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ABSTRACT BOOK

1964 -2014 Celebrating the
50TH ANNIVERSARY
OF THE UNIVERSITY OF IOANNINA

Abstract Book

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Abstracts of Oral Presentations

OP01

ACT1080

DEVELOPMENT OF A NOVEL ION-ASSOCIATED THIN-FILM EXTRACTION METHOD FOR THE DETERMINATION OF UV FILTER RESIDUES IN NATURAL WATERS

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Summary

In this work we report on a novel and expedient methodology for the extraction of organic compounds from aqueous media based on an ion-associated thin-film. A cationic surfactant and a benzesulfonic acid derivative form a suspended aggregate film upon mixing. Hydrophobic compounds are quantitatively collected in the suspended aggregate which is easily isolated as a thin film on a glass-fiber filter paper. The analytes are then back-extracted by liquid desorption into an appropriate organic solvent and analyzed directly. As an analytical demonstration, the method was optimized for the determination of organic UV filters in natural waters.

Introduction

The development of new extraction methodologies has been for many years at the forefront of analytical chemistry research. A large number of new extraction methods have been developed, mainly relying on the principles of liquid and solid state micro-extraction [1,2].

Despite their unique advantages over more traditional sample pre-treatment methods, their use in routine analysis has not been in accord with the amount of research dedicated for their development. This is attributed to several reasons such as a) the need for synthesizing the extraction medium, which confers additional costs and complexity, b) the delicate handling of the extracts, especially in liquid microextraction methods, c) the additional post-extraction steps which are often required resulting in lengthy turnaround times and lower sample throughput. As a result, routine analysis is most commonly performed with methods which are well documented and widely commercialized (e.g. SPE or SPME).

In this work we introduce a new extraction method that resembles SPE but it is simpler to perform and requires minimal equipment resources. The method is based on the formation of a suspended aggregate which extracts the target analytes and is then collected by simple filtration.

Experimental Procedure

A cationic surfactant and a benzesulfonic acid derivative are sequentially added into an aqueous solution of controlled ionic strength and mixed under stirring. The liquid suspension is then collected by suction filtration on plain filter paper. The collected film is then eluted with an organic solvent and a 20 µL aliquot is injected into the liquid chromatograph. The experimental procedure is graphically illustrated in Figure 1.

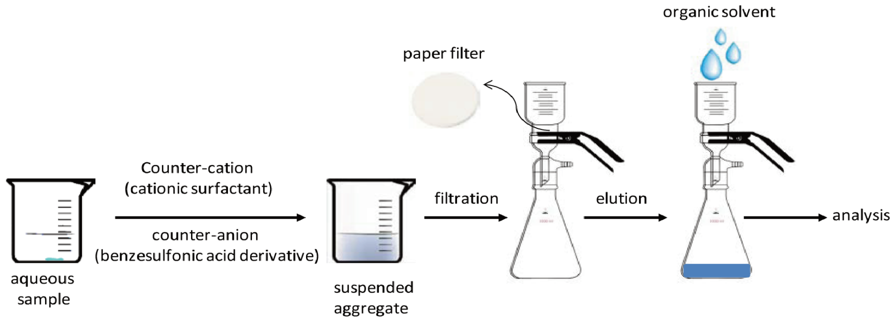

Figure 1. Experimental procedure for the ion-associated thin-film extraction of organic compounds from aqueous media.

Results and Discussion

A series of organic UV filters with variable polarity were used as model organic compounds for the optimization study. The parameters associated with their extraction were thoroughly optimized in a univariate manner, by varying one parameter at a time while keeping the rest constant. In this vein, the filter type and pore diameter, the filtration rate, the concentration of the ion-pair forming reagents, the pH, the ionic strength and the mixing time were investigated for their influence on the extraction efficiency of the target compounds. The concentration of the ion-pair reagents, the selection of the appropriate filter type and filtration speed were the most important variables. However, due to the ion-pair reaction, reduced tolerance to inorganic salts was observed (e.g. seawater).

