RISK ASSESSMENT OF PESTICIDES, IN SURFACE WATER OF LOUROS RIVER (N.W GRECEE) AFTER OFF-LINE SOLID PHASE EXTRACTION COUPLED GC-MS AND LC-MS

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ABSTRACT

Over the years there has been an increasing interest in water- quality preservation and improvement. Concerns over the contamination of water by pesticides generally arise from two scenarios, which are, concern over human health risks when water (e.g. groundwater) is used for drinking and concern over eco-toxicological effects when non target organisms (e.g aquatic organisms and amphibians) are exposed to water in their habitats [1]. Among the various water pollutants, pesticides constitute a significant category. The widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in the aquatic environment. The determination of pesticide residue in water samples is necessary for solving various environmental and biological problems.

In this study, the occurrence of thirty four multiclass pesticides as a result of an extensive monitoring survey that was carried out for a period of one year in Louros River (Epirus region, north-western Greece) is presented. Louros River flows into Amvrakikos Gulf which is protected by the Convention of Ramsar (1971). The run off basin of the Louros River has an area 800 km^2, its length is 80 km and the average annual flow rate has been estimated at 19.3 m^3s^{-1}. Thirty five water samples were collected between June 2011 and May 2012. The target pesticides were determined by Solid Phase Extraction and Gas Chromatography or Liquid Chromatography coupled with Mass Spectrometry in the selected ion mode [2]. The selection of the pesticides studied was based on their use in agricultural practices in Epirus region.

To evaluate negative impact on ecosystems, the environmental risk assessment is necessary. The protection of a specific ecosystem, such as surface water, requires the preservation of its ecological functionality. The exposure assessment involves the measured environmental concentrations derived from monitoring studies or the developing of models that can estimate the predicted environmental concentration based on factors such as application rate, distribution, bioaccumulation, and persistence. The effect assessment involves summarizing toxicant reference values (TRV) on the effects of the pesticide on representative organisms expressed as lethal concentration for the 50% of the population of organisms exposed (LC50), effect concentration for the 50% of the population of organisms exposed (EC50) [3].

Keywords: pesticides, risk assessment, solid-phase extraction, gas chromatography

1. Introduction

During the last two decades, the occurrence of pesticides in natural aquatic environments has been considered as an emerging issue because of the adverse effects they pose, both to aquatic life and humans. The use of pesticides may lead to the contamination of surface waters by drift, runoff, drainage and leaching. The pollution of water by pesticides is a topic of considerable environmental interest, owing to the increasing number of pesticides detected in water and to the establishment of strict directives in Europe, aiming the protection of water sources used for the production of drinking water [4].

There are many sources of pesticides discharge into the aquatic environment. They can come from both area sources (e.g., atmospheric precipitation or farmland) and point source. They can also be transported over long distances through the air. Pesticides that are found in water samples, belong to different structural groups, such as organophosphates, pyrethrins, carbamates, organochlorine e.t.c. Exposure to such contaminated water is harmful to the health and the life of not just humans, but also living organisms. That is why pesticides levels in water must be monitored continuously, especially in sources of water.

To achieve a practical and reliable method for the determination of pesticide residues in water samples, several sample preparation methods have been developed including liquid-liquid extraction and solid-phase extraction. However, liquid-liquid extraction is being gradually superseded by solid-phase extraction for the separation of pesticides from water in many methods as a result of the wide availability of selective sorbent materials, to avoid the need to dispose of organic solvents.

The purpose of this study was to evaluate the potential impact of the pesticides detected in Louros River (N.W. Greece) on the aquatic organisms belonging to this ecosystem. For this purpose, the occurrence and risk assessment of 34 pesticides were determined in Louros surface waters. In the present work, solid phase extraction (SPE) technique followed by gas chromatography or liquid chromatography coupled mass spectrometric analysis, was applied in order to determine a large number of pesticide residues with wide range of polarities and chemical structures in natural water samples, due to extensive monitoring survey that was conducted at along the Louros River and the Amvrakikos Gulf (Epirus region, north-Western Greece) for a period of one year. The ecological risk associated with pesticide contamination was assessed to estimate the preliminary risk posed to studied ecosystem.

