OCCURRENCE OF SOME PESTICIDES IN THE DISSOLVED WATER PHASE OF THE DANUBE RIVER AND ITS THREE MAJOR TRIBUTARIES

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Abstract

Polar organic contaminants like organophosphate insecticides (fenthion, disulfoton, azinphos-ethyl, azinphos-methyl, dimethoate, omethoate, demeton-s-methyl) and herbicide (bentazone) were analysed in the surface water of Danube River and its tributaries (Arges, Jiu and Olt). Analyses were performed by solid-phase extraction (SPE) followed by triple-quadrupole liquid chromatography mass spectrometry (LC-MS/MS). The surface water samples were found to be contaminated by disulfoton over the admissible value (7.5 - 10.7 ng/l), demeton-s-methyl (4.67-19.8 ng/l), dimethoate (0.21-2.8 ng/l). The pesticides detected with the highest frequency were omethoate and bentazon in concentrations ranging from 6.5ng/l to 16 ng/l and from 0.34ng/l to 18.8 ng/l, respectively. The highest concentrations of pesticides were recorded in Danube River in Calafat sampling point: demeton-s-methyl 19.8 ng/l, omethoate 17 ng/l.

Keywords*: Danube River, pesticides, SPE-LC-MS/MS*

9. Introduction

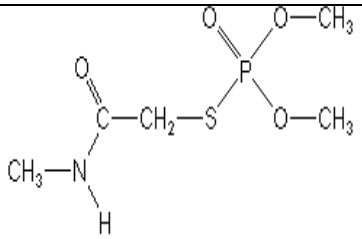
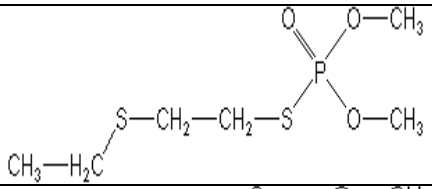
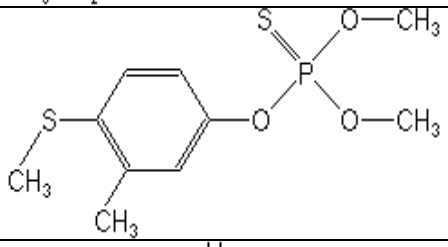
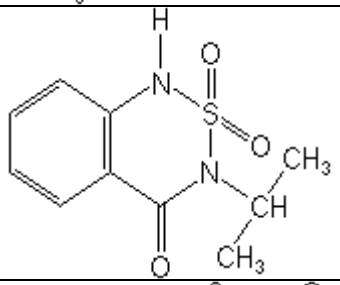
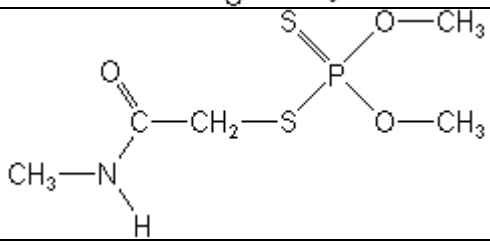
Pesticides are widely applied on vegetables, fruits and flowers during cultivation and post-harvest storage to ensure quality and to satisfy the requirements of consumers and of the trade. However, these molecules can be harmful to human, depending on the concentration level of pesticides. It is widely accepted that pollution of waste waters, surface waters, ground waters and drinking waters with pesticides is still a major problem which is due to intensive use of pesticides and is resulting from water runoff, agricultural storm-water discharges and return waters from irrigated soils [1-3].

