraction a pH 8 was chosen.

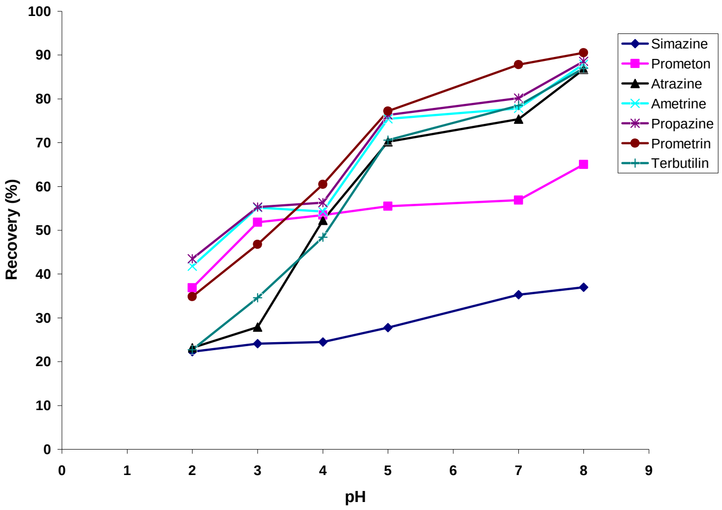

Fig. 4 Influence of pH over the extraction efficiency

II.3.4.2 Influence of the solvent extraction

Another optimized parameter for a better extraction was the solvent extraction. For this case, three solvents were assessed, namely: acetone, acetonitrile and methanol. Three soil samples in triplicate were spiked with 300 ng of pesticide. Each sample was extracted with the previously mentioned solvents. In order to increase the extraction efficiency samples were both manually agitated and subjected to ultrasonication for 15 minutes. After ultrasonication, the samples were centrifuged, then 1 mL was taken from the supernatant and placed in 10 mL of water brought to a pH 8 and extracted according to the protocol above.

The enrichment factor was found to be higher in the case of methanol, consequently, for further extractions, methanol was chosen as a solvent extraction (Table 4).

Table 4. Enrichment factor for the three tested solvents (acetone, acetonitrile, methanol)

Compound	Acetone	Acetonitrile	Methanol
Simazine	24	55	80
Prometon	80	65	96
Atrazine	46	35	99
Ametryn	70	68	98
Propazine	72	63	97
Prometryn	91	45	103
Terbutryn	93	33	97

II.3.4.3 Influence of ionic strength

Extraction efficiency is increased through salt addition. This aspect decreases the solubility of the analytes in the aqueous phase and improves their partition in the organic phase.

In order to investigate the influence of salt addition different experiments were undertaken where various sodium chloride concentrations (0.5-2.5%) were added into the aqueous phase. The graph in Fig. 5 clearly depicts how extraction efficiency increases with sodium chloride concentration until it reaches the 2 % threshold, then starting to decrease. For further studies 2 g of sodium chloride were added.

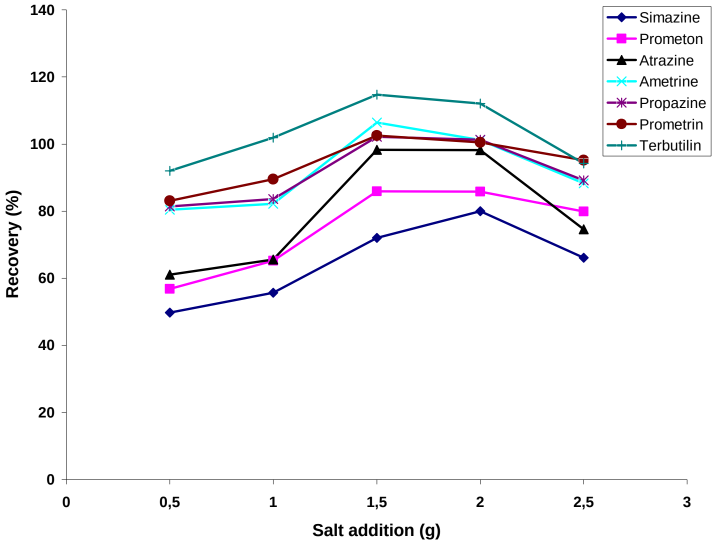

Fig. 5 Influence of ionic strength over extraction efficiency

II.3.5 DLLME-SFO method validation

Method validation has been made through the prism of accuracy, repeatability, linearity, LOD (limit of detection) and LOQ (limit of quantification).

Accuracy

For the accuracy study 3 grams of dry and grounded soil were spiked with 200 nanograms of each pesticide and the recovery of triazines after the developed extraction method was studied. Recovery was expressed according to the following formula:

Recovery (%) = $((C_{found}/C_{added})-C_{initial})$ x 100

The results show a good recovery of the analytes, above 80% which represents an adequate accuracy of the extraction method. Table 5 presents the results for each compound taken into study, while Fig. 6 presents a chromatogram of a soil sample and a chromatogram of a spiked soil sample.

Table 5. Recovery for each of the studied triazines

Compound	Initial Concentration	Added Concentration (μg/kg)	Found Concentration (μg/kg)	Recovery (%)
Simazine	nd*	1.33	0.798	60.00
Prometon	nd	1.33	1.286	96.69
Atrazine	0.32	1.33	1.681	102.33
Ametryn	nd	1.33	1.304	98.05
Propazine	0.55	1.33	1.871	99.32
Prometryn	1.53	1.33	2.889	102.18
Terbutryn	nd	1.33	1.416	106.46

