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Environmental monitoring and risk assessment of pesticide residues in surface waters of the Louros River (N.W. Greece)

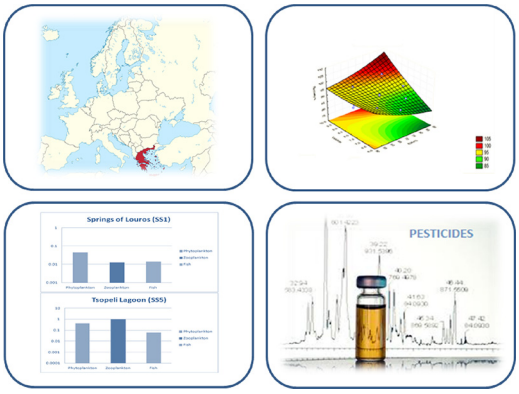
Margarita Kapsi [a], Charoula Tsoutsi [a], Anastasia Paschalidou [b], Triantafyllos Albanis [a,*]

[a] Laboratory of Analytical Chemistry, Department of Chemistry, University of Ioannina, Panepistimioupolis, Ioannina 45110, Greece
[b] Department of Forestry and Management of the Environment and Natural Resources, Democritus University of Thrace, 68200, Greece

HIGHLIGHTS

- A chemometric approach was used for the extraction optimization.
- Pesticide occurrence was studied with a combination of GC–MS/SPE and LC-ESI-MS/SPE.
- One year monitoring survey was carried out.
- Ecological risk assessment associated with detected pesticides was performed.

GRAPHICAL ABSTRACT



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ABSTRACT

Estuarine environments are being constantly stressed by new sources of pollution (e.g. pesticides) derived from activities of industry and intensive agriculture. The present study aims at quantify pesticides of three different categories (fungicides, herbicides and insecticides) in the Louros River (Epirus region, North-Western Greece). A monitoring study of 34 compounds was carried out in surface river waters from June 2011 until May 2012. Seven water sampling stations were established and 35 water samples were collected. A solid-phase extraction (SPE) method coupled with gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC-MS), depending on the compound, was developed and validated. During the monitoring study 25 pesticides were detected (13 herbicides, 9 insecticides, 3 fungicides). The most commonly encountered pesticides were quizalofop-ethyl, trifluralin and pendimethaline. Tebufenpyrad was found in all sampling stations and seasons, with the highest concentrations of 0.330 μg/L at Tsopeli Lagoon exceeding the rather low concentrations reported nationwide. Regarding the environmental risk due to the presence of target compounds in surface waters, this was estimated by calculating risk quotients (RQs) for different aquatic organisms (algae, zooplankton and fish). The results denoted a possible threat for the aquatic environment, rendering in this way the RQ method as a useful screening tool. In any case, further extensive study is needed for acetochlor, pirimiphos-methyl, endosulfan-a and azinphos-ethyl in order to better correlate their occurrence and potential toxic effects in aquatic life and humans.

* Corresponding author.
E-mail address: talbanis@uoi.gr (T. Albanis).

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1. Introduction

A wide variety of pesticides have been used in agriculture and landscape maintenance for controlling insect, bacterial and fungal pests, and for reducing competition from weeds since the middle of the 20th century (Masiá et al., 2014; Wu et al., 2010). Water pollution due to the use of pesticides in agriculture is a priority environmental issue and a cause of major global concern (Herrero-Hernández et al., 2017). Only herbicides represent about 50% of the demand in agricultural chemicals; their prolonged use involves the risk of retention in crops and soils. Besides, because of washing and leaching processes, these substances can pass to the surface and ground waters. Pesticides are primarily transported from agricultural fields to surface waters through surface run-off. The amount lost from fields and transported to surface waters depends on several factors, including soil characteristics, topography, weather, agriculture practices and chemical and environmental properties of individual pesticides (Konstantinou et al., 2006). Chemicals which are sufficiently resistant to degradation and are adequately soluble to be transported on water may reach the sea in significant amounts. Water run-off and river transport are the main processes involved in the land-sea transfer of chemicals (Albanis and Hela, 1998). The uptake of pesticides into watercourses and their propagation through biological chains highlight the importance of monitoring and understanding the fate of herbicides and their degradation products, not only in the areas where they are applied, but also in proximal areas (Tankiewicz et al., 2010; Konstantinou et al., 2006; Wackett, 2007; Gavrilescu, 2005).

Several pesticides are included in the European Union list for priority organic compounds to be monitored from discharges (European Union Directive EC/76/464), while some of them and their transformation products are classified by the IARC (International Agency for Research on Cancer) as potentially carcinogenic to humans (Richardson and Kimura, 2016). In addition a total of 91 pesticides have been listed as confirmed or possible endocrine disruptor (ED) chemicals by the Environment Agency of England and Wales, The German Environment Agency, The European Union Community Strategy for EDs, the Oslo and Paris Commission and the World Wildlife Fund (McKinlay et al., 2008). Causality of detrimental effects on wildlife as a direct consequence of exposure to many ED pesticides is established, and in some cases has been shown to have population level impacts. The European Union has introduced strict directives to protect water quality, such as the REACH Regulation (European Comission, 2006) concerning the Registration, Evaluation, Authorization and Restriction of Chemicals, while Directive 2008/105/EC, on environmental quality standards in the field of water policy, provides a detail of priority substances (33) to be controlled in water, with pesticides making up a third of the list (Herrero-Hernández et al., 2017; Dujaković et al., 2010; EU, 2008). In addition, the EU Regulations for drinking-water quality set a limit in concentration at 0.5 μg/L for the sum of all pesticides and 0.1 μg/L for each individual compound, in order to limit human risks and environmental pollution (Herrero-Hernández et al., 2017; Postigo et al., 2010; Dujaković et al., 2010; Hurtado-Sánchez et al., 2013; EC, 1998).

Pesticides ecological risk assessment is given as a function of environmental exposure and ecotoxicological effects. This is usually expressed as the ratio of the predicted environmental concentration (PEC) to predicted no-effect concentration (PNEC). PEC values are calculated using several models taking into consideration application rates, persistence, leaching, sorption and compound bioaccumulation or directly from monitoring data while PNEC values are usually calculated on the basis of critical concentrations, e.g. EC_{50}, LC_{50} and NOEC (Vryzas et al., 2009).

Several sample preparation techniques, mainly liquid liquid extraction (LLE) (Tankiewicz et al., 2010; Wu et al., 2010; Farajzadeh et al., 2014), solid-phase extraction (SPE) (Andrade-Eiroa et al., 2016a; Kuster et al., 2008; Cruzeiro et al., 2017; Bonansea et al., 2013), solid-phase micro-extraction (SPME) (Souza-Silva et al., 2015; Bonansea et al., 2013; Li et al., 2015), liquid phase micro-extraction (LPME) (Tankiewicz et al., 2010; Pinto et al., 2010; Heftmann et al., 2007; Ahmad et al., 2015) and many more, have been used for the preparation of pesticides from water and other sample matrices. Several Environmental Protection Agency (EPA) methodologies include SPE as the procedure recommended for pretreatment of organic pollutants (EPA Method 1699, 2007). SPE is the most widely used method for the extraction, changing of solvents, cleanup, concentration, fractionation of organic compounds from number of samples, but also cleaning up interferences, thus improving detection sensitivity and reducing matrix effects in Mass Spectrometry (MS) (Andrade-Eiroa et al., 2016b).

MS is recognized as a highly sensitive and specific technique suitable for use in environmental organic analysis (Cacho et al., 2017; Masiá et al., 2014; Robert et al., 2016). Gas Chromatography (GC) (Yang et al., 2010; Domínguez et al., 2016) is often used for the determination of pesticides because of its high resolution and high detector sensitivity. However, some of the pesticides cannot be analyzed via GC methods, due to their thermo-instability (poor volatility and high polarity) (Alder et al., 2006; Kuster et al., 2008). As an alternative, liquid chromatography (LC) (Dujaković et al., 2010; Hurtado-Sánchez et al., 2013; Caldas et al., 2016) equipped with ultra-violet (UV) (Polati et al., 2006; Irace-Guigand et al., 2004), fluorescence (FLD) (Fu et al., 2009; Pinto et al., 2010) and MS (Masiá et al., 2014; Caldas et al., 2016; Hao et al., 2016) detectors provide simple and rapid techniques for analysis.