Conclusions

A new expedient extraction method has been developed. The method is easy to perform with minimal laboratory equipment, does not depend on a commercial source (beyond the procurement of chemicals) and affords quantitative extraction of organic compounds from aqueous samples. The potential of using this method in the field using syringe-type filtration apparatus as well as ways to overcome the limitations associated with the use of the method in high ionic strength solutions are under investigation.

Acknowledgments

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OP02
ACT1088

TARGET AND NON-TARGET ANALYSIS OF PESTICIDE RESIDUES AND THEIR METABOLITES IN FOOD AND WATER MATRICES USING GC-MS/MS AND LC-MS/MS

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Summary

This work shows the development and validation of multi-residue methods for the detection and quantification of pesticides -parent compounds and transformation products -from different chemical classes, in various food matrices, using general extraction procedures such as QuEChERS in combination with LC -MS/MS and GC-MS/MS techniques fulfilling the performance criteria described in the European Union guidelines (SANCO Doc. No 12571/2013 [2]).

Introduction

Pesticide residue analysis on food samples is essential for the protection of human health but also to guarantee international trade and to comply with regulatory controls. More than 1000 substances of different chemical groups that are active against pests - triazoles, strobilourines, carbamates, pyrethrins, organophosphates, sulfonylureas, triazines and others - are currently used worldwide. EU adopted Regulation -396/2005 for the maximum permissible pesticide residues in food, the majority of which ranges over the concentration range 0.01-1 mg / kg. For the control of residues of these categories of plant protection substances in different types of food commodities there is the need to develop and validate large scale multi-residue methods based on general extraction procedures in combination with hyphenated instrumental analysis techniques such as - gas and liquid chromatography with mass spectrometry [1].

Results and Discussion

During LC -MS/MS and GC-MS/MS methods development, the influence of selected per case analytical operating parameters on pesticide response was thoroughly investigated in order to achieve the highest possible analytical sensitivity, accuracy and detectability in the complex food matrices. A LC-MS/MS method using QqQ-MS technology has been developed for the simultaneous analysis of 140 compounds based on SRM acquisition data. Additionally a GC-MS/MS method using Ion-Trap MS^n technology has been applied for the determination of 60 GC-amenable compounds. More than 2500 pesticide/food commodity combinations have been validated using both LC- and GC-MS/MS methods, with limits of quantification of 0.01mg/Kg in most of the cases. Recoveries in the range 70-120% with relative standard deviations less than 20-25% have been achieved. Both methods have been successfully applied for the monitoring of multiclass pesticide residues in more than 1500 food samples enhancing the capacity and the productivity of the laboratory.

Conclusion

Results of our monitoring program are included in Annual Report of European Food Safety Authority (EFSA) published on line at www.efsa.europa.eu

EFSA has just published its 2010 annual report concerning pesticides residues in food. The European Food Safety Authority (EFSA) reports that the chemical residues on the foods analysed did not pose a long-term risk to consumer health.

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OP03
ACT1120

CHEMOMETRIC OPTIMIZATION OF DISPERSIVE SUSPENDED MICROEXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN NATURAL WATERS

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Summary

A dispersive suspended microextraction (DSME) method coupled with gas chromatography-mass spectrometry (GC-MS) was developed and validated for the simultaneous determination of ten polycyclic aromatic hydrocarbons in real water samples. The optimization of the method was achieved with a 2^{7-4} Plackett –Burman design, while the significant factors were optimized using a central composite design (CCD). The parameters that were studied included the sample volume, organic solvent volume, extraction time, restoration time and organic solvent. The optimum experimental conditions for the proposed method comprised 4.3 mL of the water sample, 93 μL of toluene as the extraction solvent, a 104-s extraction time and a 10-min restoration time. The recoveries varied from 70 to 111%. Chrysene was the least recovered compound, while anthracene displayed the highest extraction efficiency. The analytical method (DSME) was shown to be linear (R^2>0.993) over the studied range of concentrations, exhibiting satisfactory precision (RSD% <10.6%) and reaching limits of detection of between 8 and 46 ng L-1.