*nd - not detected

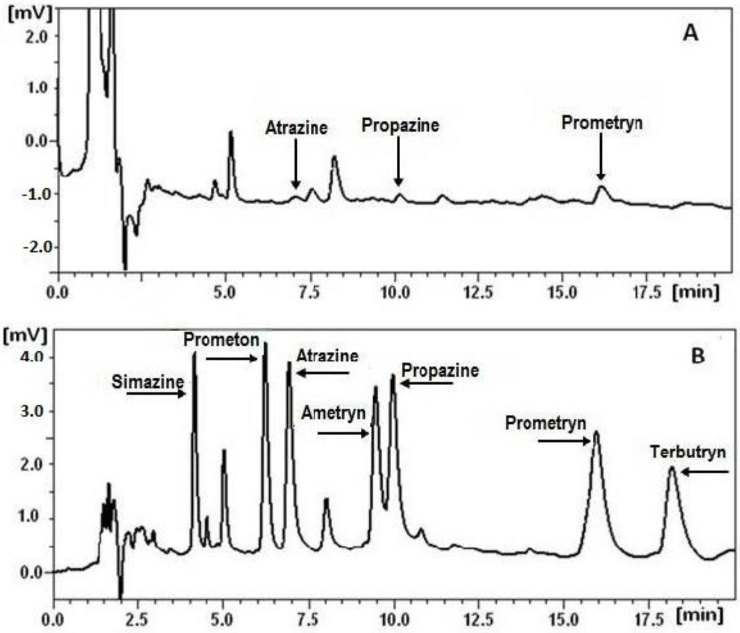

Fig. 6 Chromatograms of soil sample A (sample code 2B) and spiked soil sample B

Repeatability

Repeatability was assessed by spiking 5 samples of soil with 300 nanograms of each pesticide and extracted according to the previously described protocol. Samples were analyzed by HPLC and relative standard deviation (RSD) was estimated for each compound in each sample. Results show a good precision the values being under 8% which are considered to be in the satisfying range considering the concentration at which samples were spiked. Table 6 presents the values for each triazine compound.

Table 6. Relative standard deviation (RSD %) for the triazine compounds

Compound	**RSD % (n=5)**
Simazine	10.42
Prometon	9.51
Atrazine	6.74
Ametryn	5.91
Propazine	6.68
Prometryn	5.23
Terbutryn	7.31

Linearity

For the study of linearity 5 samples of soil were prepared according to the protocol previously described and spiked with different concentrations of pesticides. Subsequently, the samples were extracted as mentioned in the protocol and analyzed by HPLC. Linearity was graphically represented by peak area versus concentration of the analytes. Three grams of dried and grounded soil were spiked with50 ng, 100 ng, 200 ng, 300 ng, 400 ng of pesticides. The r^2 coefficient values are above 0.99 for all the analytes, demonstrating a good linear range. In Fig. 7 the linearity of each compound can be observed, while Table 7 presents the linear curve equations and r^2.

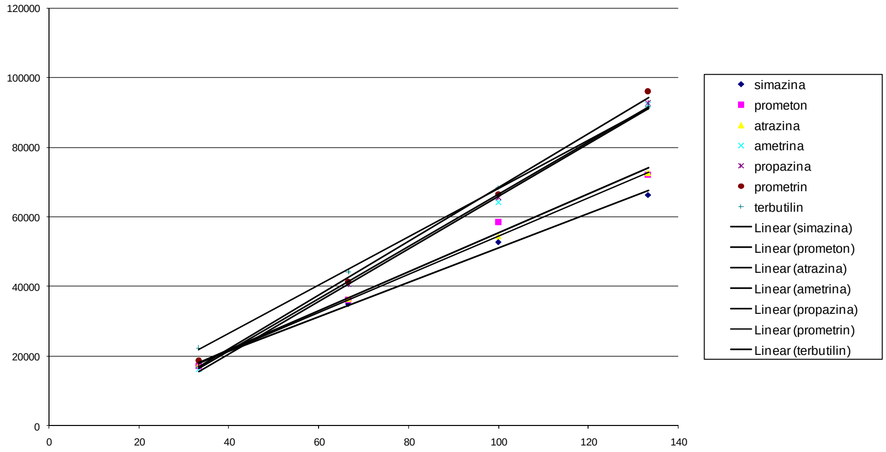

Fig. 7 Linearity of the extraction method

Table 7. Linear curve equations and r^2 values

Compound	Linear curve equations	r^2
Simazine	$y = 496.73 x + 1403.7$	0.996
Prometon	$y = 562.79 x – 800.65$	0.992
Atrazine	$y = 548.82 x – 408.91$	0.999
Ametryn	$y = 753.71 x – 9423.5$	0.998
Propazine	$y = 752 x – 8618.7$	0.999
Prometryn	$y = 771.79 x – 8691.1$	0.996
Terbutryn	$y = 694.68 x – 1235.2$	0.999

The limit of detection and limit of quantification was calculated taking into account the standard deviation (SD) of a sample contaminated with 100 ng per gram of soil pesticide and the values of the slopes of the calibration curves obtained on samples of contaminated soil. LOD and LOQ values obtained for the compounds studied are presented in Table 8.

Table 8. LODs and LOQs for triazine pesticides

Compound	SD	Slope	LOD (μg/kg)	LOQ (μg/kg)	EF
Simazine	0.08	496.73	0.54	1.63	176
Prometon	0.10	562.79	0.59	1.79	189
Atrazine	0.05	548.82	0.30	0.91	216
Ametryn	0.04	753.71	0.19	0.57	218
Propazine	0.06	752	0.25	0.74	220
Prometryn	0.05	771.79	0.21	0.62	221
Terbutryn	0.14	694.68	0.68	2.06	247

II.3.6 Assessment of triazine pesticides in soil samples

The developed method was applied in order to analyze the content of triazine pesticides in soil samples from different agricultural areas of Romania. A certain number of samples were sampled shortly after pesticides were applied on crops, while some were sampled after a few months since the spraying period with the aim to highlight the persistence of these agrochemicals in soil. The type of crop and the soil origin are presented in table 9.

Table 9. Type of crop and sampled soil origin

Sample code	Type of crop	Area (Commune/County)
1C	maize	Cojocna, Cluj
2B	maize	Mariselu, Bistrita-Nasaud
3B	wheat	Sieu-Magherus, Bistrita-Nasaud
4S	wheat	Stroiesti, Suceava
5V	wheat	Albesti, Vaslui
6V	wheat	Grivita, Vaslui
7V	vegetables	Grivita, Vaslui
8V	sun-flower	Grivita, Vaslui
9V	maize	Miclesti, Vaslui
10N	maize	Dobreni, Neamt

The samples were taken from a depth ranging between 0 and 15 cm in plastic bags and kept in a cool environment until analysis. Crops from where the soil samples were taken were mainly corn and wheat. Samples were dried at room temperature and subjected to further clean-up and analysis.

II.3.7 Results

The data obtained from analyzing the soil samples is shown in Table 10.