2. Experimental

2.1. Area description

Louros River has length of about 75 Km and an average flow about 10.6 m^3s^{-1} that ranges between 5 m^3s^{-1} in summer and 20 m^3s^{-1} in winter. It drains into the Amvrakikos Gulf, an important wetland protected under the Ramsar Convention and European Communities Legislation. The run off basin of the Louros River has an area of 800 Km^2, its length is 80 Km and the average annual flow rate has been estimated at 19.4 m^3/ s. The agricultural areas at the river watershed have a surface of 74,700 hectars and are cultivated with citrus fruits (30%), olives (22%), corn (14%) cotton (7,5%) etc [6].

2.2. Sampling

Water samples were collected from the Louros River between May 2011 and March 2012. Seven sampling stations were selected in order to cover all the possible pollution sources of the Louros River. A 2.5L volume of water was collected in glass bottles from each sampling site at medium depth. The bottles were reaching the laboratory on the same day, and were normally extracted within 48h.

2.3. Chemicals and Reagents

Pesticides standards were purchased from Sigma-Aldrich (Steinheim, Germany), purity 98-99%. Stock standard solution, was prepared at a concentration of 1–2 μg/L in methanol and was stored at −20 ◦C. The solvents used, including methanol and water LC-MS grade, Dichloromethane, Acetone, Ethyl acetate were supplied by Pestiscan (Labscan Ltd., Dublin, Ireland). C18 disks were purchased from 3M (Saint Paul, MN, USA). Sodium sulphate anhydrous was obtained from J.T. Backer.

2.4. Solid Phase Extraction

Isolation of Irgarol from sea water samples was performed off-line, using a standard 16-port SPE manifold connected to a vacuum pump. C18 disks were first activated by wetting with 10 ml acetone. Then they were washed with 10 ml ethyl acetate and were vacuum dried. Methanol (10

ml) was then percolated through the disks and without letting the disks become dry, 10 ml distilled water was added before the final water sample. The analytes were eluted with ethyl acetate/ dichloromethane (80:20). The final extract was dried over anhydrous sodium sulphate. Finally they were evaporated to a final volume of 0.1 ml under a gentle stream of N_2.

2.5. Risk Assessment

The aquatic risk assessment for the detected pesticides was assessed on the basis of the risk quotient method (RQ). The detected concentrations of the pesticides in the water bodies are divided by an effect level reported in the literature:

$$Risk\ Quotient\ (RQ) = \frac{Exposure}{Toxicity} = \frac{Water\ or\ Sediment\ Concentration}{LC\ 50 or EC50} \quad (1)$$

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/sediment sample analyzed to a certain taxonomic group. In the present study the mean reported effect level for a certain taxonomic group is 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, 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 suitably representative for aquatic insects and other invertebrates. The toxicity values for the three taxonomic groups are shown in the (Table 1) [5].

3. Results and discussion

3.1. Analytical Characteristics of the Method

The SPE method showed good linear response with R^2 values in the range of 0.990- 0.999. Values of relative recoveries located within the acceptable range of (67.32-104.99%). Limits of detection (LODs) were estimated on the basis of 3:1 signal-to noise ratios obtained with standards containing the compounds of interest at low concentration level, ranged from (7-30ng/L). The repeatability and reproducibility of measurements, expressed as relative standard deviation (RSD), was less than 10% for analytes.

3.2. Application of the Method to real samples

The pesticides with detection higher of fifty per cent in water samples were eptc, propachlor, trifluralin, chloropyriphos methyl, pirimiphos ethyl, pendimethaline, triadimenol, a-endosulfan, myclobutanil and endosulfan-sulphate, while the pesticides quinalphos and quizalofop-ethyl were detected in less than ten per cent of total water samples. Propachlor, eptc and endosulfan sulphate were was the most frequently detected pesticides with detection range between 80 and 85%. Their occurrence was observed throughout the whole survey period with the minimum detection of the winter months when dilution effects and degradation reduced concentrations.
Seasonal variations of pesticide detection in Louros River water samples, corresponding to pesticide application periods, were observed. Pesticide detection tended to be more frequent and levels more elevated during the late spring and summer months (Table 1).