Introduction

The contamination of surface and ground waters with PAHs concerns many researchers around the world. Numerous analytical methods based on extraction techniques have been developed in recent years. In 2011, Yang *et al.* [1] developed a new extraction method called dispersive suspended microextraction (DSME). The method is based in a high speed agitation of a small amount of organic solvent with an aqueous sample. In this method, two critical steps are involved: the extraction step and the restoration step. During the extraction step, target analytes are extracted into the extraction solvent, while during the restoration step, the organic and aqueous phases are separated. After the separation of the two phases, the target analytes are injected into GC-MS for further analysis.

Apparatus

Analyses were performed using a Trace GC Ultra instrument (Thermo Scientific, Austin, Texas, USA) coupled to an ISQ mass spectrometer controlled by a computer running XCalibur software. The separation was performed using a TR-5 column with a film thickness of 0.25 μm (30 m×0.25 mm i.d., Thermo Fisher Scientific, Austin, Texas, USA). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}.

Dispersive suspended microextraction (DSME) procedure

For the DSME procedure, 4.3 mL of aqueous sample was placed in a 10-mL vial. A 93-μL aliquot of toluene was added as the extraction solvent to the surface of the aqueous sample. A magnetic micro stirring bar was placed in the bottom of the vial and the sample vial was placed on a magnetic stirrer to stir the sample.

The SDME procedure first involved an extraction step, followed by a restoration step. In particular, the stirring speed was set at 1500 rpm (extraction speed) for 104 s (extraction time) and then was reduced to 800 rpm (restoration speed). In the extraction step, a cloudy solution was formed, and the analytes in the water sample were extracted into fine toluene droplets. When the stirring speed was adjusted to 800 rpm, the restoration step began. At this stirring speed, a gentle vortex was formed and the toluene droplets began to coalesce. After 10 min (restoration time), the toluene had separated from the aqueous phase, and an organic droplet was formed in the bottom center of the vortex. Finally, 1.5 μL of the organic phase was collected with a 10 μL micro syringe and injected into the GC-MS for further analysis.

Experimental design

To evaluate the main factors affecting the efficiency of the DSME method, a design with two steps (screening and optimization) was used to screen the optimal experimental conditions. For this purpose, the STATISTICA 7.0 statistical package was used to generate the experimental matrix and to evaluate the results.

Results and discussion

The figures of merit of the proposed method were calculated to evaluate the analytical performance of the method under the optimum conditions. The detection limit of the method ranged from 8 to 46 ng L^{-1}, while the quantification limits ranged between 24 and 138 ng L^{-1}. The intra-day repeatability ranged between 3.7 and 10.6%, while the inter-day reproducibility ranged between 3.3 and 10.9%. Recoveries varied from 70 to 111% while the linear determination coefficients ranged between 0.993 and 0.999.

Conclusions

In the present study, for the first time, directly suspended microextraction (DSME) coupled with GC-MS was evaluated for the simultaneous determination of PAH residues in real water samples. This simple, rapid and inexpensive extraction method fulfilled analytical validation criteria. The sensitivity and linear dynamic range were relevant to environmental water analysis, and precise and accurate measurement of the compounds of interest in surface waters was achieved.

Acknowledgments

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

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OP04
ACT1118

EVALUATION OF A MULTIRESIDUE METHODOLOGY FOR THE DETERMINATION OF PESTICIDES RESIDUES IN NATURAL WATERS. A CASE STUDY: LOUROS RIVER (EPIRUS REGION)

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Summary

The aim of this work was to develop an efficient method on the basis of solid phase extraction (SPE) technique [1] for the determination of 34 multiclass pesticides in natural waters in combination with chemo-metric tools (experimental design, response surface methodology and desirability profile). The optimized method was applied in a case-control study carried out on a Louros River (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.