Table 10. Concentrations of triazine pesticides in soil samples

Sample code	Simazine (µg/kg)	Prometon (µg/kg)	Atrazine (µg/kg)	Ametryn (µg/kg)	Propazine (µg/kg)	Prometryn (µg/kg)	Terbutryn (µg/kg)
1C	3.65	4.04	6.70	5.86	9.19	7.15	7.53
2 B	5.86	0.81	nd	nd	nd	12.48	8.48
3 B	4.40	nd	nd	nd	7.05	nd	22.83
4 S	1.34	nd	nd	2.71	nd	nd	nd
5 V	nd	nd	nd	nd	nd	16.74	nd
6 V	nd	nd	nd	nd	nd	9.42	nd
7 V	nd	nd	nd	nd	nd	nd	2.30
8 V	nd	nd	nd	nd	nd	4.33	nd
9 PN	nd	nd	nd	nd	nd	25.94	nd
10 PN	nd	nd	nd	nd	nd	44.80	nd

* nd - under the limit of detection

The results show that the main compounds detected were simazine, prometryn and terbutryn with concentrations ranging between 1.34-4.40 µg/kg, 7.15-44.80 µg/kg, 2.30-22.83 µg/kg, respectively. Prometryn was detected in 70% of the samples, while simazineand terbutryn were detected in 40% of the samples.

According to the EU legislation which specifies the MRLs for triazine pesticides to be 0.01 mg/kg, in most of the samples no breaches were observed, with the exception of four soil samples in the case of prometryn and one sample in the case of terbutryn, where the 10 ppb limit was breached.

It can be observed that the most significant breaches are in the case of the soil samples that originate from maize crops (1C - total pesticide concentration, 2B, 9V, 10N). This aspect could be explained by the fact that triazine pesticides are applied especially on maize crops.

II.3.8 Conclusions

The miniaturized extraction method for the analysis of seven triazine pesticides was applied successfully in order to detect the aforementioned agrochemicals in soil samples. The limits of quantification ranged between 0.57 - 2.06 µg/kg, the linearity coefficient gave a value of $r^2 > 0.991$ and the enrichment factor had values higher than 176.

DLLME-SFO can be a fast and viable approach for the extraction of the triazine compounds.

II.4 Studies regarding the incidence of triazine pesticides in food matrices (raw cow milk, honey) sampled from Cluj County

Due to the pesticides mobility and persistency in the environmental compartments, these compounds can easily be transferred into food products, thus representing a continuous source of exposure for the population. The studies pertaining to the present paper are focused on two staple food products, namely cow milk and honey. The target compounds were isolated from the food matrices according to specific optimized protocols and further analyzed with HPLC. In the following subchapters we will present the detailed protocols employed for this study, as well as the sampling areas.

II.4.1 The incidence of triazine pesticides in honey

Honey is a highly used product in society. Due to the numerous groups of substances it contains, the beneficial properties of honey make this an important product on the national and international market. Studies that have assessed the quality of honey concluded that the agricultural sector can contaminate this product (Barganska et al., 2013; Panseri et al., 2014).

The present study focused on assessing the incidence of triazines in honey samples directly collected from the producer from areas with different types of agriculture. In table 11 there is presented the type of analyzed honey and the year of production.

Table 11. Type of the sampled honey and its production year

Sample Code	**Type of honey**	**Sampling location (village/county)**	**Year of production**
2C	polyfloral	Boju-Catun, CJ	2012, July
3C	acacia	Boju-Catun, CJ	2013, May
4T	colza	Topolog, TL	2013, May
5T	sun-flower	Topolog, TL	2013, June
7V	linden	Lipovat, VS	2013, June
8V	acacia	Lipovat, VS	2013, June
9V	acacia	Doagele, VS	2013, July
10V	linden	Doagele,VS	2013, July
11V	acacia and linden	Barlad, VS	2013, May
12V	linden	Pogana, VS	2013, July
13V	polyfloral	Trestiana, VS	2013, August
14B	polyfloral	Magura-Ilvei, BN	2013, September

II.4.2 Experimental section

Reagents and materials

A standard containing a mixture of seven triazine pesticides (simazine, prometon, atrazine, ametryn, prometryn and terbutryn) of >98% purity was purchased from Sigma-Aldrich. Acetonitrile and methanol of HPLC grade were purchased from Merck (Germany). The water was prepared using a Milli-Q system from Millipore (USA).

The honey samples were collected in 100 mL sterilized polyethylene bottles and kept at room temperature until further analysis.

Apparatus and chromatographic conditions

Chromatographical conditions for this type of analysis were the same as in the case of the soil samples. Hence, for the honey samples the HPLC system was a Shimadzu model, equipped with a 10 LC pump, 10 LSD UV/Vis detector and a manual injection valve with a loop of 5 µL. For the detection of the triazine pesticides the wavelength was set at 220 nm. Separation of the analytes was undertaken on a reverse-phase Nova Pak C18 column (300 x 3.9 mm, 4 µm). The mobile-phase was a mixture of acetonitrile: monopotassium phosphate (25 mM) (40:60, *v/v*) in isocratic elution mode at a flow of 1.2 mL/min.

Solid-phase extraction procedure

Isolation and pre-concentration of the triazine compounds from the honey samples was undertaken by solid-phase extraction using EC (end capping) C18 cartridges from Phenomenex (USA). In order to activate the solid phase, conditioning of the cartridges was carried out in three steps:

- 5 mL of Milli-Q water;
- 10 mL methanol;
- 5 mL Milli-Q water.

For the extraction of the pesticides from the honey samples a slightly modified method of Albero et al. (2004) was employed: 10 g of honey were dissolved in 40 mL of Milli-Q water and methanol (70:30, *v/v*) and subjected to ultrasonication for 15 minutes. The obtained solution was passed through the cartridge at a flow of 2 mL/min for the retention of the pesticides. After this process, another 5 mL of Milli-Q water was passed through the cartridge in order to remove any interferences, followed by passing air for 5 minutes to remove any remaining water from the cartridge. The retained compounds were eluted with 3 mL of methanol and collected in a vial. The extract was subjected to evaporation under nitrogen and the residue was diluted in 0.5 mL methanol and further analyzed with HPLC.

II.4.3 Solid-phase method validation

Due to the fact that the extraction method was slightly modified the accuracy was studied by evaluating the level of recovery of the target compounds from spiked samples. 10 g of honey were spiked with 500 ng of each herbicide and subjected to the extraction and analysis protocol mentioned above. The obtained recovery levels for the triazine pesticides are presented in Table 12.