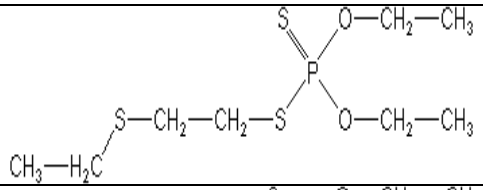
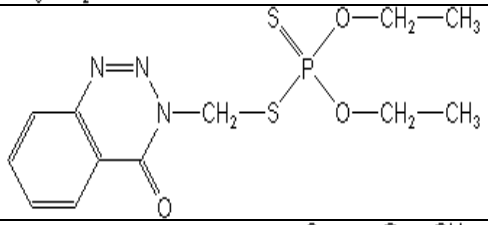
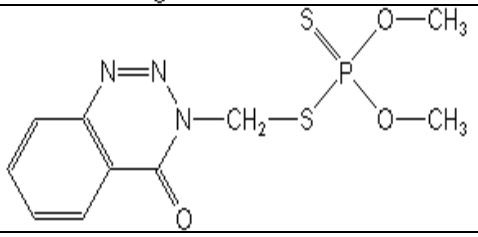
The 1038 Romanian Government Decision established maximum allowable values for the selected pesticides in river and ground water (0.1ng/ml for azinphos-ethyl, azinphos-methyl, dimethoate, omethoate, demeton-s-methyl, 0.03ng/ml for fenthion and 0.004ng/ml for disulfoton) [4]. The most widely used analytical techniques for the determination of organophosphate insecticides in water samples are based on solid phase extraction (SPE) followed by liquid chromatography coupled with mass spectrometry (LC-MS) [5-7] or gas chromatography coupled with mass spectrometry (GC-MS) [8-10, 12]. Growing concern for Danube water quality is mainly determined by the fact that it is an important source of drinking water for riparian population. Up to now the occurrence of pesticides in the Danube River basin has been investigated by some studies [9, 11].

In this paper we used a previously developed solid-phase extraction followed by triple-quadruple liquid chromatography mass spectrometry (SPE-LC-ESI-

MS/MS) method for simultaneous detection of seven organophosphate insecticides (fenthion, disulfoton, azinphos-ethyl, azinphos-methyl, dimethoate, omethoate, demeton-s-methyl) and one herbicide (bentazon) from Romanian surface water samples (Danube River and three major tributaries: Arges, Jiu, Olt) [3]. The main objective of this research was to investigate the occurrence of some priority pesticides in the Romanian surface rivers. Chemical structures of the selected pesticides are presented in Table 1 with their molecular formula, molecular weight and CAS number.

Table 1. Chemical structure, molecular formula, molecular weight, IUPAC chemical name of the target pesticides

Compounds/ IUPAC chemical name	**Chemical structure**	**Molecular formula/ Molecular weight (g/mol)**	**CAS number**
Omethoate 2-(Dimethoxyphosphoryl)sulfanyl]-*N*-methyl-acetamide		$C_5H_{12}NO_4PS$/ 213.2	1113-02-6
Demethon-s-metyl O,O-Dimethyl *S*-2-(ethylsulfanyl)ethyl phosphorothioate		$C_6H_{15}O_3PS_2$/ 230.3	919-86-8
Fenthion *O,O*-Dimethyl *O*-[3-methyl-4-(methylsulfanyl)phenyl] phosphorothioate		$C_{10}H_{15}O_3PS_2$/ 278.33	55-38-9
Bentazon 3-Isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide		$C_{10}H_{12}N_2O_3S$ / 240.28	25057-89-0
Dimethoate *O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] dithiophosphate		$C_5H_{12}NO_3PS_2$/ 229.26	60-51-5

Disulfoton *O*,*O*-Diethyl *S*-2-(ethylsulfanyl)ethyl phosphorodithioate		$C_8H_{19}O_2PS_3$/ 274.404	298-04-4
Azinphos-ethyl/ (Diethoxyphosphinothioylsulfanylmethyl)-1,2,3-benzotriazin-4-one		$C_{12}H_{16}N_3O_3PS_2$/ 345.38	2642-71-9
Azinphos-methyl/ *O*,*O*-Dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]dithiophosphate		$C_{10}PN_3H_{12}S_2O_3$ 317.324	86-50-0

10. Materials and Methods

Sampling sites and sample collections

The collection of the samples was performed in February 2014. Samples were collected from 10 locations along the Romanian part of the Danube River and from 2 locations from each of the main tributaries, one location being close to their confluence with Danube River, as shown in Fig. 1. All samples were taken from a depth of approximately 0.5 m at 2 m from the river bank. Water samples were collected in 500mL amber PET bottles, previously rinsed with water sample at the sampling site. After collection, the samples were kept at 4°C until arrival to the laboratory and pretreated by solid phase extraction within 48h. Location data, GPS coordinates and description are given in Table 2. Before the analysis the surface waters were vacuum filtered through 0.45µm glass fiber filters.