The present work uses a combination of SPE and GC–MS/ LC-MS as an analytical tool for the screening of 34 pesticides residues in surface waters, including river, lake and sea water. The objectives of this study were: (1) to establish a single extraction procedure using SPE that will allow the multi-residue determination of selected compounds belonging to different chemicals groups in surface waters; (2) to combine this sample preparation step with the use of GC–MS/ LC-MS using the selected ion-monitoring mode (SIM) for the qualification and quantification of the target analytes; (3) to apply the methodology developed for the routine analysis of natural water samples in the framework of an extended water quality monitoring survey that included 7 different sampling stations at Louros River basin (North-Western Greece), during a period of one year; and (4) to assess the ecotoxicological risk in Louros River for three taxonomic groups (algae, zooplankton, fish). The novelty of this work lies, on the one hand, in the chemometric approach used for the optimization of the SPE method by means of experimental design and response surface methodology. On the other hand, the study of 34 pesticides of different action groups simultaneously determined was enlightening for the Epirus region. To the best of our knowledge, this is the first study reporting the occurrence of pesticides in the aquatic region of Epirus and especially in the River Louros, in such an extensive and comprehensive manner. In addition, the collection of a year data (survey monitoring) is essential for the assessment of changes in the fluvial system of Louros River. This area is intensely subjected to anthropogenic activity and the aim of this work has been to provide a better understanding of the fate of pesticides in the environment.

2. Experimental

2.1. Chemicals and reagents

Pesticides were obtained from Sigma-Aldrich (St. Louis, Missouri, USA), and were of high purity grade (>96%), with the only exception that of ethoprophos (93.1%). Acetone and methanol were supplied by Carlo Erba (Milan Italy), methanol (LC-MS grade), water (LC-MS grade) and dichloromethane were purchased by Fisher Scientific (Leicestershire, UK), and ethyl acetate was supplied by Pestiscan (Labscan, Ltd., Dublin, Ireland). All solvents and reagents were analytical

Table 1
Physical-chemical properties of the target pesticides.

Compound	Molecular formula	Group	Solubility in water (mg L^{-1})	LogD (pH 7.4)	Vapor pressure (mPa)	Log Koc
Eptc	$C_9H_{19}NOS$	Herbicide	370	2.80	4500	2.47
Molinate	$C_9H_{17}NOS$	Herbicide	1100	2.34	500	2.27
Propachlor	$C_{11}H_{14}ClNO$	Herbicide	580	2.39	30.6	1.9
Ethoprophos	$C_8H_{19}O_2PS_2$	Insecticide	1300	3.22	78	1.84
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	Herbicide	0.221	4.60	9.5	3.94
Atrazine	$C_8H_{14}ClN_5$	Herbicide	35	2.20	0.039	2.0
Terbuthylazine	$C_9H_{16}ClN_5$	Herbicide	6.6	2.48	0.12	
Disulfoton	$C_8H_{19}O_2PS_3$	Insecticide	25	3.03	7.2	3.12
Dimethenamid-P	$C_{12}H_{18}ClNO_2S$	Herbicide	1450	2.92	2.5	2.54
Chloropyriphos-methyl	$C_7H_7Cl_3NO_3PS$	Insecticide	2.74	4.07	5.6	3.50
Acetochlor	$C_{14}H_{20}ClNO_2$	Herbicide	282	3.50	0.022	2.19
Pirimiphos-methyl	$C_{13}H_{24}N_3O_3PS$	Insecticide	93	2.96	0.68	2.47
Metolachlor	$C_{15}H_{22}ClNO_2$	Herbicide	530	3.48	1.7	2.07
Pendimethaline	$C_{13}H_{19}N_3O_4$	Herbicide	0.33	4.82	1.94	4.24
Quinalphos	$C_{12}H_{15}N_2O_3PS$	Insecticide	17.8	3.30	0.346	3.16
Triadimenol-A	$C_{14}H_{18}ClN_3O_2$	Fungicide	62	3.28	0.02	2.47
Endosulfan-α	$C_9H_6Cl_6O_3S$	Insecticide	0.32	2.60	8.3	4.06
Endosulfan-β	$C_9H_6Cl_6O_3S$	Insecticide	0.32	2.60	8.3	4.03
Endosulfan-sulfate	$C_9H_6Cl_6O_4S$	Insecticide	0.48	−5.59	0.83	3.71
Myclobytanil	$C_{15}H_{17}ClN_4$	Fungicide	132	3.66	0.198	2.71
Azinphos-ethyl	$C_{12}H_{16}N_3O_3PS_2$	Insecticide	4.5	3.96	0.32	3.17
Quizalofop-ethyl	$C_{19}H_{17}ClN_2O_4$	Herbicide	0.31	4.39	0.04	2.73
Acetamiprid	$C_{10}H_{11}ClN_4$	Insecticide	2950	1.11	0.000173	2.30
Buprofezin	$C_{16}H_{23}N_3OS$	Insecticide	0.46	3.87	0.042	3.72
Fenpyroximate	$C_{20}H_{33}NO$	Insecticide	0.023	5.00	0.01	◊
Fluometuron	$C_{10}H_{11}F_3N_2O$	Herbicide	111	2.20	0.125	◊
Iprodione	$C_{13}H_{13}Cl_2N_3O_3$	Fungicide	12.2	2.29	0.0005	2.84
Methoxyfenozide	$C_{22}H_{28}N_2O_3$	Insecticide	3.3	4.75	0.00148	2.60
Spirodiclofen	$C_{21}H_{24}Cl_2O_4$	Insecticide	0.05	6.62	0.0003	4.49
Tetramethrin	$C_{19}H_{25}NO_4$	Insecticide	1.83	3.09	2.1	3.15
Triadimefon	$C_{14}H_{16}ClN_3O_2$	Fungicide	70	3.97	0.02	2.47
Tebufenozide	$C_{22}H_{28}N_2O_2$	Insecticide	0.83	5.35	0.000156	
Tebufenpyrad	$C_{18}H_{24}ClN_3O$	Herbicide	2.39	4.10	0.0016	3.77
Triadimenol	$C_{14}H_{18}ClN_3O_2$	Fungicide	72	3.28	0.0005	2.87

Data predicted with www.chemspider.com and PPDB (https://sitem.herts.ac.uk/aeru/ppdb/en/).
◊ logD at pH 7.4 for Endosulfan sulfate 3.16.

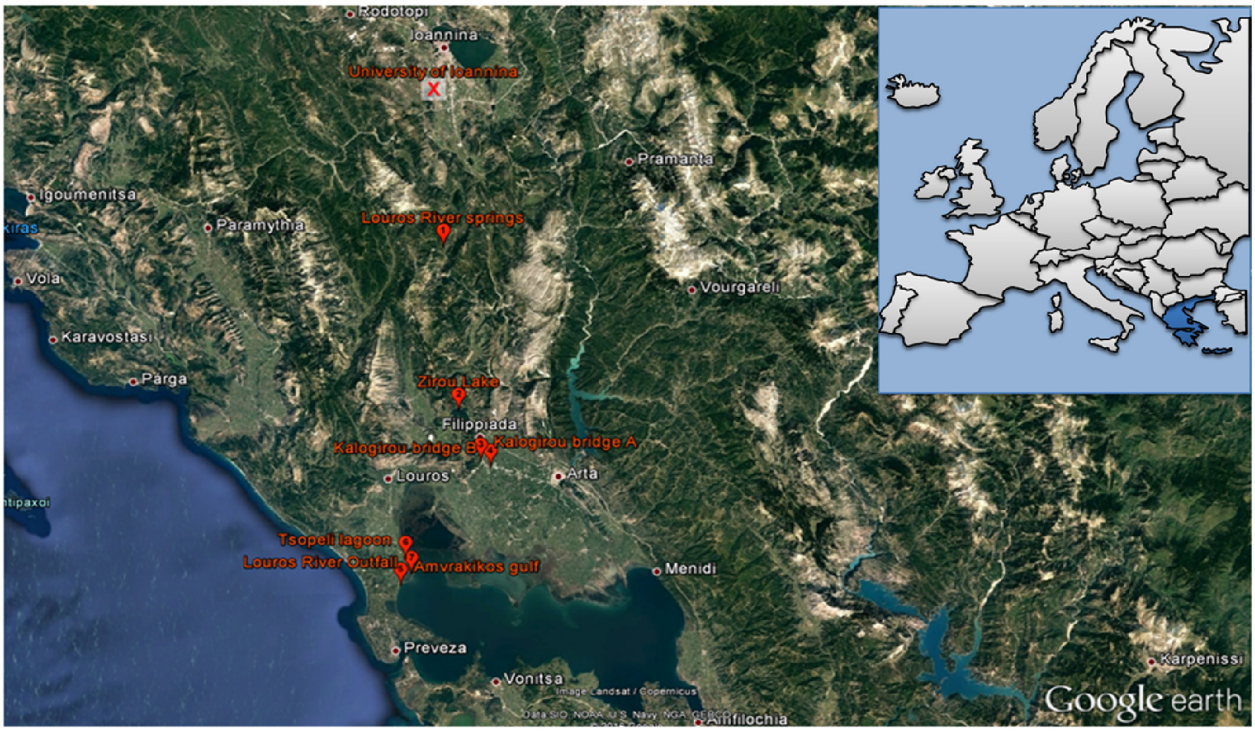

Fig. 1. Area of description.