Table 12. Recovery levels for the honey samples

Herbicide	Initial amount (ng)	Added amount (ng)	Found amount (ng)	Recovery (%)
Simazine	-	500	446	89
Prometon	-	500	446	89
Atrazine	-	500	483	97
Ametryn	-	500	480	96
Propazine	-	500	437	87
Prometryn	-	500	439	88
Terbutryn	-	500	460	92

*- mean value of three replicates

II.4.4 Results

The results highlight the presence of triazines in the honey samples. In fig. 8 the juxtaposed chromatogram of a contaminated honey sample (sample code 9V) and the chromatogram of the triazine standard is presented.

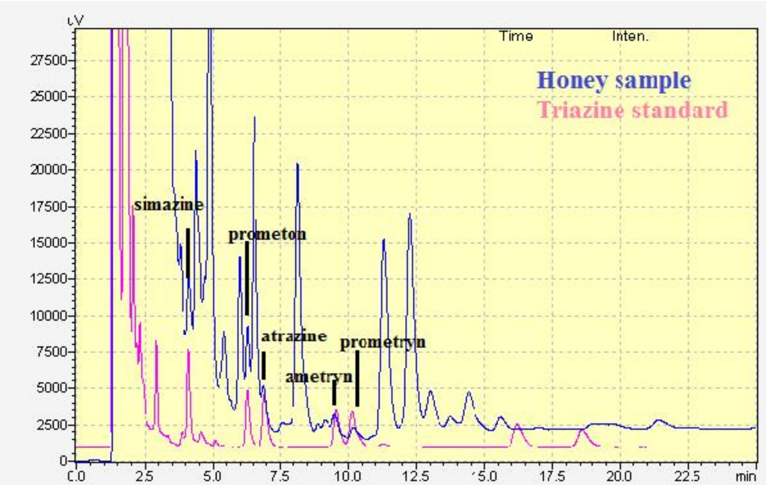

Fig. 8. Chromatogram of tested triazines in a honey sample (sample code 9V) and the triazine standard (1µg mL^{-1})

The results for all the honey samples are shown in table 13.

Table 13. Concentrations of triazine pesticides in honey samples

Sample code	**Concentration (µg/kg)**							
	Simazine	Prometon	Atrazine	Ametryn	Propazine	Prometryn	Terbutryn	Total
2 C	nd	nd	1.19	nd	5.65	nd	nd	6.84
3 C	nd	nd	4.97	nd	nd	nd	nd	4.97
4 T	50.20	nd	nd	nd	nd	nd	nd	50.20
5 T	34.59	26.08	11.18	16.34	13.92	nd	nd	102.11
7 V	80.49	nd	nd	nd	nd	nd	34.58	115.07
8 V	58.56	54.96	nd	nd	nd	nd	nd	113.52
9 V	3.80	7.80	75.97	60.29	88.74	nd	15.00	251.6
10 V	116.05	31.66	nd	nd	20.04	nd	nd	167.75
11 V	82.05	17.15	nd	nd	4.78	nd	nd	103.98
12 V	32.73	nd	nd	nd	nd	nd	47.94	80.67
13 V	490.0	507.5	nd	nd	nd	nd	nd	997.5
14 B	nd	nd	nd	nd	nd	nd	nd	-

*nd - not detected

The predominant detected compounds were simazine, atrazine and prometon with concentrations ranging between 3.80-490.0 µg/kg. From the total of samples, simazine is found in 75% of the samples, prometon is found in 50% of the samples, and atrazine is found in 33% of the honey samples.The limit imposed by EU regulations regarding the MRLs of triazine pesticides in honey is set at 0.01 mg/kg. It can be observed that the majority of the samples exceed the 10 ppb limit. This aspect could be explained due to the fact that the samples where breaches are recorded pertain to Moldavia (7V, 8V, 9V, 10V, 11V, 12V, 13V) and Dobrogea (4T, 5T) where the agricultural activity is modern and intensive and where pesticides are applied. High concentrations of triazine (8V, 9V, 11V) pertain to acacia honey samples, fact that could be explained by the blossoming of the acacia tree (May - June) in the period when pesticides are applied on crops.

In Transylvania area the concentration of triazines does not record any breaches, samples 2C and 3C having concentrations ranging between 4.97 - 6.84 µg/kg, while in sample 14B the target compounds were not detected.

II.4.5 Conclusions

The proposed method for the extraction of seven triazine pesticides from honey samples proved to be efficient. Recovery levels ranging between 87-97% are considered to be satisfying. In the areas where intensive agriculture is practiced the pesticide residues exceed the maximum level, while in the areas where agriculture does not use pesticides in an aggressive way , triazine concentrations do not exceed the allowable limit set by the EU.

II. 5 Incidence of triazine pesticides in milk

Studies regarding pesticide residues from different food matrices represent a major interest due to the fact that these agrochemicals can contaminate the food chain, subsequently posing various human health risks.

The present study includes the results of the triazine pesticides analyses in raw cow milk collected directly from various producers in Cluj County.

Due to the fact that foodstuffs contain a complex matrix which include proteins, lipids, sugars and other compounds with large molecular structures, extraction of trace compounds is often troublesome. For this reason, food samples require additional steps in order to eliminate proteins and extract the fat. These steps can require long periods of time and can cause analyte loss or contamination of the extract.

The study pertaining to this paper includes beside the actual milk sample analyses a study concerning the optimization of an extraction method of triazine pesticides based on QuEChERS.

II.5.1 Experimental section

Reagents and Materials

The reagents and materials utilized in the present study are similar with the purity and manufacturers of the previous studies, namely the triazine mix standard, acetonirtile, methanol, sodium chloride, monopotassium phosphate, Milli-Q water. In addition, hexane and ethyl acetate (Merck, Germany), as well as three adsorbents: C18 EC silicagel, Strata X (Phenomenex, USA) and florisil (Carlo Erba, Italy).

Instrumentation and chromatographical conditions

Analysis of the triazine compounds were undertaken on a HPLC, model Shimadzu, equipped with a 10 LC pump, 10 LSD UV/Vis detector. For the detection of the pesticides a 220 nm wavelength was set. Separation of the analytes was carried out on a reverse phase column Nova Pak C18 (300 x3.9 mm, 4µm). Centrifugation of the samples was made on an Eppendorf system, model 5804 (Austria). Elution of the compounds was made in an isocratic mode employing a mixture of acetonitrile: monopotassium phosphate (25 mM) (40:60, *v/v*) at a flow of 1.2 mL/min.