For most of the pesticides detected the decrease in rainfall in summer results in an increase in pesticide concentrations at this time of year, in addition to the fact that the summer period comes just after their application and most pesticides have soil half-lives of several weeks.

Table 1: Maximum detected concentration and seasonal variation of the Louros River, at seven stations, in the period from June 2011 to May 2012.

Pesticide detected	Maximum detected concentration	Mean detection (%) Seven stations		
		Winter	Spring	Summer
Eptc	0.1338	70.00%	78.57%	92.86%
Molinate	0.1441	14.28%	57.14%	64.28%
Propachlor	0.1390	71.42%	92.86%	85.71%
Ethoprophos	0.0440	28.57%	42.86%	35.71%
Trifluralin	0.0836	100.00%	71.42%	78.57%
Atrazine	0.0761	0.00%	35.71%	28.57%
Disulfoton	0.0047	0.00%	28.57%	0.00%
Acetochlor	0.1040	85.71%	14.29%	35.71%
Dimethenamid	0.0667	28.57%	50.00%	21.43%
Pirimiphos-ethyl	0.0641	28.57%	57.14%	71.43%
Metolachlor	0.0788	71.42%	92.86%	100.00%
Pendimethaline	0.1220	85.71%	57.14%	92.86%
Quinalphos	0.1412	0.00%	71.43%	14.29%
Triadimenol	0.1624	100.00%	64.28%	92.86%
Endosulfan-a	0.1012	100.00%	57.14%	100.00%
Endosulfan-b	0.1853	85.71%	64.29%	71.42%
Endosulfan-sulfate	0.0543	100.00%	78.57%	78.57%
Myclobutanil	0.0448	71.42%	78.57%	92.86%
Azinphos-ethyl	0.8205	57.14%	71.43%	14.28%
Quizalofop-ethyl	0.1002	100.00%	85.71%	100.00%

3.3. Deterministic Approach Results (RQ)

The Risk Quotients were calculated by dividing the maximum detected concentration in Table 1 and the toxicant values from the literature using Eq.(1). The risk quotients of the representative species in Louros river ranges from 0.0000014 to 0.45. Comparing to literature reported levels of concern (i.e., RQ≥ 1 high risk, 0.1≤ RQ≤1 medium risk, 0.01 ≤ RQ≤ 0.1 low risk) [1]. Concerning the springs of Louros, under High risk assessment is phytoplankton and zooplankton. At Zirou lake, under high risk assessment is zooplankton and under Medium risk assessment phytoplankton and fish. Concerning the Kalogirou bridge (A), under High risk assessment for phytoplankton and zooplankton, under Medium risk assessment for fish. The same trends were observed in Kalogiroy bridge (B), the fourth sample station. Additionally in the Tsopeli lagoon, under High risk assessment is phytoplankton and zooplankton and under Medium risk assessment is fish. Furthermore in Amvrakikos gulf, under High risk assessment for the three taxonomic groups. Finally, cconcerning the outfall of Louros, under High risk assessment is phytoplankton and zooplankton and under Medium risk assessment is fish. It is clear that pesticide pose lower risk to higher animals creatures, and low risk to higher animals. The relative contributions of pesticides to the total ecological risk were calculated to identify pesticides that pose higher risk to a specific organism. Azinphos-ethyl, acetochlor, endosulfan-a and pendimethaline are the greatest hazards to the species in the Louros River. As a result, in order to protect the ecosystem of Louros river, there is need to focus on the contaminations azinphos-ethyl, acetochlor, endosulfan-a and pendimethaline.

4. Conclusions

The present SPE-GC-MS/LC-MS method provided good repeatability and reproducibility range, high extraction efficiency and low LODs. The performance results confirm the usefulness of the proposed methodology for the routine analysis of multiclass pesticides in natural waters. It possesses the advantages of SPE (fast, simple, highly sensitive) and could be potentially extended to other classes of pesticides. The pesticides detected in river water samples belong to different chemical or/and active groups. Twenty two multiclass pesticides were detected during an extensive monitoring survey that was carried out for a period of one year in Louros River (Epirus region, north-western Greece) using SPE coupled to GC/MS. Seasonal variations of pesticide detection in Louros River water samples, corresponding to pesticide application periods, were observed.

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