Table 2
Physicochemical characteristics of selected environmental waters.

Sampling station	pH	Conductivity (μS/cm)	Salinity ‰	TDS (mg L^{-1})
SS1	7.2	317.2	0.0	347.8
SS2	7.2	289.6	0.0	310.4
SS3	7.2	1990	0.8	788.8
SS4	7.4	832.4	0.2	504
SS5	7.2	36,388	25	1789.4
SS6	7.5	28,552	23.9	1570.4
SS7	7.2	3064	1.64	604.8

grade. An individual stock solution of each compound was prepared (2000 mg L^{-1}) in methanol, and two standard mixture solutions, one for LC-ESI-MS and one for GC–MS, of all target analytes were prepared in the same solvent at a concentration of 50 mg L^{-1} and were stored in the dark at −20 °C. Calibration solutions were prepared in LC methanol by appropriate dilution of the above standard solutions. C18 disks were purchased by Agilent Technologies (Santa Clara, California, USA) and sodium sulfate by Merc KGaA (Darmstadt, Germany). Table 1 lists the physicochemical properties of the pesticides investigated. Glass fiber filters (1 μm) and nylon membrane filters (0.45 μm) were purchased from Whatman (United Kingdom).

2.2. Data analysis

A design with two steps (screening and optimization) was used to evaluate and screen the optimal experimental conditions. The main factors affecting the efficiency of the SPE method were the extraction disks, the sample volume, the elution solvent volume, the ratio ethyl acetate: dichloromethane, the pH, the addition of methanol and last but not least, the addition of salt. For this purpose, the STATISTICA 7.0 (StatSoft Inc., Tulsa, USA) statistical package was used to generate the experimental matrix and to evaluate the results.

An experimental Plackett–Burman design was created to determine the main factors affecting the extraction efficiency (expressed as recoveries). This design was applied to evaluate the main effects of the following seven real factors: pH (V_1), elution solvent volume (V_2), ratio ethyl acetate: dichloromethane (V_3), addition of methanol (V_4), volume of sample (V_5), addition of salt (V_6) and sorbent of extraction disks (V_7). Overall, the experimental design included eight experiments with one replication for each experiment. Thus, in total, 16 experiments were carried out.

2.3. Area description

Louros is a river in the Epirus Region, at the North Western part of Greece (Fig. 1). Its springs are in mountain Tomaros and its outfall is in Amvrakikos Gulf which is protected by the Convention of Ramsar (1971) (Konstantinou et al., 2006; Albanis et al., 1995; Albanis and Hela, 1998). Amvrakikos Gulf is the recipient of a runoff basin with total area of 4400 km^2. The total average annual runoff from rainfall into the Amvrakikos Gulf is estimated at 28×10^8 m^3. The run off Basin of Louros River has an area of 952 km^2, its length is 73.52 km and the average annual flow rate has been estimated at 10.6 m^3 s^{-1}. The summer flow is used for irrigation of a section of the Arta plain, which covers a total area of 5500 ha. The lowlands of the Arta plain, on the eastern side of Amvrakikos Gulf, consist of saline soils, and beyond them stretch the agricultural areas with a surface of 74,700 ha (30% citrus fruit trees, 22% olive trees, 9% alfalfa, 14% corn and 7.5% cotton, etc.) (Albanis et al., 1995).

Table 3
Summary data for each pesticide, indicating the minimum, maximum, average concentrations, LOQs of the target compounds and the percentage of positive detections.

Group	Compound	Minimum concentration μg/L	Maximum concentration μg/L	Average concentration μg/L	LOQ μg/L	Positive detections% N = 35
Herbicide	Eptc	0.045	0.157	0.033	0.040	42.85
	Molinate	0.051	0.144	0.023	0.046	22.85
	Propachlor	0.114	0.139	0.081	0.030	65.71
	Trifluralin	0.082	0.084	0.051	0.020	60.0
	Atrazine	0.075	0.077	0.013	0.033	17.14
	Terbuthylazine	0.075	0.087	0.022	0.026	25.71
	Dimethenamid-P	0.048	0.067	0.064	0.017	28.57
	Acetochlor	0.102	0.105	0.026	0.026	25.71
	Metolachlor	0.074	0.077	0.047	0.033	62.85
	Pendimethaline	0.095	0.340	0.085	0.026	68.57
	Quizalofop-ethyl	0.099	0.114	0.083	0.040	73.9
	Tebufenpyrad	0.127	0.337	0.215	0.079	82.85
	Fluometuron	0.172	0.172	0.005	0.059	2.85
Insecticide	Disulfoton	n.d	n.d	0.000	0.033	0.00
	Chloropyriphos-methyl	n.d	n.d	0.000	0.026	0.00
	Pirimiphos-methyl	0.064	0.064	0.026	0.020	40.00
	Tebufenozide	n.d	n.d	0.000	0.059	0.00
	Fenpyroximate	0.098	0.139	0.022	0.092	17.14
	Methoxyfenozide	n.d	n.d	0.000	0.079	0.00
	Quinalphos	0.140	0.142	0.028	0.040	20.00
	Spirodiclofen	n.d	n.d	0.003	0.073	2.85
	Tetramethrin	n.d	n.d	0.000	0.056	0.00
	Endosulfan-α	0.030	0.241	0.039	0.026	45.71
	Endosulfan-β	0.044	0.205	0.610	0.020	51.42
	Endosulfan-sulfate	0.038	0.194	0.071	0.033	54.28
	Azinphos-ethyl	0.075	0.250	0.034	0.017	31.42
	Acetamiprid	n.d	n.d	0.000	0.066	0.00
	Buprofezin	n.d	n.d	0.000	0.050	0.00
	Ethoprophos	0.041	0.044	0.013	0.033	31.43
Fungicide	Triadimenol-A	0.127	0.255	0.089	0.099	60.00
	Triadimenol	n.d	n.d	0.000	0.050	0.00
	Iprodione	0.117	0.265	0.068	0.073	37.14
	Triadimefon	n.d	n.d	0.000	0.099	0.00
	Myclobytanil	0.037	0.063	0.020	0.043	45.71