II.5.2 Optimization of QuEChERS extraction method

The extraction method employed in this study was adapted after Li et al. (2013). The first step consisted of testing different types of sorbents: C18 EC, Florisil and Strata X. Afterward, milk samples were contaminated with 100 ng of each pesticide and subjected to extraction as follows:

In a conical vial fitted with a screw cap were added 0.3 g of sodium chloride and 5 mL of milk. Afterward, 8 mL of solvent extraction were added, containing hexane and ethyl acetate (1:1, *v/v*). The mixture was vigorously shaken for 5 minutes, then it was subjected to centrifugation for 10 minutes. After centrifugation the supernatant which contains the organic phase and the target compounds was transferred in another vial in which a certain type of adsorbent was added (0.08 g) and again subjected to centrifugation for 5 minutes at 4500 rpm. After the sedimentation of the adsorbent the extract was filtered through a filter with a porosity of 0.45 µm and subjected to evaporation. The remaining residue was resumed with 100 µL of MeOH and injected into the HLPC system.

The results referring to the efficiency of the tested adsorbents are shown in fig. 9.

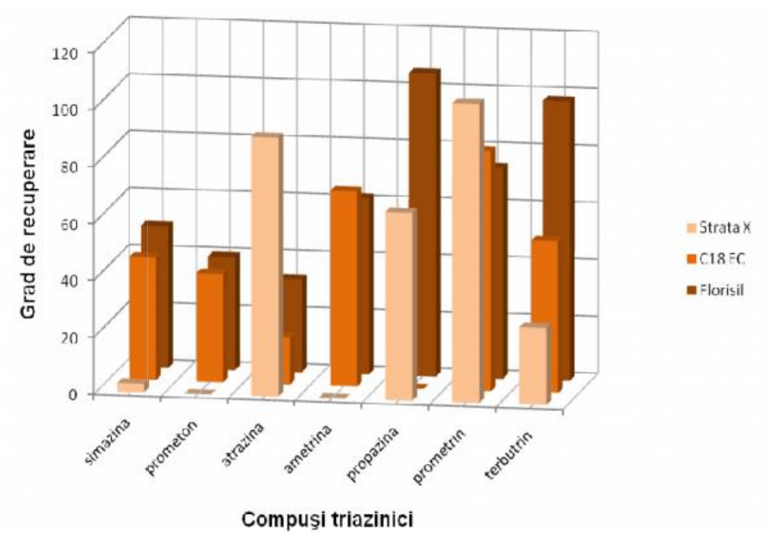

Fig. 9 Efficiency of tested adsorbents

As it can be observed from the previously presented figure the best efficiency is rendered by florisil which will be employed in processing the milk samples.

II.5.3 Results

Cow milk was directly collected from various producers (Table 14) in 500 mL polyethylene bottles and kept in the freezer until analysis. For the analysis, the samples were defrosted by placing the bottles in cold water for 30 minutes, shaken to homogeneity and subjected to the extraction process. The procedure for extracting the milk samples was as follows:

In a conical tube fitted with a screw cap was added 5 ml of milk and 0.3 g NaCl. Subsequently, 8 mL of extraction solvent was added and shaken vigorously for 5 minutes. After centrifugation, the supernatant which contains the organic phase and the compounds of interest was transferred to another test tube in which 0.08 g of florisil was added and subjected again to centrifugation for 5 minutes at 4500 rpm. The extract was then filtered and evaporated to dryness, resumed with 100 µL methanol and injected into the HPLC system.

This method yields a good purification level and a satisfying selectivity of the target compounds (Fig. 10).

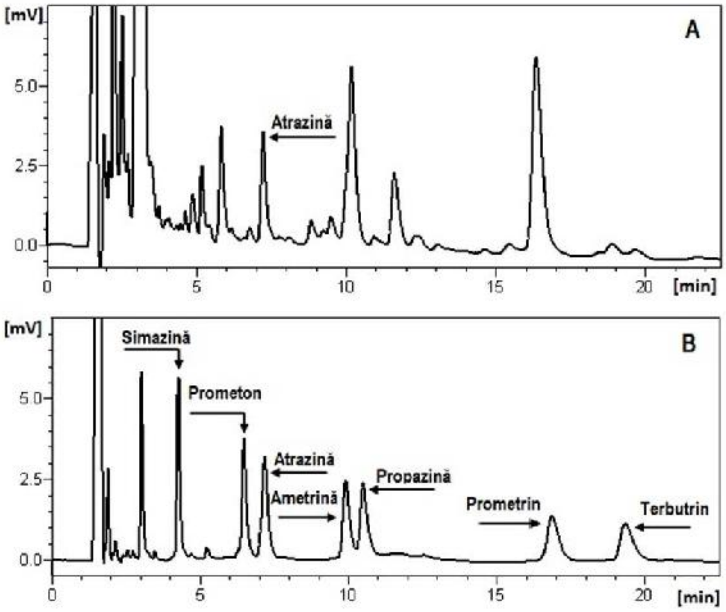

Fig. 10 Chromatogram of a milk sample (sample code P9) extracted with the optimized QuEChERS method

Table 14. Origin of the collected milk sample

Sample code	Sampling area (village, commune, county)	Collection period
P1	Boj-Cătun, Cojocna, Cj	19.07.2014
P2	Sănduleşti, Sănduleşti, Cj	19.07.2014
P3	Jucu de Sus, Jucu, Cj	17.07.2014
P4	Apahida, Apahida, Cj	17.07.2014
P5	Apahida, Apahida, Cj	17.07.2014
P6	Aruncuta, Suatu, Cj	18.07.2014
P7	Boj-Cătun, Cojocna, Cj	19.07.2014
P8	Ştrucuţ, Ceanu Mare, Cj	19.07.2014
P9	Ştrucuţ, Ceanu Mare, Cj	19.07.2014
P10	Fânaţe, Ceanu Mare, Cj	19.07.2014

The results obtained following the analyses of the milk samples regarding the content of triazine pesticides are presented in table 15.