Introduction

Contamination of water resources by pesticides residues is one of the major challenges for the preservation and sustainability of the environment. Their extensive use in world- wide agricultural practice in addition to industrial emission during their production has led to substantial occurrence of pesticide residues and their metabolites in water [2]. Pesticides are among the most dangerous environmental pollutants because of their stability, mobility and long-term effects on living organisms [3]. Thus, pesticide residue analysis in environmental samples has received increasing attention in the last few decades, resulting in many environmental monitoring programs for a broad range of pesticides.

Experimental Part

SPE using C18 extraction disks followed by gas chromatography (GC-MS) and liquid chromatography (LC-MS) were used for the determination of various pesticides in natural waters. An experimental 2^{7-4} Plackett–Burman design was used to screen the important variables that significantly influenced pesticide extraction. Each variable was examined at two levels. From the Pareto chart extraction disk, volume of elution, the volume of sample and the ratio EtAc/DCM with a positive effect were the most significant factors affecting the extraction process. These factors were further investigated using a central composite design (CCD). The final step was to find the optimal conditions for the independent factors that optimize the response of the dependent factor. In order to do that, a desirability surface response was developed by the CCD model in 3D plots. The optimized method was applied in a case-control study carried out on a Louros River (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.

Analytical Characteristics of the Method

The optimized analytical methodology is linear over the studied range of concentrations, with correlation coefficients between 0.991 and 0.999. Limits of detection (LODs), on the basis of 3:1 signal-to noise ratio, ranged from 0.005 to 0.020 μg/L with GC-MS and 0.015 to 0.030 hh μg/L with LC-MS, depending on the compound. The intra-day repeatability and inter-day reproducibility of the target compounds, both expressed as relative standard deviation ranged from 1.6 %to 4.9% and from 2.3% to 4.9% respectively.

Application of the Method to real samples

The optimized method was applied in a case-control study carried out on Louros River (Epirus region, north-western Greece) for a period of one year. 42% of the pesticides, belonging to different chemical classes, were detected. 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. 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.

Conclusions

The present multiresidue methodology was successfully employed for the determination of pesticides residues in water samples. It possesses the advantages of SPE (fast, simple, highly sensitive) and could be potentially extended to other classes of pesticides, as a useful tool for monitoring purposes.

Acknowledgments

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

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OP05
ACT1031

DEVELOPMENT OF A GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PHENOXY ACIDS AND PHENOLS IN WATER SAMPLES AS SILYL DERIVATIVES

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Summary

A rapid and cost-efficient solid phase extraction (SPE) method was developed for the simultaneous determination of two acidic groups of compounds, phenoxy acids and phenols, for the routine monitoring of the final water at Thessaloniki Drinking Water Treatment Plant. Their similar polarity allows them to be extracted on the same solid phase extraction sorbent thus reducing cost and pretreatment time. Aiming to monitor these compounds at trace levels, a single step derivatisation with *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoracetamide (MTBSTFA) was used for both groups but at different temperature conditions. [1,2] These two groups were analyzed separately with gas chromatography-mass spectrometry (GC/MS).

Introduction

Phenoxy acids have been extensively used as herbicides in agriculture. Due to their polarity and high solubility in water, they often enter into both ground and surface water by infiltration or natural drainage. [3] They are of primary interest in drinking water monitoring, since E.U. directive limits them to a maximum parametric value of 100 ng/L for each compound. [4]

Phenols are used in large quantities in chemical production of plastics, drugs, colors, explosives, pesticides, detergents, stabilizers and antioxidants. They are formed as degradation products of biocides, ammunition wastes or even as decomposition products of wood and leaves. [5,6] E.U. directive sets a limit of 500 ng/L for the total concentration of all phenols and an individual limit of 100 ng/L for pentachlorophenol which is used as a pesticide.[4]