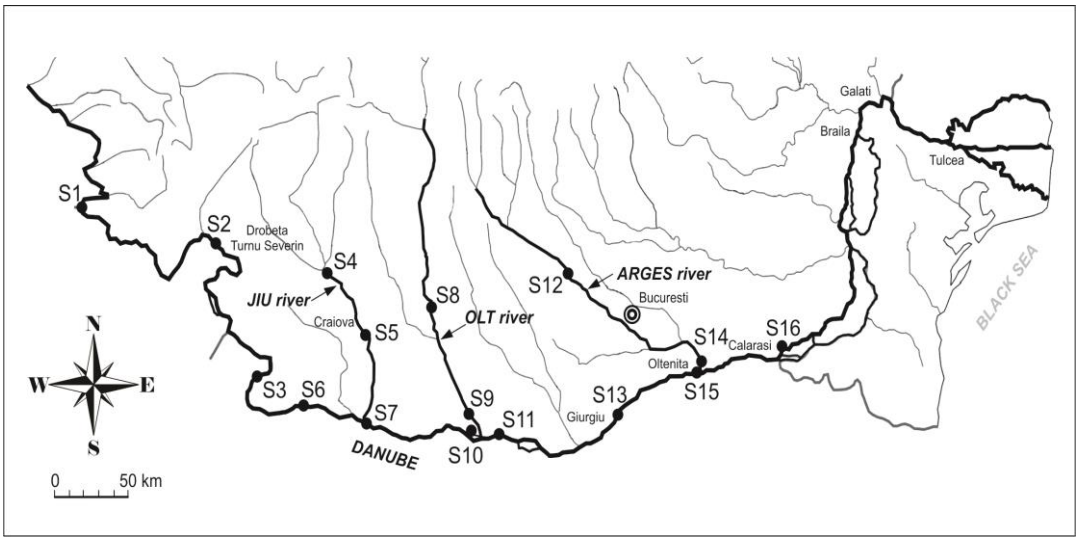


Fig. 1. Map of the study area in the Danube River basin, location and codes of the sampling points

Table 2. Location data, GPS coordinates and description

Sampling point code	*Site name*	*GPS coordinates*	*Description*
S1	Danube	44°47'32.61"N 21°23'20.07"E	Bazias
S2	Danube	44°40'7.40"N 22°33'10.74"E	Gura Vaii, upstream Drobeta -Turnu Severin
S3	Danube	43°57'50.94"N 22°54'15.72"E	Calafat
S4	Jiu River	44°34'8.32"N 23°27'18.14"E	Filiasi, upstream Craiova
S5	Jiu River	44°15'18.48"N 23°47'25.08"E	Podari, downstream Craiova
S6	Danube	43°51'24.84"N 23°17'18.79"E	Rast, upstream of the confluence with Jiu River
S7	Danube	43°45'11.32"N 23°56'30.69"E	Bechet, downstream of the confluence with Jiu River
S8	Olt River	44°23'29.63"N 24°21'4.84"E	Downstream Slatina
S9	Olt River	43°48'41.71"N 24°42'27.71"E	Izbiceni, upstream of the confluence with Danube
S10	Danube	43°42'22.72"N 24°44'4.01"E	Islaz, upstream of the confluence with Olt River
S11	Danube	43°43'2.69"N 24°48'56.65"E	Turnu Magurele, downstream of the confluence with Olt River
S12	Arges River	44°28'45.25"N 25°40'47.87"E	36 km upstream Bucharest
S13	Danube	43°52'37.50"N 25°58'49.92"E	Giurgiu, upstream of the confluence with Arges River
S14	Arges River	44° 6'38.09"N 26°38'15.33"E	upstream of the confluence with Danube River
S15	Danube	44° 3'51.89"N 26°38'45.49"E	Oltenita, downstream of the confluence with Arges River
S16	Danube	44° 8'15.69"N 27°20'8.26"E	Calarasi