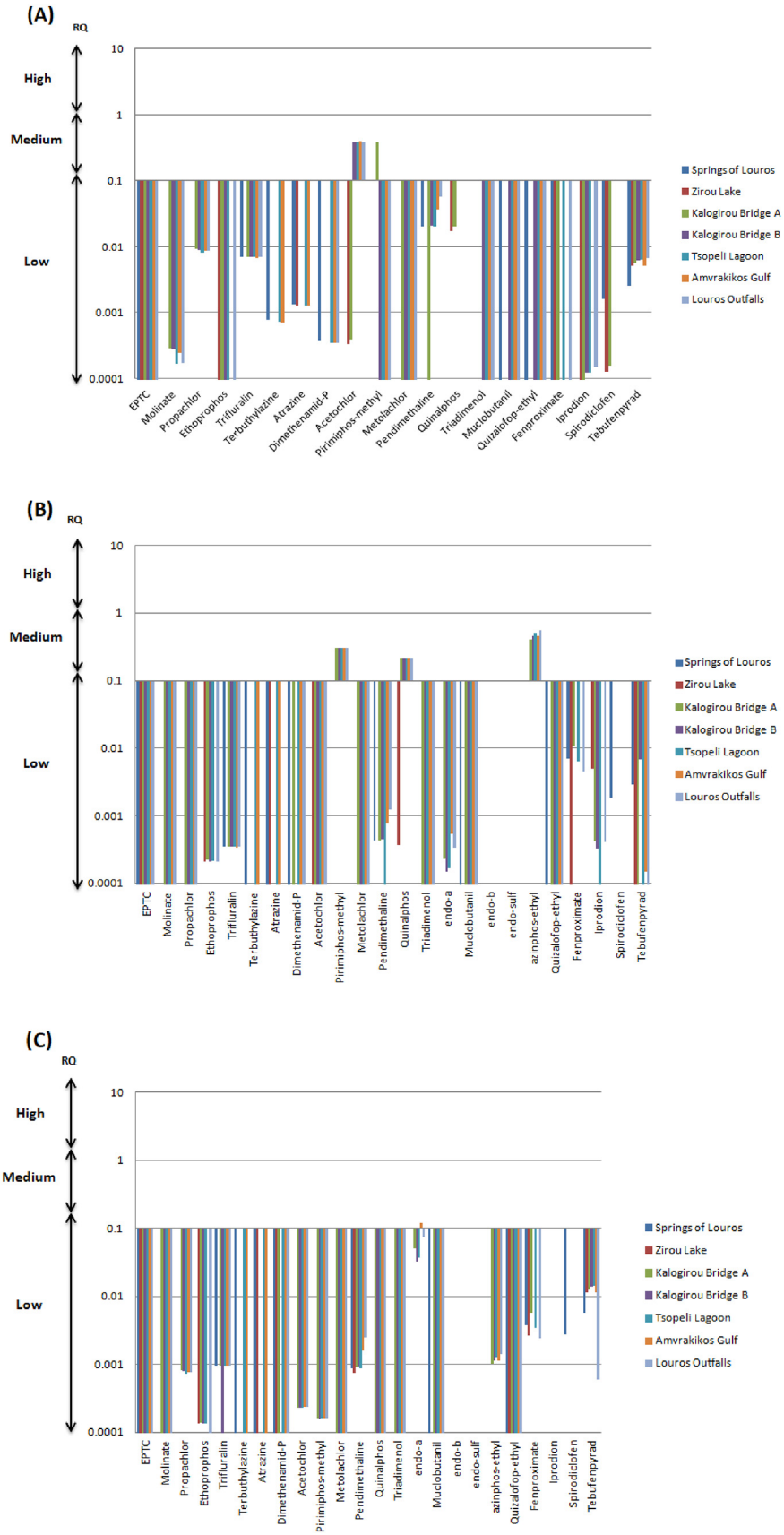

Fig. 2. Risk quotients for pesticides in each sampling station were estimated for algae (A), zooplankton (B) and fish (C).

2.4. Sampling

Seven sample stations were established in order to cover all possible pollution sources of the Louros River (Fig. 1) and were located near points where tributaries meet the main river course. The exact sample stations were SS1: Springs of Louros, SS2: Zirou Lake, SS3: Kalogirou Bridge A, SS4: Kalogirou Bridge B, SS5: Tsopeli Lagoon, SS6: Amvrakikos Gulf and SS7: Louros River Outfalls (Hereafter for brevity reasons they will be referred to as SS1–SS7, respectively). Totally, 35 water samples were collected from the main flow of the Louros River between June 2011 and May 2012. The samples were collected into amber glass bottles (volume 2.5 L) from each sampling station in the mid-depth of the water column. All samples were collected with the aid of Niskin sampler. Their physicochemical characteristics were measured in-situ and their mean value is given in Table 2. After being filled with water, the bottles were sealed with screw caps and were lined with aluminum foil. The bottles were placed in a portable cooler filled with ice and were transported to the laboratory on the same day. The samples were extracted within the next 48 h.

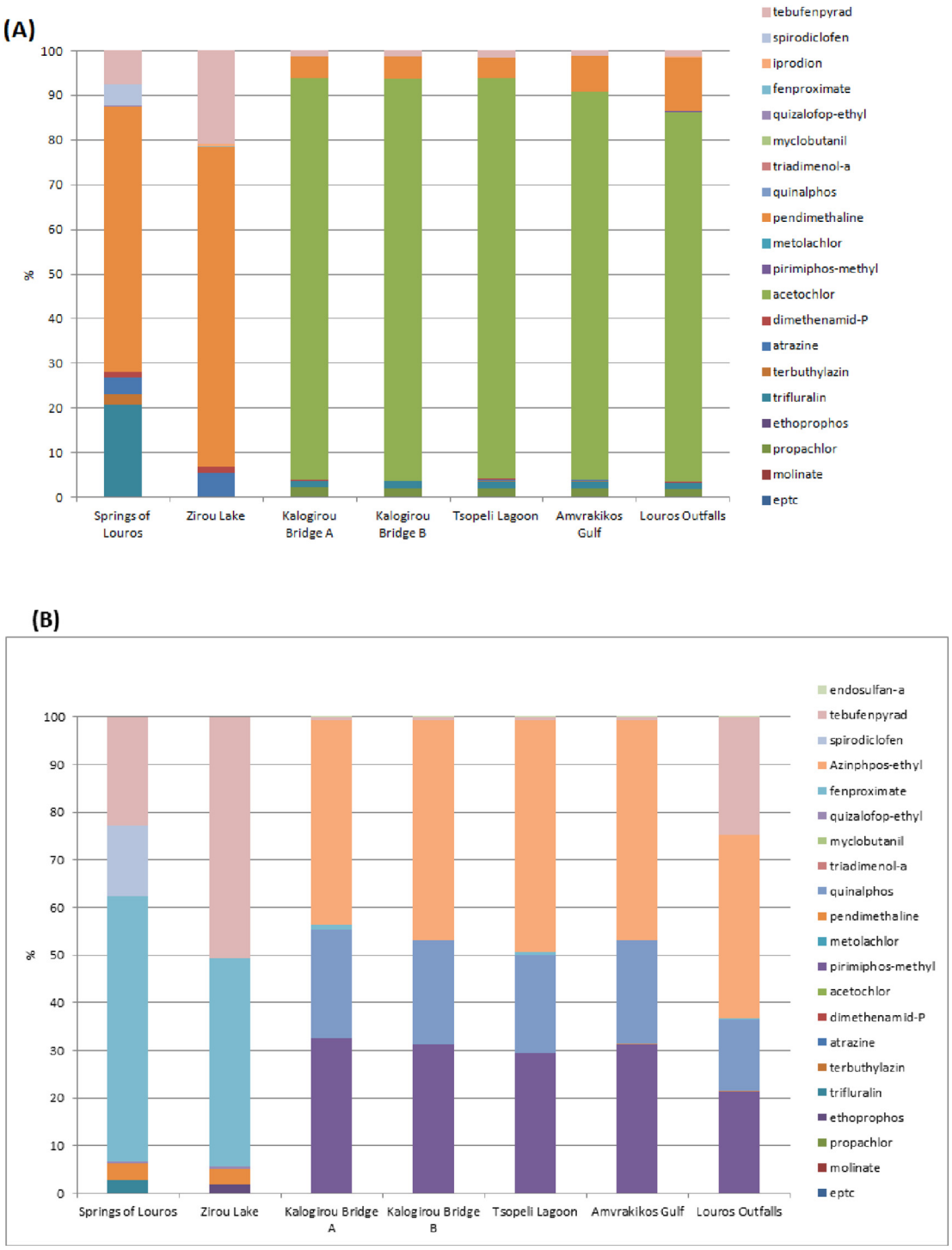

Fig. 3. The contribution of detected compounds in total acute toxicity was estimated for algae (A), zooplankton (B) and fish (C).