Materials and Methods

In this project, the development of the method was carried out using ultrapure water samples acidified to pH<2 and spiked with the selected compounds at various ng/L levels. Solid phase extraction was carried out using Styrene Divinyl Benzene extraction disks (SDB-XC) instead of cartridges which allow easier extraction of large volume samples (e.g. 1-2L samples). Elution was performed using two consecutive 5mL fractions of ethyl acetate (EtOAc), one 5mL fraction of EtOAc : methanol (CH_3OH) (95%:5%). The eluent was dried over anhydrous Na_2SO_4 and concentrated to 500μL. [4,7] Derivatisation prior to GC/MS analysis was different for the two groups analyzed. For phenoxy acids, 250μL of the concentrate were mixed with 50μL of MTBSTFA in a 1,5mL GC/MS vial. The vial was capped and shaken for 1 min and after 10 min the final product was injected for analysis. For phenolic compounds, 50μL of the concentrate were mixed with 50mL MTBSTFA in a 1,5mL GC/MS vial which was capped and heated to boiling point for 1 min. After this step, the vial was placed in a dessicator for 60 min and then was analyzed. GC/MS methods were different for phenoxy acids and phenols so two different runs were necessary for each sample.

Results and Discussion

This study included 2 phenoxy acids (MCPA and 2,4-D) which were detected in a previous survey [8] in the inlet water of Thessaloniki Drinking Water Treatment Plant from Aliakmonas River and 11 phenols which are classified as "priority pollutants' by the US Environmental Protection Agency including pentachlorophenol. [9] Two internal standards were used for the quantification of the results, one for each group of compounds. The response factors (Rfs) were determined with standards in EtOAc in various concentrations (25-500 ng/L). MDLs, accuracy and precision were determined analyzing six fortified blanks in a relatively low concentration different for each group (25ng/L for phenoxy acids and 100 ng/L phenols respectively).

The MDLs, accuracy and precision were well within the E.U. directive for both phenoxyacids and phenols (25% of the parametric value given for each group). For phenoxyacids, MDLs were around 14%, accuracy was 8,7%-11,4% and precision was 5,5%-5,8% of the parametric value (100 ng/L) and for phenols MDLs ranged from 4%-14%, accuracy from 4%-25% and precision from 2%-5% of the parametric value (500 ng/L).

This method has been used for routine analyses of the pre-mentioned groups of compounds in Thessaloniki Drinking Water Treatment Plant for the last two years. During that time, the concentrations of the analytes in the samples were below the limits of E.U. directive.

Conclusions

A new cost and time efficient routine method has been developed for the simultaneous determination of acidic organic micropollutants such as phenoxy acids and phenols in water samples which is in accordance with the E.U. directive.

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OP06
ACT1044

DISPERSIVE LIQUID-LIQUID MICRO EXTRACTION METHOD USING MAGNETIC IONIC LIQUIDS FOR THE DETERMINATION OF PHENOLIC ENDOCRINE DISRUPTERS AND ACIDIC PHARMACEUTICALS IN AQUEOUS MATRICES

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Summary

Phenolic endocrine disrupting compounds are commonly found in the environment as a result of their applications in industry. Because of their high toxicity, the regulatory agencies have established strict limitations for their accepted concentrations in water intended for human consumption and usage. Also, pharmaceuticals have become recognized as relevant environmental contaminants in the last decade. A number of studies have revealed that pharmaceuticals are ubiquitous in the aquatic environment, mainly because some compounds are not efficiently removed during wastewater-treatment processes reaching surface, ground and consequently drinking water systems. In this study, a novel method is proposed for their determination in aqueous matrices based on the principles of dispersive liquid-liquid micro extraction and the employment of magnetic ionic liquids, as extractants.