According to the date in table 15, the predominant compound detected in the milk samples was atrazine, with concentrations ranging between 1.91 - 19.46 µg/L. The incidence of atrazine in the raw milk samples is 80%. The next compounds detected in 30% of the samples are simazine, ametryn and terbutryn. Prometon is detected in just 10% of the samples, while propazine and prometryn are not detected at all.

The maximum residue level in the case of milk is set by the E.U. at 0.01 mg/L. It is worth mentioning that in the E.U. database concerning the triazine pesticide residues present in milk, atrazine and simazine are the only compounds that have a MRL set, despite the fact that deleterious effects on human health of the other triazine representants were observed (Moretti et al., 2002).

Table 15. Concentration of triazine pesticides in the collected milk samples

Sample code	**Simazine (µg/L)**	**Prometon (µg/L)**	**Atrazine (µg/L)**	**Ametryn (µg/L)**	**Propazine (µg/L)**	**Prometryn (µg/L)**	**Terbutryn (µg/L)**	**Total (µg/L)**
P1	0.23	nd	1.91	4.13	nd	nd	6.47	12.74
P2	1.21	nd	nd	nd	nd	nd	nd	1.21
P3	nd*	nd	1.92	4.32	nd	nd	4.93	11.17
P4	nd	nd	4.07	nd	nd	nd	nd	4.07
P5	nd	nd	19.46	nd	nd	nd	nd	19.46
P6	nd	nd	2.44	nd	nd	nd	nd	2.44
P7	2.10	1.23	nd	5.18	nd	nd	6.34	14.85
P8	nd	nd	4.58	nd	nd	nd	nd	4.58
P9	nd	nd	17.65	nd	nd	nd	nd	17.65
P10	nd	nd	11.68	nd	nd	nd	nd	11.68

*nd - not detected

Concentrations in the analyzed samples breach the set MLR only in the case of atrazine - samples P5, P9, P10. The collected milk samples originate from pasture areas which are assigned to farmers. Milk sampling was carried out close to the post-emergence application period of pesticides on maize crops. Therefore, these results demonstrate the mobility of the target compounds and their movement through the food chain.

II.5.4 Conclusions

In the present study a fast and efficient extraction method was optimized based on QuEChERS methodology. The method was applied for the extraction of seven triazine pesticides in raw milk samples. The majority of the studied compounds were detected in the collected samples, with the exception of propazine and prometryn. Atrazine exceeds the maximum residue levels, aspect which demonstrates the influence of the atmospheric transfer and deposition in areas which are not targeted by the application of pesticides.

III. General conclusions

Triazine compounds pertain to the vast class of pesticides and the subclass of herbicides. In the E.U. the majority of triazine representants were banned, with the exception of terbutylazine which is approved for usage until 2021, while the situation of prometon it is not specified in the case of approved active substances. However, triazine pesticides are the most used category of agrochemicals on the american and asian continent. Considering their toxicity and their mobility in environmental factors, analyses concerning the incidence of triazines in the environment, as well as in the food chain, ensure the balance of ecosystems and food safety.

In the scientific literature there are numerous studies that have detected the presence of triazine pesticides in underground and surface water, in various types of soil, vegetables and food products. These contaminations can trigger imbalances of the ecosystems, which can present great remediation difficulties. Regarding the effects on human health, toxicological studies have shown that triazines can act as endocrine disrupters, causing problems with the reproductive system and developmental difficulties.

Within this context, the present paper focused on the incidence of seven triazine pesticides (atrazine, simazine, prometryn, prometon, ametryn, terbutryn, propazine) in major environmental compartments, such as underground and surface water, soil, as well as their incidence in raw milk and honey samples directly sampled from producers in various parts of Romania.

Quantification of the pesticides was carried out by high performance liquid chromatography coupled with UV/Vis. Optimization of the method consisted of investigating different types of columns and various mobile phases. Experimental data has shown that the best results were obtained with NovaPak C18 column (30 cm x 3.9 mm, 4 µm), wavelength set at 220 nm, and the mobile phase was a mix of acetonitrile: monopotassium phosphate (60:40, *v/v*). In order to validate the method various parameters were taken into study, such as: repetability, linearity, limit of detection (LOD), limit of quantification (LOQ). The validated method rendered a satisfying repetability and linearity, with limits of detection and quantification in the order of µg/L, thus meeting the requirements for validation.

Regarding the well waters, experimental data has shown the presence of triazine pesticides in the collected samples with concentrations ranging between 3.07-8.07 µg L^{-1} . According to the Directive 98/83/EC with respect to water quality for human consumption the found concentrations exceed the maximum allowable limit. Communities in the rural areas wich consume these waters for a long period of time could be exposed to certain risks, therefore upgrading drinking water systems is an action that is imperiously required.

In the case of surface waters collected from Somesul Mic Basin and the Upper Tisza Basin, the investigated samples have revealed the presence of triazine pesticides only in Somesul Mic Basin, the found concentrations ranging between 0.08 - 5.29 µg/L. According to Directive 2013/39/EU, where the MAC is mentioned for atrazine, simazine and terbutryn (2 µg/L, 4µg/L, respectiv 0.34 µg/L), the found concentrations do not exceed this threshold, with the exception of atrazine at the confluence of Somesul Mic with Somesul Mare, where the concentration was 5.29 µg/L. Since at the aforementioned point (Dej after meeting Somesul Mare) the hydrographic input is significant, thus collecting all the pesticide residues from different tributaries, therefore the presence of triazines is not surprising.

For the soil samples regarding the incidence of triazine pesticides a modern and fast extraction method was elaborated. Parameters such as, salt addition, pH, and type of solvent extraction were optimized, in order to obtain a good efficiency. The investigated validation parameters (accuracy, repetability, linearity, limit of detection, limit of quantification) rendered satisfying values, demonstrating the efficiency and the viability of the method. Dispersive liquid-liquid microextraction based on the solidification of floating organic droplet (DLLME-SFO) provided a good linearity with a correlation coefficient ranging between 0.992 and 0.999 and enrichment factors ranging between 176 and 247. The presence of triazine pesticides was detected in all the collected samples, but the majority of the concentrations do not exceed the maximum limit of 0.01 mg/kg imposed by the E.U., with the exception of four samples. Triazines are generally applied on maize crops, and a correlation can be observed between the areas with high concentrations and the crop type.