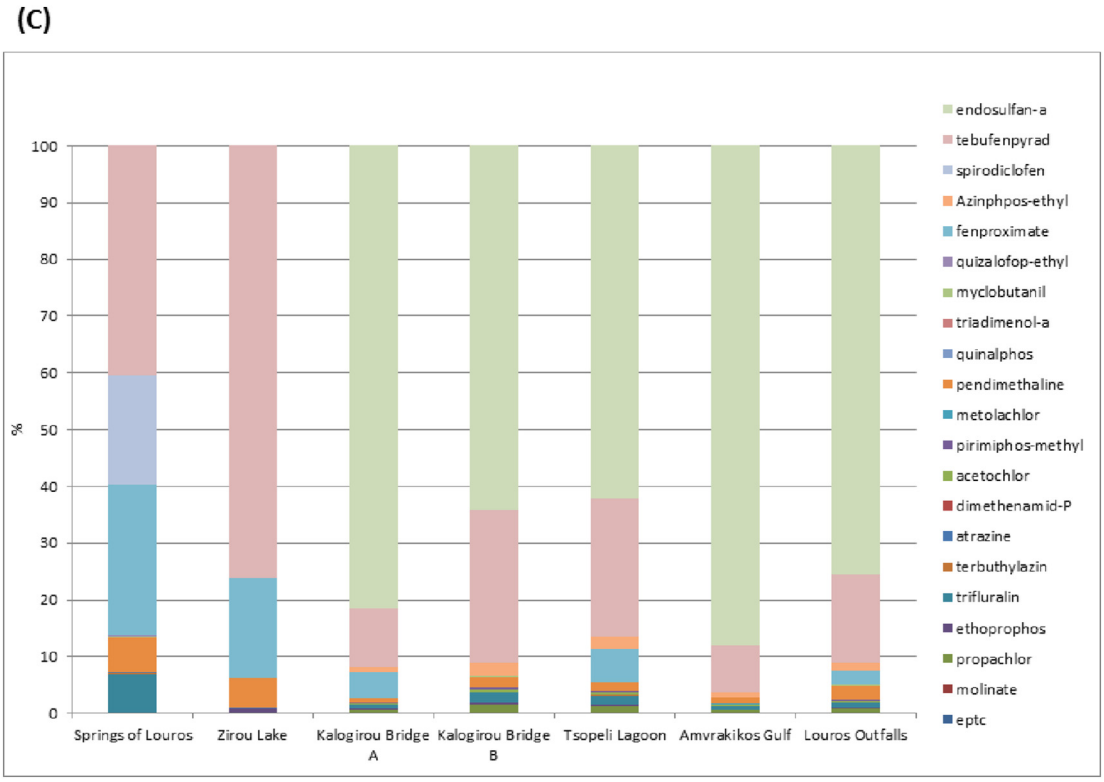

Fig. 3 (*continued*).

2.5. Solid-phase extraction (SPE) procedure

The water samples were vacuum-filtered through 1 μm glass fiber filters GF/B (Whatman, UK) prior to analysis, in order to remove the suspended Solid-matter, avoiding in this way any potential interference during the analysis. At a first stage, different extraction disks were tested, to determine which yielded better recoveries, namely SDB-RPS [Poly(styrenedivinylbenzene)sulphonated] and C18 (Octadecyl) (Erger and Schmidt, 2014; Andrade-Eiroa et al., 2016a). Target analytes were isolated and pre-concentrated from water samples using Empore C18 disks connected to a vacuum pump. For SPE extraction, the evaluation of disks was assessed. Prior to extraction, C18 disks were preconditioned with 10 mL of acetone, followed by 10 mL of ethyl acetate, 10 mL of methanol and 10 mL of deionized water. Before the disk dried out, water samples (230 mL) were passed through the SPE disks, at a flow rate of approximately 10 mL/min, using a vacuum manifold that maintained a constant pressure differential between the inlet and the outlet of the disk. Once the total sample was percolated, disks were rinsed with 2 × 5 mL of deionized water. Afterwards, the disks were dried under vacuum for 10 min to remove residual water, and analytes were eluted with 9 mL of ethyl acetate/dichloromethane (85:15), drop-by-drop, at flow rate of 1 mL/min. The final extract was dried over anhydrous sodium sulfate. The extracts were evaporated to dryness under a gentle stream of nitrogen, reconstituted in 0.1 mL methanol and stored at −20 °C prior to chromatographic analysis.

2.6. Apparatus

2.6.1. Gas chromatography

The analyses were performed using a Trace GC Ultra instrument (Thermo Scientific, Austin, Texas, USA) coupled with an ISQ mass spectrometer controlled by a computer running X-Calibur software. The separation was performed using a DB-5-MS column with a film thickness of 0.25 μm (30 mm × 0.25 mm i.d., Thermo Fisher Scientific, Austin, Texas, USA). Helium (purity > 99.999 vol%, Air Liquid, Greece) was used as the carrier gas at a flow rate of 1 mL/ min. The GC oven temperature program was as follows: initial temperature of 55 °C, 5 °C min^{-1} to 200 °C, 1 °C min^{-1} to 210 °C (held for 2 min), and finally 20 °C min^{-1} to 270 °C (held for 16 min). The injector was set at 220 °C in the splitless mode and the injection volume was 1 μL. The temperatures of the ion source and the interface were set at 240 °C and 290 °C, respectively. The mass spectrometer was operated in the electron ionization mode at ionization energy of 70 eV. In the selected-ion monitoring (SIM) acquisition mode, the target ions were monitored at different time-windows defined by the corresponding retention times. The quality criteria adopted for the retention times of the analytes, as well as the relative intensities of the selected ions were within the tolerances established by the 2002/657/EC directive concerning the performance of analytical methods and the interpretation of results (2002/657/EC, 2002). The retention times, as well as the identification and quantification ions selected for the target compounds are shown in Table S1. Fig. S1 depicts a typical SPE/GC–MS chromatogram obtained from a spiked (1 μg/L) natural water sample.

2.6.2. Liquid chromatography

The analysis was carried out using an SPD 20A UV–Vis detector coupled in series with the LC–MS 2010EV mass selective detector, equipped with an atmospheric pressure ionization source electrospray (ESI) interface. The chromatographic column used for analyte separation was a C18, 150 × 4.6 mm with 5 μm particle size (Restek, USA). The injection volume was set at 20 μL. The samples were analyzed using the ESI interface in positive (PI) ionization mode. For the analysis, a gradient elution was performed by a binary gradient composed of solvent A (methanol with 0.1% formic acid) and solvent B (water LC-MS) according to the following program: Initial conditions 90% B, decreased to 40% in 15 min, decreased to 10% in 5 min, returned to the initial

conditions after 2 min and re-equilibration time was set at 3 min. The total run analysis lasted 40 min, returned to the initial conditions after 2 min and re-equilibration time was set at 3 min. The total run analysis lasted 50 min. Column temperature was set at 40 °C and the flow rate was 0.5 mL/ min. The drying gas was operated at a flow of 10 L min^{-1} at 200 °C. The nebulizing pressure was 100 psi, the capillary voltage was 4500 V and the fragmentation voltage was set at 5 V. For each compound the precursor molecular ion, $[M + H]^+$ for positive ESI and at least one confirming ion in the selected-ion monitoring (SIM) mode was acquired. The most abundant ion was used for quantification, except for tetramethrin and triadimenol (Table S2). Tetramethrin and triadimenol did not show any precursor molecular ion response, so the most intense signal was at *m/z* 306 and 394 respectively. The identification of target compounds was performed by matching the retention time (within 2.5%) and mass spectrum with standards. Fig. S2 depicts a typical SPE/LC–MS chromatogram obtained from a spiked (1 μg/ L) natural water sample.

2.7. Risk quotients (RQ) and ecotoxicological risk assessment

In the scientific literature, many approaches have been suggested for the evaluation of the health risks from exposure to various mixtures of chemicals. However, there is no internationally accepted procedure. The risk of quotient (RQ) (Papadakis et al., 2015; Palma et al., 2009; Ccanccapa et al., 2016) comprises a useful tool for characterizing the potential ecological risk of various contaminants in aquatic ecosystems, and has been developed to quantify the risk exposures of chemicals to specific species. RQ is the quotient of the measured or estimated environmental concentration (exposure) divided by a toxicant reference value (TRV). It is a single-value estimate for screening-level risk assessment at early stage and the RQ of a single pesticide *i* is calculated using worst case assumptions i.e. cases when the highest concentration of the target compound is detected, as follows in Eq. (1):

$$RQ_i = \frac{exposure}{toxicity} + \frac{MEC_i}{TRV_i} + \frac{MEC_i}{LC_{50} \text{ or } EC_{50}} \quad (1)$$

where MEC *i* is the measured environmental concentration of pesticide *i*, and TRV *i* is the toxic reference value (LC_{50} – half lethal concentration for the 50% of the population of the tested species or EC_{50} – effect concentration for the 50% of the population of the tested species) of pesticide. For a mixture of *n* kinds of pesticides, the risk quotient of the mixture (RQ *m*) is calculated as the sum of RQ *i* as follows in Eq. (2):

$$RQ_m = \sum_{i=1}^{n} RQ_r = \sum_{i=1}^{n} \frac{MEC_i}{TRV_i} \quad (2)$$

The sum of risk quotients of each pesticide detected provides a preliminary indication of the total ecological risk to the specific representative species. For risk analysis a commonly used risk ranking criterion is applied. When the ratio between the exposure concentration and the predicted no-effect concentration equals to or exceeds 1, then an ecological "high risk" is suspected (RQ ≥ 1). Similar criteria proposed by Carazo-Rojas et al. (2018) describe that when 0.01 < RQ < 0.1 "low risk" is suspected and when 0.1 < RQ < 1 "medium risk" is suspected.