Introduction

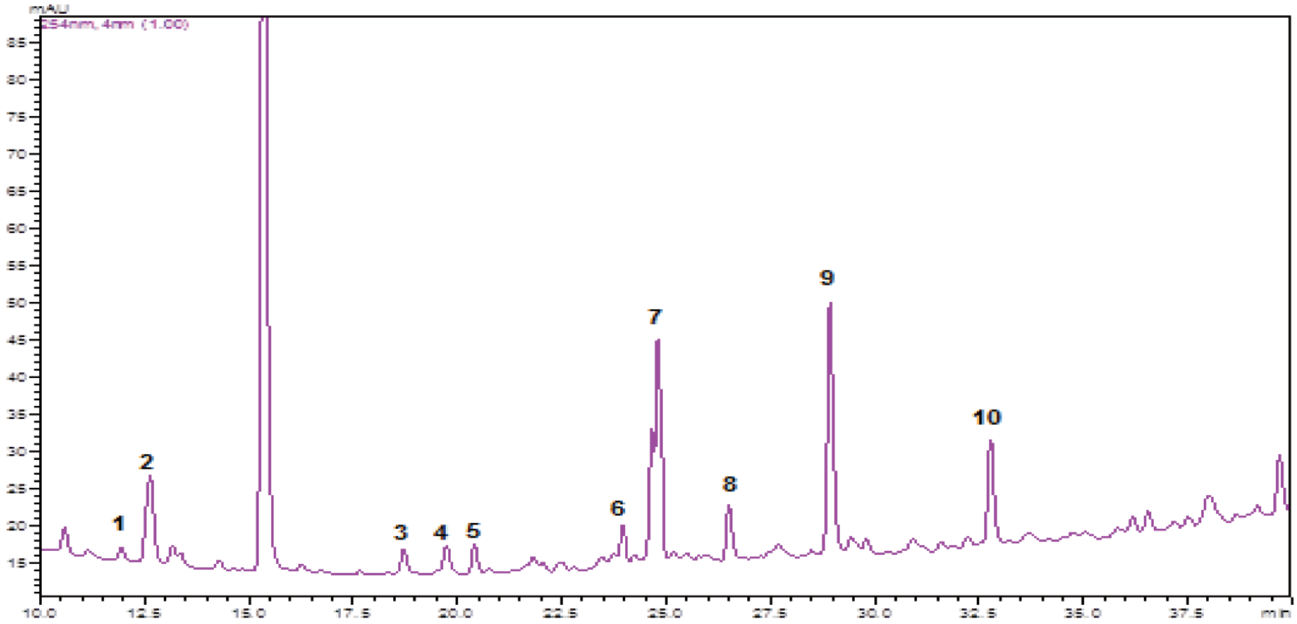
Phenolic compounds are widely encountered in several industrial processes. They are used as precursors for the synthesis of pesticides and drugs and also in the manufacturing process of preservatives, anti-oxidants and paper [1]. Researches have confirmed that the phenolic compounds exhibit a high degree of toxicity. Considering their widespread application and usage, it is inevitable that part of them may end up in the aquatic environment with the industrial waste waters. Pharmaceuticals in their native form or their metabolites are continually being introduced into sewage waters, mainly indirectly by excreta, through disposal of unused or expired drugs, or directly in discharges from pharmaceutical-manufacturing plants. Several pharmaceuticals have been detected at trace level in wastewaters throughout Europe, and some have been measured at sub-trace contents in rivers and drinking water sources. From all the pharmaceuticals reported in literature, the classes of non-steroidal acidic anti-inflammatory drugs, such as *o*-acetylsalicylic acid, ibuprofen and diclofenac are the most frequently mentioned as environmental contaminants. Therefore, the presence of both categories in the environment can be hazardous and even fatal for the living organisms. For this reason, regulatory agencies such as the European Union and the US Environmental Protection Agency include these compounds in their list as priority pollutants. So far, different methods have been proposed for the analysis of aqueous samples. Ionic Liquids (IL's) are organic salts that do not crystalize in room temperatures. They are liquids composed solely of cations and anions [2]. IL's have low vapour pressures, thermal stability and high solvation interactions both with polar and non-polar compounds. They are non-toxic and easily synthesized in a one-step procedure. Therefore, they can be considered as a ''green alternative solvent'' compared to the classical organic solvents [3]. Magnetic IL's are more preferable because of their magnetic properties but they have not been used so far for analytical purposes. We propose an effective method using dispersive liquid-liquid micro extraction with magnetic ionic liquids and analysis with a HPLC-DAD. Dispersive liquid-liquid micro extraction brings benefits compared to the classical techniques. The droplet of the extraction liquid is dispersed in the aquatic sample in fine tiny droplets maximizing the surface area available for the extraction and the pre-concentration of the analytes [4].