In the case of honey samples, the extraction was carried out by SPE. The elaborated method was subjected to validation, where the recovery is over 80%. The results of the honey samples have demonstrated that in the areas where the agricultural activity is intensive - in the case of this thesis, Moldova and Dobrogea - the concentrations detected here are the highest (50.2 - 997.54 µg/kg) and exceed the limit of 0.01 mg/kg. In the areas where the agricultural activity is not so aggresive, triazine concentrations do not exceed the previously mentioned limit.

Milk samples were extracted with a modern extraction method which is based on an optimized QuEChERS method (quick, easy, cheap, effective, rugged and safe). Three types of sorbents were tested, florisil was subsequently used for further processing the milk samples. Experimental data has shown the presence of triazines with total concentrations ranging between 2.44 - 19.46 µg/L. Three milk samples exceed the maximum allowable limit. These samples originate from meadow areas, and the collecting period coincided with the post-emergence application of the pesticides on crops. The results show the ability of triazines to move in the environmental compartments and to influence the food chain.

The studies present in this thesis could found the basis of future investigational programmes, to examine the exposure of rural communities from sensitive areas, where centralized water systems are not yet existing, and the long term consumption of contaminated water could lead to various chronic effects. Moreover, the lack of proper information of farmers regarding the correct application of pesticides increases the risk of finding these pesticides in other matrixes, such as legumes. Therefore, the investigation of triazine pesticides in various legumes susceptible to contamination, such as tomatoes or potatoes, could form the basis of other studies.

Published articles

1. Assessment of triazine herbicides content in honey samples by solid-phase extraction and HPLC analysis, Andreea Drăguş, Mihail Simion Beldean-Galea, Lucrare acceptată spre publicare în Universitatis Babeş-Bolyai Seria Chemia, Impact Factor = 0.136;

2. Determination of triazine herbicides in soil samples by ultrasound-assisted dispersive liquid-liquid microextraction based on solidification of floating organic droplet followed by high performance liquid chromatography analysis, Andreea Drăguş, Mihail Simion Beldean-Galea, Virginia Coman, Lucrare acceptată spre publicare în Revue Roumaine de Chimie, IF = 0.418;

3. Assesing Impacts of Triazine Pesticides Use In Agriculture Over the Well Water Quality, Andreea Dragus, Mihail Simion Beldean-Galea, Radu Mihaiescu, Tania Mihaiescu, Dumitru Ristoiu, Lucrare publicată în Environmental Engineering and Management Journal, Februarie 2012, Vol. 11, Nr. 2, pp. 319-323, ISSN: 1582-9596, Impact Factor = 1.435;

4. Metode de analiză a unor pesticide şi cuantificarea acestora în probe de apă Andreea-Bianca Drăguş, Lucrare publicată în volumul de lucrări din cadrul workshop-ului: *„Tendinţe şi cerinţe de interdisciplinaritate în cercetare. Prezentarea rezultatelor obţinute de doctoranzi”*, 25 Ian 2013, Editura POLITEHNIUM IAŞI ISBN 978-973-621-408-0;

5. Pesticides in the environment and human health, Andreea-Bianca Dragus, Dumitru Ristoiu,
Lucrare publicată în Ecoterra- Journal of Environmental Research and Protection, 2012, nr. 31, pp. 44-47, Jurnal indexat BDI; cu ISSN 1584-7071.

Conferences participation

1. *Assessement of triazine pesticides content in honey samples by solid phase extraction and HPLC analysis*, Andreea Drăguş, Mihail Simion Beldean-Galea, Dumitru Ristoiu, the 10th ELSEDIMA International Conference, 18-19 Sept. 2014, Cluj-Napoca, România;

2. *Triazine herbicides and their assessement on well waters,* Andreea Drăguş, Mihail Simion Beldean-Galea, Radu Mihaiescu, Tania Mihaiescu, Dumitru Ristoiu, 9th Edition of the ELSEDIMA International Conference, 25-27 Oct. 2012, Cluj-Napoca, Romănia

3. *Impact evaluation of triazine pesticides used in agriculture over the well water quality,* Andreea Drăguş, Mihail Simion Beldean-Galea, Radu Mihăiescu, Tania Mihăiescu, Dumitru Ristoiu, 6th International Conference Environmental Engineering and Management, Balaton, 1-4 Sept. 2011, Ungaria;

4. *Pesticides in the environment and human health,* Andreea-Bianca Drăguş, Dumitru Ristoiu, ENVIRONMENT&PROGRESS, Environment – Research, Protection and Management, 11 Noiembrie, 2011, Cluj-Napoca, România;

5. *The Impact of the Herbicide Glyphosate: A review,* Andreea Drăguş, Dumitru Ristoiu, National Symposium Environment & Progress, 25 Oct., 2013, Cluj-Napoca, România;

6. *Agricultural influence on water quality (original article in romanian) Influenţa agriculturii asupra calităţii apei,* Andreea-Bianca Drăguş, Mihail Simion Beldean-Galea, Carmen Roba, Dumitru Ristoiu, Ecouniversitaria, 20-22 Mai, 2011, Bistriţa-Năsăud, România.