Instrumentation

For the LC analysis, an Agilent 1260 HPLC system with a binary pump was used. This was equipped with a C18 analytical column of 100mm×2.1 mm and 3.5 µm particle size (Agilent Zorbax Eclipse Plus C18). The mobile phases, A and B, were ultrapure water with 0.1% formic acid and acetonitrile, respectively. A gradient program was used with the mobile phase, combining solvent A (with 0.1% formic acid in ultrapure water) and solvent B (acetonitrile) as follows: from 10 to 100% B in 6 min, isocratically till 11 min with 100%B. Then the system was equilibrated for 7 min prior to the next injection. The column temperature was kept at 25 °C. The flow rate was constant, 0.25ml/min during the whole process and a volume of 5µl of standard solutions and sample extract was injected in every case. All the analytes were eluted within 11 min. The LC system was connected to a triple quadrupole mass spectrometer Model 6410 Agilent (Agilent Technologies, Waldbronn, Germany) equipped with electrospray ionization (ESI) source, operating in positive and negative ion mode. The optimal MS parameters were as follows: gas temperature, 300^0C; gas flow, 10 L/min; nebulizer gas, 60 psi; capillary voltage, 4500 V. Nitrogen

was served as the nebulizer and collision gas. The analyses were done in the positive ion mode for disulfoton, omethoate, dimethoate, fenthion, demethon-s-methyl, azinphos-methyl azinphos-ethyl and in negative mode for bentazon. MS/MS signal acquisition was performed in Multiple Reaction Monitoring (MRM) mode. For each analyte, two signals were monitored, corresponding to the transition between the precursor ion of the protonated molecule $[M+H]^+$ and deprotonated molecule (only for bentazon, $[M-H]^-$) of the two most abundant product ions. The most abundant one was used for quantification while the other one was used for confirmation. Instrument control and data processing were carried out by means of MassHunter software from Agilent Technologies. MRM transitions, the optimum collision energies and cone voltages selected for each transition are indicated in Table 3.

Table 3. Retention times and MS/MS parameters for the analysis of target pesticides

Compound	***Polarity***	***t_R (min)***	***MRM transitions (m/z)***	***Fragmentor voltage (V)***	***Collision energy (eV)***	***Dwell time (ms)***
Omethoate	ESI +	1.898	214→125 214→183	80 80	15 25	200 200
Demeton-s-methyl	ESI +	6.799	231→89 231→61	80 80	15 15	100 100
Dimethoate	ESI +	6.796	230→199 230→125	80 80	15 25	100 100
Bentazon	ESI -	8.100	239→197 239→175	125 125	15 15	200 200
Azinphos-methyl	ESI +	8.841	318 →132 318 →160	70 70	10 10	200 200
Azinphos-ethyl	ESI +	9.436	346 →132 346 →160	70 70	10 10	50 50
Disulfoton	ESI +	10.136	275→89 275 →61	60 60	10 30	50 50
Fenthion	ESI +	9.743	279 →169 279 →247	60 60	10 15	50 50