Materials and methods

Two different magnetic ionic liquids were synthesized using a one-step procedure and used without further purification. For the dispersive liquid-liquid microextraction a water sample is stirred in a glass beaker with the aid of a magnetic stirrer. One drop of the magnetic ionic liquid is added and the stirring is continued for 30 minutes. Then, by attaching a commercial magnet at the walls of the beaker, the magnetic IL's droplets concentrate around the magnet and bulk water is discarded. After washing with double distilled water, the magnetic IL is resuspended in 300 μL of a mixture ACN- water (3:1) and injected into an HPLC-DAD system.

Results and Discussion

Experiments have shown that the magnetic IL's are capable of extracting and preconcentrating seven endocrine phenolic compounds and three acidic pharmaceuticals in water samples.



HPLC chromatogram with DAD, at 260nm. Peak assignment: 1) salicylic acid, 2) 3-nitrophenol, 3) bisphenol A, 4) β-estradiol, 5) 4 *tert*-butylphenol, 6) 2,3,5-trichlorophenol, 7) dichlofenac, 8) ibuprofen, 9) irgasan, 10) octylphenol.

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OP07
ACT1060

EVALUATING THE RESPONSE OF PASSIVE SBSE TOWARDS CONCENTRATION PEAKS OF PESTICIDES IN FRESH-WATER

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Summary

Passive samplers can integrate fluctuating concentrations of organic micropollutants in the aquatic environment, and allow the determination of reliable estimates of average concentrations. In agricultural watersheds, most passive samplers cannot however integrate high and transient concentrations of pesticides in small rivers. Passive stir bar sorptive extraction (SBSE) is a novel and simple passive sampling technique suited for highly fluctuating micropollutant concentrations. The aim of this study was to evaluate, under controlled laboratory conditions, the response of SBSE stir bars exposed first to concentration peaks, then to low concentrations of 20 pesticides. Results show that passive SBSE allows a fast integration of the concentration peaks of the pesticides. Also, large to no elimination of the pesticides were observed for the SBSE stir bars plunged in unspiked water, whereas partial elimination to additional accumulation were observed for the SBSE stir bars plunged in spiked water. These results provide a clear insight on the behavior of passive SBSE during concentration peaks of the pesticides and its potential performances during field applications.

Introduction

Representative monitoring of organic micropollutants such as pesticides in aquatic systems is a challenging issue. Passive sampling allows the integration of temporal variations of concentrations and the determination of realistic estimates of average concentrations in surface waters [1]. Flood-related concentration peaks represent a major pathway for the transport of pesticides in dynamic rivers located in small agricultural watersheds [2]. Although they can integrate variations of concentrations, most passive samplers are not reactive enough to integrate these high and transient concentrations of pesticides. Stir bar sorptive extraction (SBSE) is a solvent free extraction technique for hydrophobic compounds in water samples. It is composed of a magnet enclosed in a glass tube coated with a thick film of polydimethylsiloxane [3]. Recently, we developed passive SBSE as a passive sampling technique suited for the monitoring of water bodies with highly fluctuating concentrations of pesticides [4]. The aim of the present study was to evaluate the response of SBSE stir bars exposed to concentration peaks then to low concentration levels of 20 selected pesticides.

Experimental

We selected 20 pesticides and metabolites frequently quantified in rivers located in agricultural watersheds. Two laboratory kinetic experiments were conducted at constant concentrations for 7 days. Both experiments consisted in plunging 30 SBSE stir bars in a glass tank filled with water spiked with high concentrations of the pesticides (from 2 to 800 µg L^{-1}, depending on the pesticide), for 2, 4 or 6 h (with 10 stir bars per scenario). Then, for each scenario, 7 stir bars were plunged in a glass tank continuously supplied with unspiked water or with water spiked at concentrations ranging from 0.2 to 80 µg L^{-1}. The pesticides accumulated in the SBSE stir bars were desorbed by liquid desorption and determined by ultrahigh performance liquid chromatography coupled with tandem mass spectrometry (LD-UHPLC-MS/MS) [5].