3. Results and discussion

3.1. Experimental design

Analysis of variance (ANOVA) was performed to examine whether the studied experimental factors were significant in the performance of the method proposed. An effect was considered significant when it was above the standard error at the 95% confidence level ($p < 0.05$). According to the Pareto chart and analysis of variance, three factors were found to be significant and were evaluated in the central composite design for further analysis (Fig. S3). More specifically, the elution solvent volume ($p = 0.010$) and the ratio ethyl acetate: dichloromethane ($p = 0.043$) had a positive effect, while the sample volume ($p = 0.008$) had a negative effect. According to the results obtained in the investigated experimental domain (Fig. S4), pH ($p = 0.116$), addition of methanol ($p = 0.606$), addition of salt ($p = 0.407$) and sorbent of extraction disks ($p = 0.260$) had no significant impact on the extraction yield and, therefore, they were kept fixed for further analysis. In particular, C18 was chosen as the extraction sorbent, pH was adjusted at 7, while on the other hand there was no addition of methanol and salt in aqueous samples.

3.1.1. Central composite design

Following the results of the previous design, the following step was to optimize the analytical method for the remaining three factors by employing a central composite design (CCD). Seventeen experiments were required in this design with three central points. The conditions set in each experiment are listed in Table S3.

The main effects, interaction effects, and quadratic effects were evaluated through analysis of variance (ANOVA) at a spiked concentration of 1 μg/ L. The lack of fit, which measures the failure of the model to represent the data in the experimental domain at points that are not included in the regression, was also evaluated and shown to be not significant (p value = 0.061), indicating the good response of the model. A summary of the ANOVA is given in Table S4. The Pareto chart (Fig. S5) shows linear effects, quadratic effects and interaction between factors.

The reflected interaction between the sample volume (Vs) and the elution solvent volume (Ve) (Fig. S4) showed that the highest extraction efficiency (recoveries) was obtained at a sample volume of 200–300 mL and at the highest levels of elution solvent volume. This observation was also confirmed by other research groups (Albaseer et al., 2010; Primel et al., 2012; Soriano et al., 2001). The interaction between the elution solvent volume (Ve) and the ratio of ethyl acetate: dichloromethane (EtAc %) (Fig. S4) showed that the highest extraction efficiency was obtained at the highest levels of both Ve and EtAc %.

The overall optimal extraction conditions were calculated by the software (99.9% average recovery of the target analytes) to comprise a 230 mL sample volume, 9 mL of elution solvent volume, 85:15 of ethyl acetate: dichloromethane ratio, C18 as extraction disks, pH 7, and 0% addition of methanol or salt.

The enrichment factors (EFs), were used to evaluate the extraction yield under different experimental conditions. The (EFs) were defined as the ratio between the analyte concentration in extraction solvent after the extraction process and the concentration of analyte in the water before the extraction process taking into account the recovery of each compound. Enrichment factors (EFs) were in the range of 1548–2415. For instance, the enrichment factor of dimethenamid-P was 2415 at the low concentration level while the recovery was 104.99% and the preconcentration factor (PCF) 2300 (Table S5). In addition, for ethoprophos the Enrichment factor was 1733, taking into account the recovery at the low concentration level 75.33% and the preconcentration factor (PCF) 2300.

3.2. Analytical performance of SPE

The Limit of detection and quantification (LOD and LOQ) of the proposed method were based on a signal-to-noise ratio of 3 ($S/N \geq 3$) and 10 ($S/N \geq 10$), respectively. The detection limit of the method ranged from 5 to 30 ng/ L, while the quantification limits ranged between 17 and 99 ng/ L (Table S5). The intra-day repeatability ranged between 1.6 and 4.9%, while the inter-day reproducibility ranged between 2.3 and 4.9% (Table S5). The trueness of the method was evaluated by recovery studies. The recoveries were calculated at three different concentrations. The water samples were spiked at concentrations of LOQ, 5

× LOQ and 15 × LOQ for each compound. Table S5 shows the results from these concentration levels.

3.3. Real sample analysis

Descriptive statistics (minimum, maximum, average) as well as the LOQs of the method and the positive detections for each compound in the 35 samples are displayed in Table 3 by category, i.e., herbicides, insecticides and fungicides. During the monitoring study a number of 25 pesticides were detected (Table 3): 13 herbicides, 9 insecticides, 3 fungicides. Herbicides were the most frequently detected pesticides, followed by the insecticides and the fungicides. The most abundant herbicides detected, were tebufenpyrad > quizalofop ethyl > pendimethaline > propachlor > metolachlor > trifluralin > eptc > dimethenamid-P > acetochlor > terbuthylazine > atrazine > fluometuron. Regarding insecticides endosulfan-sulfate > endosulfan-β > endosulfan-α > pirimiphos-methyl > ethoprophos > azinphos-ethyl > quinalphos > fenpyroximate > spirodiclofen. Finally, for fungicides the order was triadimenol-A > myclobytanil > iprodione. The frequency of detection and the mean concentrations of these compounds indicate that they are probably the most used ones and readily transported into the Louros River. This is due to the higher application rate of herbicides compared to the other pesticide classes, their relatively high polarity and their persistence (Stehle et al., 2013) and this has been also reported in other recent monitoring studies (Herrero-Hernández et al., 2013; Moschet et al., 2014).

Tebufenpyrad was found in all sampling stations and seasons during the whole monitoring survey with its highest concentration (0.330 μg/L) detected at SS5 (Tsopeli Lagoon) in March of 2012. It was detected far more frequently than any other pesticide (82.85% detection frequency) and that can be attributed to the fact that some of the major crops in the area citrus fruits, olives, corn, alfalfa and cotton (Albanis et al., 1995). Similarly, quizalofop-ethyl was found in all sampling stations during the whole campaign (except for the SS4, during March of 2012) and its highest concentration (0.114 μg/L) was recorded in May of 2012 at SS6 (Outfalls of Louros River). This finding indicates the continuous and intensive use of tebufenpyrad and quizalofop-ethyl throughout the year. On the other hand, 9 pesticides (disulfoton, chloropyriphos-methyl, tebufenozide, methoxyfenozide, tetramethrin, acetamiprid, buprofezin, triadimenol, triadimefon) were not detected in any of the sampling stations. Their low water solubility and surface runoff combined with their moderate –to- low persistence in soil due to both biotic and abiotic degradation could explain the low concentrations detected despite its widespread use (Lewis et al., 2016). Interestingly, the herbicide atrazine was detected in 17.4% of the samples with average concentration 0.013 μg/L. The use of terbuthylazine has increased in recent years replacing atrazine, and has been detected in natural waters (Gonçalves et al., 2007). In this work its positive detection was 25.71%. However, atrazine concentrations in earlier works have always been higher than terbuthylazine concentrations, contrary to the results obtained here (Table 2) possibly due to the fact that atrazine was banned in the EU in 2004 and finally retired from the market in 2007 (Decision 2004/248/CE). Azinphos-ethyl was found in 31.42% of the samples with average concentration of 0.034 μg/L (Table 3). Both atrazine (banned in 2004) and azinphos-ethyl (banned on 2006) were not registered in Greece during the monitoring period, suggesting illegal use, according to Directive 91/414, (EEC, 2002). In a recent survey performed by the Greek ministry of agriculture, almost 30% of the farmers stated that they either used or they were aware of other farmers using illegal pesticides (Ministry of Agricultural Development and Food, 2014). Thus, it is apparent that the detection of atrazine and azinphos-ethyl could be the result of illegal pesticides use.

It is noted that in previous monitoring studies (Albanis et al., 1995; Albanis and Hela, 1998) that took place in the same study field from March 1992 to February 1993 4 herbicides atrazine, metolachlor, molinate and trifluralin were also detected with mean concentrations ranging from 13.8 ng/L to 69.6 ng/L.

Regarding the seasonality of pesticide occurrence, during the wet season (such as the months of March, May, and November) the rain water washes away herbicides and insecticides used in the farming process and then transfers them into the Louros River through surface runoff. According to McGrath et al. (2008), the timing and intensity of precipitation events strongly control pesticide migration into rivers and ground waters, as these meteorological phenomena are known to trigger rapid flows processes, such as surface runoff and preferential flow. This is probably the main reason for the increased pesticides concentrations detected during the wet season in the area studied here. In addition, the sum of concentrations of the pesticides detected was recorded higher at Amvrakikos Gulf and Louros Outfalls as these two sampling stations constitute the recipient of Louros River.

3.4. Ecotoxicological risk assessment of pesticides

The aquatic risk assessment for the pesticides detected was assessed on the basis of the risk quotient method (RQ). The pesticides concentrations detected in the water bodies were divided by an effect level reported in the literature: This approach provides an estimate of the contribution of the compound of interest (expressed as toxic units, TU) to the total toxicity of the water sample analyzed to a certain taxonomic group (Hela et al., 2005).