Water sample preparation and SPE extraction

The pre-concentration applied to the water sample is based on the off-line SPE procedure described in a previous study with some changes [3]. All water samples were filtered using glass fiber filters to remove particles large than 0.45µm and then kept at 4^0C until analysis. The pH of the samples was adjusted to 4.5 using hydrochloric acid. Solid phase extraction was carried out on a Chromabond® SPE vacuum manifold for 12 cartridges from Macherey-Nagel GmbH (Düren, Germany). Methanol was used to elute the pesticides from the OASIS HLB SPE cartridge (60 mg sorbent/3mL cartridge, Waters, USA). For SPE extraction, prior to sample application, the cartridge was conditioned with 6mL of methanol and 10mL of LC-grade water at pH 4.5. A volume of 500mL of each sample at pH 4.5 was passed through the SPE cartridge at 10mL/min flow rate. After the sample was passed through the cartridge, the adsorbent was, first washed with LC-grade water and then was dried with a nitrogen stream for 10 min. Then, the elution was done with 5ml of methanol. The elution fraction was collected and dried using a gentle stream of

nitrogen and the residue was reconstituted with 1mL methanol and analyzed by LC-MS/MS.

11. Results and Discussion

Analysis of pesticides from real water samples

Sixteen real surface water samples influenced by agriculture were analysed by the developed SPE-LC-MS/MS method. Figures 2a-2c shows the pesticide concentrations measured in the Rivers: Danube, Arges, Olt and Jiu. We represented graphicaly only the pesticide concentrations higher than limit of detection in more than three collection points. Also in figure 3 an LC–MS/MS chromatogram of one surface water sample is depicted for the identification and quantification of the pesticides.

All the pesticides concentrations were situated below the admissible values in conformity with 1038 Romanian Government Decision with the exception of disulfoton that presented concentrations higher than 4 ng/l in seven samples (fig 2c). The disulfoton concentration values ranged from 7.5 ng/l in Arges River, in upstream of confluence with the Danube (S14) to 10.7 ngl in Jiu River, in Podari section (S5). In the Danube River, the disulfoton was determined in two samples at concentrations of 8.3ng/l in Gura Vaii point (S2) and 10 ng/l in Calafat point (S3). The omethoate, bentazon, dimethoate and demeton-s-methyl concentrations slightly decreased along the Danube River except the last two sampling points (Oltenita and Calarasi) where only the omethoate and bentazon increased slightly.

Omethoate and bentazon were the most frequently detected pesticides, as it was found in all surface water samples in concentration range of 6.5-16 ng/l and 0.34-18.8 ng/l, respectively. The bentazon values are similar with the concentrations reported by Loos et al. in Danube River (average of 5ng/l in Danube and 22ng/l in Tributaries Rivers) [9] and by Vassilakis et al. in Havgas River from Greece (10-24ng/l) [10]. Also, the bentazon concentrations determined in Danube and his tributaries rivers are lower than the values reported in Mediteranean Valencian area of Spain (maximum level 420ng/l) [6].

Fenthion insecticide was detected only in one Danube water sample in Bazias section (S1) at concentration of 1.8ng/l. The absence of fenthion in waste water samples discharged in river was observed by other researchers in Spain [5]. Also, azinphos-ethyl was detected in two surface water samples: in Danube River in the Bazias section (0.68ng/l) and in Olt River in Slatina upstream (8.89ng/l). The azinphos-methyl was determined only in one Danube sample in Bazias section (1.3ng/l). This value is similar to the previously reported concentration of 4ng/l for Danube River [9]. The highest pesticides concentrations were determined in Danube River in Clafat point (S3): demeton-s-methyl 19.8 ng/l, omethoate 17ng/l.

The dimethoate concentrations ranged from 0.21 ng/l in Olt River in Slatina downstream (S8) to 2.8ng/l in Danube River in Bazias point. After Bazias, all the dimethoate concentrations decreased along the Danube. The dimethoate and omethoate concentrations are lower than the results reported by other researchers for the Quadalquivir River Basin-Spain (4.46-69.26ng/l for dimethoate and 7.71-11.71ng/L for omethoate) [7].The presence of these compounds in surface water might be explained due to surface water runoff,

which can carry pesticides from area such as agricultural fields and residential properties into, rivers and lakes. The presence of pesticides in water represents a real risk to aquatic system and finally to humans, taking into account that the Danube River and its tributaries are important sources for drinking water production in some Romanian cities.