Bibliography

1. Accinelli, C., Dinelli, G., Vicari, A., Catizone, P., 2001, *Atrazine and metachlor degradation in subsoils*, Biology and Fertility of Soils 33:495-500
2. Ackerman, F., 2007, *The Economics of Atrazine*, International Journal of Occupational and Environmental Health, 13:441–449
3. Barganska, Z., Slebioda, M., Namiesnik, J., 2013, *Pesticide residues levels in honey from apiaries located in Northern Poland*, Food Control, Vol. 31, pp. 196-201.
4. Bottoni, P., Grenni, P., Lucentini, L., Barra Caracciolo, A., 2013, *Terbutylazine and other triazines in Italian water resources*, Microchemical Journal 107, pp. 136-142.
5. Boyd, R., 2000, *Herbicides and herbicide degradates in shallow groundwater and the Cedar River near a municipal well field, Cedar Rapids, Iowa*, Science of the Total Environment, Vol. 248, pp. 241-253
6. Burnside, O., 1996, *Biologic and economic assessment of benefits from use of phenoxy herbicides in the United States*, National Agricultural Pesticide Impact Assessment Program
7. Dagnac, T., Bristeau, S., Jeannot, R., Mouvet, C., Baran, N., 2005, *Determination of chloro-acetanilides, triazines and phenylureas and some of their metabolites in soils by pressurised liquid extraction, GC–MS/MS, LC–MS and LC–MS/MS*, Journal of Chromatography A, Vol. 1067, Issue 1-2, pp. 225-233.
8. Directiva 2013/39/UE, de modificare a Directivelor 2000/60/CE şi 2008/105/CE în ceea ce priveşte substanţele prioritare din domeniul politicii apei, publicată în Jurnalul Oficial al Uniunii Europene nr. 226 din 25.8.2013.
9. Directiva nr. 83/98/CE, privind calitatea apei destinată consumului uman, Publicată in Jurnalul Oficial al Comunităţii Europene nr. 330 din 05/12/1998.
10. Donna, A., Crosignani, P., Robutti, F., Betta, P.G., Bocca, R., Mariani, N., Ferrario, F., Fissi, R., Berrino, F., 1989, *Triazine herbicides and ovarian epithelial neoplasms*, Scandinavian Journal of Work, Environment & Health, 15, pp. 47-53
11. Drăguş, A., Beldean-Galea, M.S., 2015a**,** *Assessment of triazine herbicides content in honey samples by solid-phase extraction and HPLC analysis*, Universitatis Babeş-Bolyai Seria Chemia.
12. Drăguş, A., Beldean-Galea, M.S., Mihăiescu, R., Mihăiescu, T., 2012b, *Assesing Impacts of Triazine Pesticides Use In Agriculture Over the Well Water Quality*, Environmental Engineering and Management Journal, Vol. 11, No. 2, pp. 319-323.
13. Drăguş, A.B, Ristoiu, D., 2012a, *Pesticides in the environment and human health*, Ecoterra - Journal of Environmental Research & Protection, no. 31, pp. 44-47.
14. Du Prez, L.H., Jansen van Rensburg, P.J., Jooste, A.M., Carr, J.A., Giesy, J.P., Gross, T.S., Kendall, R.J., Smith, E.E., Van Der Kraak, G., Solomon, K.R., 2005, *Seasonal exposures to triazine and other pesticides in surface waters in the western Highveld corn-production region in South Africa*, Environmental Pollution, Vol. 135, Issue 1, pp. 131-141.

15. Funari, E., Barbieri, L., Bottoni, P., Del Carlo, G., Forti, S., Giuliano, G., Marinelli, A., Santini, C., Zavatti, A., 1998, *Comparison of the leaching properties of alachlor, metolachlor, triazines and some of their metabolites in an experimental field*, Chemosphere, Vol. 36, Issue 8, pp. 1759-1773.
16. Gfrerer, M., Martens, D., Gawlik, B.M., Wenzl, T., Zhang, A., Quan, X., Sun, C., Chen, J., Platzer, B., Lankmayr, E., Kettrup, A., 2002, *Triazines in the aquatic systems of the Eastern Chinese Rivers Liao-He and Yangtse*, Chemosphere, Vol. 47, Issue 4, pp.455-466.
17. Gilbert, G. Stephen, *A small dose of toxicology. The health effects of common chemicals*, 2nd Edition, Health World Press, 2012.
18. Kettles, M.A., Broning, S.R., Prince, T.S., Horstman, S.W., 1997, *Triazine herbicide exposure and breast cancer incidence: An ecological study of Kentucky counties*, Environmental Health Perspectives, 105, pp. 1222-1227
19. Koskinen, C. William, Banks, Philip, A., 2008, *The triazine herbicides*, Elsevier, pp: 355-385.
20. LeBaron, H.M., *Single Pesticide Volume: The Triazine Herbicides*, Volume 32, Springer New York, 1970, Pages 311-353.
21. Li, Ke-Bin, Cheng, Jing-Tao, Wang, Xiao-Fang, Zhou, Ying, Liu, Wei-Ping, 2008, *Degradation of herbicides atrazine and bentazone applied alone and in combination in soils*, Pedosphere 18 (2): 265-272.
22. Mast, M.A., Foreman, W.T., Skaates, S.V., 2007, *Current-use pesticides and organochlorine compounds in precipitation and lake sediment from two high-elevation national parks in the Western United States*, Archives of Environmental Contamination and Toxicology, Vol. 53, Issue 3, pp. 294-305
23. Moretti, M., Marcarelli, M., Villarini, M., Fatigoni, C., Scassellati-Sforzolini, G., Pasquini, R., 2002, *In vitro testing for genotoxicity of the herbicide terbutryn: cytogenetic and primary DNA damage*, Toxicology in Vitro, v. 16, p. 81-88.
24. Nakamura, S., Daishima, S., 2005, *Simultaneous determination of 64 pesticides in river water by stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry*, Analytical and Bioanalytical Chemistry, 382: 99-107.
25. Panseri, S., Catalano, A., Giorgi, A, Arioli, F., Procopio, A., Britti, D., Chiesa, L.M., 2014, *Occurence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources*, Food Control, Vol. 38, pp. 150-156.
26. Prado, B., Duwing, C., Hidalgo, C., Muller, K., Mora, L., Raymundo, E., Etchevers, J.D., 2014, *Transport, sorption and degradation of atrazine in two clay soils from Mexico: Andosol and Vertisol*, Geoderma, Vol. 232-234, pp. 628-639
27. Quednow, K., Puttmann, W., 2007, *Monitoring terbutryn pollution in small rivers of Hesse, Germany*, Journal of Environmental Monitoring, Vol. 9, Issue 12, pp. 1337-43
28. REPORT EH08-01, 2008: *Sampling for pesticide residues in California well water*, 2007 Update of the Well Inventory Database, C. Nordmark, J. Dias, M. Clayton, J. Troiano, M. Pepple.

29. Shah, J., Jan, M.R., Ara, B., Shehzad, F.N., 2011, *Quantification of triazine herbicides in soil by microwave-assisted extraction and high-performance liquid chromatography*, Environmental Monitoring and Assessment, 178: 111-119.
30. Wang, D.-Q., Yu, Y.-X., Zhang, X.-Y., Zhang, S.-H., Pang, Y.-P., Zhang, X.-L., Yu, Z.-Q., Wu, M.-H., Fu, J.-M., 2012, *Polycyclic aromatic hydrocarbons and organochlorine pesticides in fish from Taihu Lake: Their levels, sources, and biomagnification*, Ecotoxicology and Environmental Safety, Vol. 82, pp. 63-70.