Specifically, the mean reported effect level for a certain taxonomic group was used. The toxicity assessment is a composite of the toxicology of selected pesticides for characteristic species of the aquatic ecosystem at three environmental levels (e.g. algae, zooplankton, and fish) according to directive 414/91/EEC. Rainbow trout (*Raphidocelis subcapitata*) was selected as the more representative fish species for the Greek rivers and lakes. *Daphnia magna* was selected for the zooplankton category, as it is representative of aquatic insects and other invertebrates. The Pesticide Properties Data Base (PPDB) (Lewis et al., 2016) was used for the toxicological endpoint due to its comprehensive data on algae, zooplankton and fish toxicity and because it is extensive in the number of compounds it covers. Results for the RQ values for algae, zooplankton, and fish for each sampling station are reported in Fig. 2.

According to the results obtained for the three levels of aquatic life, for algae, all compounds pose low risk ($0.01 < RQ < 0.1$) at all stations except for acetochlor in sampling stations SS4–SS7 that show medium risk ($0.1 < RQ < 1$). Similarly, pirimiphos-methyl displays medium risk in SS3. Regarding zooplankton, all compounds pose low risk except for pirimiphos-methyl, endosulfan-a and azinphos-ethyl in SS4–SS7. Last but not least, fish are under medium risk by endosulfan-a at SS2, while the rest of the compounds show no risk for the specific taxonomic group in all sampling stations. The figure, demonstrates the risk that is posed from the pesticides in all sampling stations.

Concerning the percentage of contribution of each compound to the total toxicity, for algae it is apparent that in SS1 acetochlor contributes 50% and tebufenpyrad 15%. On the other hand the contribution in SS2 is calculated 80% and 22%, respectively. In the rest of the sampling stations, acetochlor contributes >80% to the total toxicity. Moving on to the zooplankton, the relative contribution of the pesticides detected is different, as fenpyroximate affects zooplankton in SS1 and SS2, with the percentages reaching 55% and 45% respectively. Tebufenpyrad contributes to the total toxicity by 50% in SS2 and only 23% in SS1. For the rest of the sampling stations, similar trends are observed as, 3 pesticides contribute almost 98% to the total toxicity. At SS3–SS5 azinphos-ethyl, fenpyroximate and pirimiphos-methyl contribute 46%, 20% and 33% respectively. A small decrease to the contribution of azinphos-ethyl is observed as tebufenpyrad contributes 20% to the total toxicity. Finally, regarding fish, acetochlor contributes >50% to the total toxicity at sampling stations SS3–SS7. Remarkable is the contribution of azinphos-ethyl in SS1 and SS2. In these two sampling stations fenpyroximate contributes 28% and 14%, respectively. Fig. 3 illustrates the %contribution of

all compounds in all sampling stations regarding the three selected species.

The aforementioned results highlight the importance of the study in protecting aquatic ecosystem. However, it should be born in mind that this estimation of RQs is rather simplistic, as the calculations are made for each compound separately, without taking under consideration the fact that in the aquatic environment pesticides are present in a big variety of various classes which may lead to toxicity risks that did not result in single compounds.

4. Conclusions

In the present study, solid-phase extraction (SPE) coupled with GC–MS and LC-MS was evaluated for the simultaneous determination of pesticides residues in real water samples. Real Sample analysis showed that the detection of pesticides as well as the level of concentrations of the selected compounds was increased along the flow of the River due to human activity, with the herbicides dominating in pesticide concentrations. Additionally, the seasonality of pesticide occurrence confirmed the impact of meteorology on their transport and propagation. The ecotoxicological approach used to assess the hazard of pesticides at maximum concentration reflected a potential risk, especially for zooplankton. On the whole this study highlighted the need for monitoring studies in aquatic environments using advanced analytical methods combined with ecotoxicological risk assessment to produce baseline data for implementation of pollution control measures, especially as evidence of illegal use of pesticides was identified in the specific area. In future work, more substances and more sampling stations need to be included in the analyses so that prioritization of contaminants can be feasible.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.185.

References

2002/657/EC, C.D., 2002. Commission Decision 2002/657. 96/23/Ec Comm. Decis. 29.
Ahmad, W., Al-Sibaai, A.A., Bashammakh, A.S., Alwael, H., El-Shahawi, M.S., 2015. Recent advances in dispersive liquid-liquid microextraction for pesticide analysis. TrAC Trends Anal. Chem. 72, 181–192.
Albanis, T.A., Hela, D.G., 1998. Pesticide concentrations in Louros River and their fluxes into the marine environment. Int. J. Environ. Anal. Chem. 70, 105–120.
Albanis, T.A., Danis, T.G., Hela, D.G., 1995. Transportation of pesticides in estuaries of Louros and Arachthos rivers (Amvrakikos Gulf, N. W. Greece). Sci. Total Environ. 171, 85–93.
Albaseer, S.S., Nageswara Rao, R., Swamy, Y.V., Mukkanti, K., 2010. An overview of sample preparation and extraction of synthetic pyrethroids from water, sediment and soil. J. Chromatogr. A 1217, 5537–5554.
Alder, L., Greulich, K., Kempe, G., Vieth, B., 2006. Residue analysis of 500 high priority pesticides: better by GC–MS or LC-MS/MS? Mass Spectrom. Rev. 25, 838–865.
Andrade-Eiroa, A., Canle, M., Leroy-Cancellieri, V., Cerdà, V., 2016a. Solid-phase extraction of organic compounds: a critical review. Part II. TrAC Trends Anal. Chem. 80, 655–667.
Andrade-Eiroa, A., Canle, M., Leroy-Cancellieri, V., Cerdà, V., 2016b. Solid-phase extraction of organic compounds: a critical review (Part I). TrAC Trends Anal. Chem. 80, 641–654.
Bonansea, R.I., Amé, M.V., Wunderlin, D.A., 2013. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquía River basin (Argentina). Chemosphere 90, 1860–1869.
Cacho, J.I., Campillo, N., Vinas, P., Hernandez-Cordoba, M., 2017. In situ ionic liquid dispersive liquid-liquid microextraction coupled to gas chromatography–mass spectrometry for the determination of organophosphorus pesticides. J. Chromatogr. A 1559, 95–101.
Caldas, S.S., Rombaldi, C., De Oliveira Arias, J.L., Marube, L.C., Primel, E.G., 2016. Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. Talanta 146, 676–688.
Carazo-Rojas, E., Pérez-Rojas, G., Pérez-Villanueva, M., Chinchilla-Soto, C., Chin-Pampillo, J.S., Aguilar-Mora, P., Alpízar-Marín, M., Masís-Mora, M., Rodríguez-Rodríguez, C.E., Vryzas, Z., 2018. Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. Environ. Pollut. 241, 800–809.
Ccanccapa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the Ebro River basin: occurrence and risk assessment. Environ. Pollut. 211, 414–424.
Cruzeiro, C., Amaral, S., Rocha, E., Rocha, M.J., 2017. Determination of 54 pesticides in waters of the Iberian Douro River estuary and risk assessment of environmentally relevant mixtures using theoretical approaches and *Artemia salina* and *Daphnia magna* bioassays. Ecotoxicol. Environ. Saf. 145, 126–134.
Domínguez, I., Romero González, R., Arrebola Liébanas, F.J., Martínez Vidal, J.L., Garrido Frenich, A., 2016. Automated and semi-automated extraction methods for GC–MS determination of pesticides in environmental samples. Trends Environ. Anal. Chem. 12, 1–12.
Dujaković, N., Grujić, S., Radišić, M., Vasiljević, T., Laušević, M., 2010. Determination of pesticides in surface and ground waters by liquid chromatography-electrospray-tandem mass spectrometry. Anal. Chim. Acta 678, 63–72.
EC, 1998. Council directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off. J. Eur. Communities 330 (32), 1–23.
EEC, 2002. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on market. URLS. eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A31991L0414.
EPA Method 1699, 2007. Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS. U.S. Environ. Prot. Agency, Off. Water, Washington, DC, USA, pp. 1–96.
Erger, C., Schmidt, T.C., 2014. Disk-based solid-phase extraction analysis of organic substances in water. TrAC Trends Anal. Chem. 61, 74–82.
EU, 2008. Directive 2008/105/EC. Off. J. Eur. Union 006, 235–248 (WWW Document).
European Comission, 2006. Regulation (EC) 1907/2006 of the European Parliament and of the Council of 18 December 2006 - REACH. Off. J. Eur. Union 396–849.
Farajzadeh, M.A., Khoshmaram, L., Nabil, A.A.A., 2014. Determination of pyrethroid pesticides residues in vegetable oils using liquid-liquid extraction and dispersive liquid-liquid microextraction followed by gas chromatography-flame ionization detection. J. Food Compos. Anal. 34, 128–135.
Fu, L., Liu, X., Hu, J., Zhao, X., Wang, H., Wang, X., 2009. Application of dispersive liquid-liquid microextraction for the analysis of triazophos and carbaryl pesticides in water and fruit juice samples. Anal. Chim. Acta 632, 289–295.
Gavrilescu, M., 2005. Fate of pesticides in the environment and its bioremediation. Fate Pestic. Environ. Its Bioremediation. 5. https://doi.org/10.1002/elsc.200520098.
Gonçalves, C.M., Esteves da Silva, J.C.G., Alpendurada, M.F., 2007. Evaluation of the pesticide contamination of groundwater sampled over two years from a vulnerable zone in Portugal. J. Agric. Food Chem. 55, 6227–6235.
Hao, C., Noestheden, M.R., Zhao, X., Morse, D., 2016. Liquid chromatography–tandem mass spectrometry analysis of neonicotinoid pesticides and 6-chloronicotinic acid in environmental water with direct aqueous injection. Anal. Chim. Acta 925, 43–50.
Heftmann, E., Lambropoulou, D.A., Albanis, T.A., 2007. Liquid-phase micro-extraction techniques in pesticide residue analysis. J. Biochem. Biophys. Methods 70, 195–228.
Hela, D.G., Lambropoulou, D.A., Konstantinou, I.K., Albanis, T.A., 2005. Environmental monitoring and ecological risk assessment for pesticide contamination and effects in Lake Pamvotis, northwestern Greece. Environ. Toxicol. Chem. 24, 1548–1556.
Herrero-Hernández, E., Andrades, M.S., Álvarez-Martín, A., Pose-Juan, E., Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., 2013. Occurrence of pesticides and some of their degradation products in waters in a Spanish wine region. J. Hydrol. 486, 234–245.
Herrero-Hernández, E., Rodríguez-Cruz, M.S., Pose-Juan, E., Sánchez-González, S., Andrades, M.S., Sánchez-Martín, M.J., 2017. Seasonal distribution of herbicide and insecticide residues in the water resources of the vineyard region of La Rioja (Spain). Sci. Total Environ. 609, 161–171.
Hurtado-Sánchez, M.C., Romero-González, R., Rodríguez-Cáceres, M.I., Durán-Merás, I., Frenich, A.G., 2013. Rapid and sensitive on-line solid phase extraction-ultra high performance liquid chromatography–electrospray-tandem mass spectrometry analysis of pesticides in surface waters. J. Chromatogr. A 1305, 193–202.
Irace-Guigand, S., Aaron, J.J., Scribe, P., Barcelo, D., 2004. A comparison of the environmental impact of pesticide multiresidues and their occurrence in river waters surveyed by liquid chromatography coupled in tandem with UV diode array detection and mass spectrometry. Chemosphere 55, 973–981.
Konstantinou, I.K., Hela, D.G., Albanis, T.A., 2006. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. Environ. Pollut. 141, 555–570.
Kuster, M., López de Alda, M.J., Barata, C., Raldúa, D., Barceló, D., 2008. Analysis of 17 polar to semi-polar pesticides in the Ebro river delta during the main growing season of rice by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. Talanta 75, 390–401.
Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. Int. J. 22, 1050–1064.
Li, S., Lu, C., Zhu, F., Jiang, R., Ouyang, G., 2015. Preparation of C18 composite solid-phase microextraction fiber and its application to the determination of organochlorine pesticides in water samples. Anal. Chim. Acta 873, 57–62.
Masiá, A., Blasco, C., Picó, Y., 2014. Last trends in pesticide residue determination by liquid chromatography-mass spectrometry. Trends Environ. Anal. Chem. 2, 11–24.