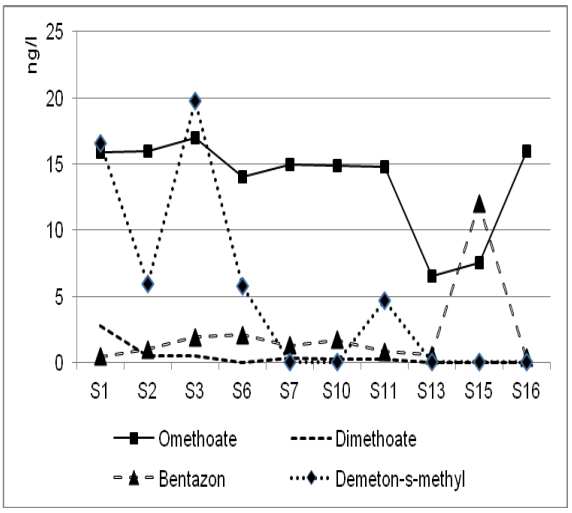


Fig. 2a. Pesticide concentrations detected in Danube River

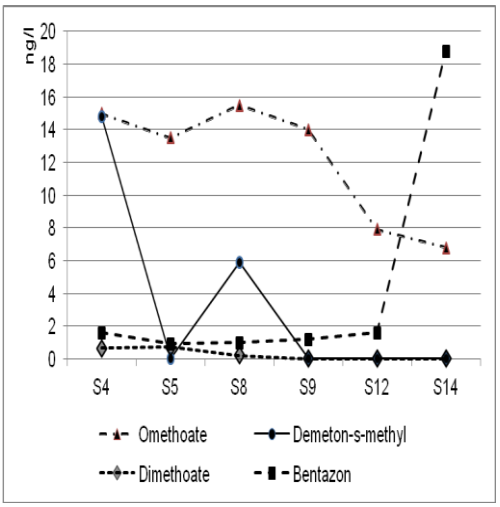


Fig. 2b. Pesticide concentrations detected in Arges, Olt and Jiu rivers

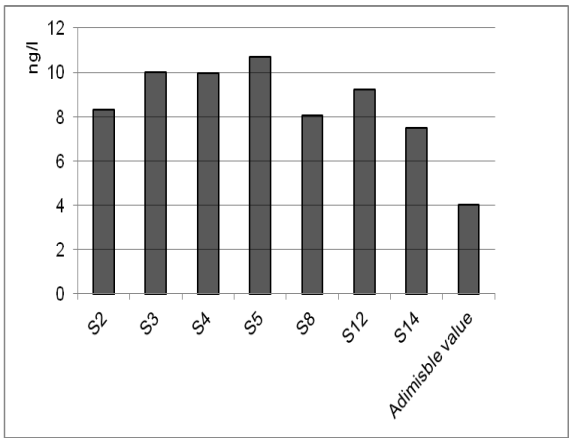


Fig. 2c. Disulfoton concentrations detected in the Danube, Arges, Olt and Jiu rivers

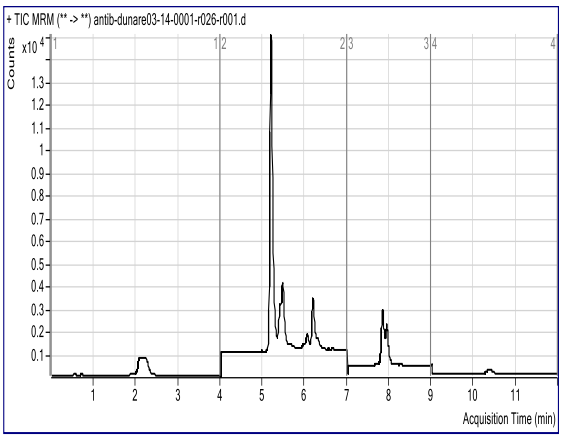


Fig. 3. LC–MS/MS chromatogram of Danube River sample

12. Conclusions

A sensitive and selective LC-ESI-MS/MS method was applied after SPE extraction to investigate the occurrence of 7 organophosphate insecticides and one herbicide in the surface water along the Romanian side of the Danube River and three of its main tributaries (Jiu, Olt, and Arges Rivers). The surface water samples were found to be contaminated by disulfoton over the admissible value (7.5 - 10.7ng/l), demeton-s-methyl (4.67-19.8ng/l), dimethoate (0.21-2.8ng/l). Also, omethoate and bentazon were the most frequently detected pesticides, as it was found in all surface water samples in concentration range of 6.5-16ng/l and 0.34-18.8ng/l, respectively.

Acknowledgements

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References

1. A. DRAGUS, M. S. BELDEAN-GALEA, R. MIHAIESCU, T. MIHAIESCU, D. RISTOIU, (2012), Assessing impacts of triazine pesticides use in agriculture over the well quality*, Environmental Engineering and Management Journal*, **11**, 2, 319-323
2. L. FERENCZ, A. BALOG, (2010), A pesticide survey in soil, water and foodstuffs from central Romania*, Carpathian journal of earth and environmental sciences*, **5**, 1, 111 - 118
3. V. I. IANCU, J. PETRE, L. CRUCERU, (2011), Determination of some organophosphorus insecticides and urea herbicides from water by HPLC, *Journal of Environmental Protection and Ecology,* **12**, 3, 833-840
4. Decision no. 1038, (2010), "*Decision regarding the approval of measures against pollution with chemical substances*", Published in the Official Gazette, Part I no. 746 of 09/11/2010 for amending and completing of the Government Decision no. 351/2005 regarding approval of the Program for the phasing out of discharges, emissions and losses of priority hazardous substances, Government of Romania
5. J. CAMPO, A. MASIA, C. BLASCO, Y. PICO, (2013), Occurrence and removal efficiency of pesticides in sewage treatment plants of four Mediterranean Rivers Basins, *Journal of Hazardous Materials,* **263P**, 146-157
6. J. M. MARIN, J.V. SANCHO., O.J. POZO, F.J. LOPEZ, F. HERNANDEZ, (2006), Quantification and confirmation of anionic, cationic and neutral pesticides and transformation products in water by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry**,** *Journal of Chromatography A*, **1133**, 204–214
7. A. MASIA, J. CAMPO, P. VÁZQUEZ-ROIG, C. BLASCO, Y. PICO, (2013), Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River Basin (Spain), *Journal of Hazardous Materials*, **263P**, 95– 104
8. M. CULEA , I. FENESAN, S. COBZAC, S. GOCAN, M. CHIRIAC, N. PALIBRODA, (1996), Trace analysis of triazines and organophosphorus pesticides in water, *Fresenius Journal of Analytical Chemistry*, **355**, 748–749
9. R. LOOS, G. LOCORO, S. CONTINI, (2010), Occurrence of polar organic contaminants in the dissolved water phase of the Danube River and its major tributaries using SPE-LC-MS2 analysis, *Water Research*, **44**, 2325-2335
10. I. VASSILAKIS, D. TSIPI, M. SCOULLOS, (1998), Determination of a variety of chemical classes of pesticides in surface and ground waters by off-line solid-phase extraction, gas chromatography with electron-capture and nitrogen-phosphorus detection, and high-performance liquid chromatography with post-column derivatization and fluorescence detection*, Journal of Chromatography A*, **823**, 49-58
11. F. VOSNIAKOS, V. GABRIELA, J. PETRE, L. CRUCERU, M. NICOLAU, M. MITRITA, V. IANCU, I. CRUCERU, L.A. VARGA, M. GOLUMBEANU, (2008), Assessment of the Quality of the Danube Delta Aquatic Ecosystem – Surface water and Sediment, *Journal of Environmental Protection and Ecology*, **9**, no. 2, 255-271
12. A. NEZIRI , I. MALOLLARI , E. LAHO , A. PAMBUKU, (2014), , Persistent Organic Pollutants in the Buna River Basin Ground Waters (Albania), *Journal of Environmental Protection and Ecology* , **15**, No 2, 405–411