McGrath, G.S., Hinz, C., Sivapalan, M., 2008. Modeling the effect of rainfall intermittency on the variability of solute persistence at the soil surface. Water Resour. Res. 44.
McKinlay, R., Plant, J.A., Bell, J.N.B., Voulvoulis, N., 2008. Endocrine disrupting pesticides: implications for risk assessment. Environ. Int. 34, 168–183.
Ministry of Agricultural Development and Food, 2014. Results From the Data Collection on Professional Pesticide Use (in Greek).
Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C., Hollender, J., 2014. How a complete pesticide screening changes the assessment of surface water quality. Environ. Sci. Technol. 48, 5423–5432.
Palma, P., Kuster, M., Alvarenga, P., Palma, V.L., Fernandes, R.M., Soares, A.M.V.M., López de Alda, M.J., Barceló, D., Barbosa, I.R., 2009. Risk assessment of representative and priority pesticides, in surface water of the Alqueva reservoir (south of Portugal) using on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. Environ. Int. 35, 545–551.
Papadakis, E.N., Vryzas, Z., Kotopoulou, A., Kintzikoglou, K., Makris, K.C., Papadopoulou-Mourkidou, E., 2015. A pesticide monitoring survey in rivers and lakes of northern Greece and its human and ecotoxicological risk assessment. Ecotoxicol. Environ. Saf. 116, 1–9.
Pinto, M.I., Sontag, G., Bernardino, R.J., Noronha, J.P., 2010. Pesticides in water and the performance of the liquid-phase microextraction based techniques. A review. Microchem. J. 96, 225–237.
Polati, S., Bottaro, M., Frascarolo, P., Gosetti, F., Gianotti, V., Gennaro, M.C., 2006. HPLC-UV and HPLC-MSn multiresidue determination of amidosulfuron, azimsulfuron, nicosulfuron, rimsulfuron, thifensulfuron methyl, tribenuron methyl and azoxystrobin in surface waters. Anal. Chim. Acta 579, 146–151.
Postigo, C., López de Alda, M.J., Barceló, D., Ginebreda, A., Garrido, T., Fraile, J., 2010. Analysis and occurrence of selected medium to highly polar pesticides in groundwater of Catalonia (NE Spain): an approach based on on-line solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry detection. J. Hydrol. 383, 83–92.
Primel, E.G., Caldas, S.S., Escarrone, A.L.V., 2012. Multi-residue analytical methods for the determination of pesticides and PPCPs in water by LC-MS/MS: a review. Cent. Eur. J. Chem. 1216, 2958–2969.
Richardson, S.D., Kimura, S.Y., 2016. Water analysis: emerging contaminants and current issues. Anal. Chem. 88, 546–582.
Robert, C., Brasseur, P.Y., Dubois, M., Delahaut, P., Gillard, N., 2016. Development and validation of rapid multiresidue and multi-class analysis for antibiotics and anthelmintics in feed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 33, 1312–1323.
Soriano, J.M., Jimenez, B., Font, G., Molto, J.C., 2001. Analysis of carbamate pesticides and their metabolites in water by solid phase extraction and liquid chromatography: a review. Crit. Rev. Anal. Chem. 31, 19–52.
Souza-Silva, É.A., Jiang, R., Rodríguez-Lafuente, A., Gionfriddo, E., Pawliszyn, J., 2015. A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis. TrAC Trends Anal. Chem. 71, 236–248.
Stehle, S., Knäbel, A., Schulz, R., 2013. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal. Environ. Monit. Assess. 185, 6295–6310.
Tankiewicz, M., Fenik, J., Biziuk, M., 2010. Determination of organophosphorus and organonitrogen pesticides in water samples. TrAC Trends Anal. Chem. 29, 1050–1063.
Vryzas, Z., Vassiliou, G., Alexoudis, C., Papadopoulou-Mourkidou, E., 2009. Spatial and temporal distribution of pesticide residues in surface waters in northeastern Greece. Water Res. 43, 1–10.
Wackett, L.P., 2007. Environmental fate of pesticides. Environ. Microbiol. 9, 3150–3151.
Wu, J., Lu, J., Wilson, C., Lin, Y., Lu, H., 2010. Effective liquid-liquid extraction method for analysis of pyrethroid and phenylpyrazole pesticides in emulsion-prone surface water samples. J. Chromatogr. A 1217, 6327–6333.
Yang, X.B., Ying, G.G., Kookana, R.S., 2010. Rapid multiresidue determination for currently used pesticides in agricultural drainage waters and soils using gas chromatography–mass spectrometry. J. Environ. Sci. Health, Part B 45, 152–161.