Results and discussion

We briefly present the results of two pesticides with different behaviors.

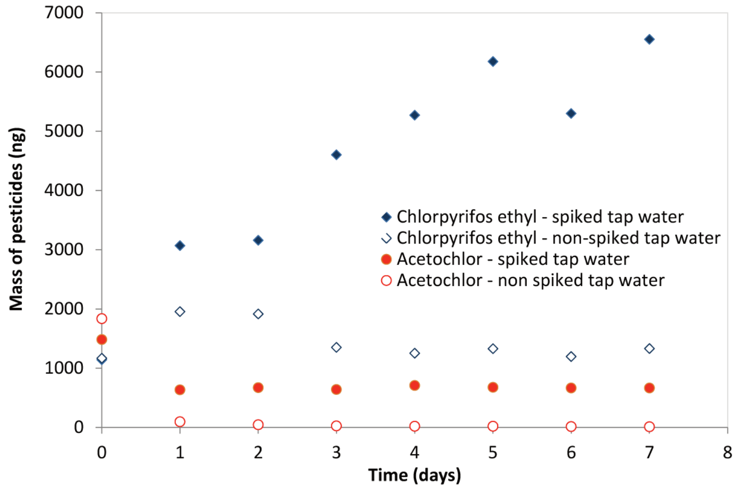

Figure 1. Masses of chlorpyrifos-ethyl and acetochlor in the SBSE stir bars plunged in unspiked water and spiked water for 7 days, after exposition to a concentration peak for 2 h

Fast accumulations of acetochlor and chlorpyrifos-ethyl were observed, as 1200 to 1800 ng of these pesticides were determined in the stir bars exposed to a 2-h concentration peak (day 0 in Figure 1). Then, acetochlor was mostly eliminated from stir bars plunged in unspiked water, whereas chlorpyrifos-ethyl remained in the stir bars. Moreover, elimination of acetochlor by half and additional accumulation of chlorpyrifos-ethyl (6-fold increase) were observed for stir bars plunged in spiked water. Further details on the results of this study will be developed in the communication.

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OP08
ACT1075

DETERMINATION OF THE ANTIDIABETIC DRUG METFORMIN AND ITS TRANSFORMATION PRODUCT GUANYLUREA IN WASTEWATERS IN GREECE

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Summary

In the present work, the occurrence and investigation of the antidiabetic drug metformin and its transformation product guanylurea, in eight Wastewater Treatment Plants (WWTPs) in Greece, was studied. Influent and effluent samples were collected over the four seasons of one year (along 2010-2011). They were obtained from eight WWTPs, from various cities (Ioannina City, Ioannina Hospital, Arta, Preveza, Agrinio, Grevena, Kozani, Veroia), in N.W. Greece (Epirus, Macedonia, Aitoloakarnania). An SPE procedure was applied followed by LC-ESI-MS in order to determine the occurrence of the two compounds in wastewaters. For further confirmation of positive findings of metformin and guanylurea, liquid chromatography coupled to a high resolution Orbitrap mass spectrometer was used (UHPLC/LTQ-Orbitrpap-MS). Next, the removal efficiencies across various types of WWTPs were assessed. Finally, hazard posed by pharmaceuticals in effluent wastewaters was assessed towards different aquatic organisms, (algae, daphnids and fish) through the calculation of risk quotients.

Introduction

Pharmaceutical compounds are characterized recently as "new environmental contaminants" or so called as "emerging contaminants", not only due to their persistence in the environment and incomplete degradation during conventional WWTPs, but also due to their possible effects or impacts in wildlife and humans [1, 2]. As far as pharmaceuticals concern, substantial data exists regarding their occurrence and ecotoxicology. Most of the analytical methods published focus their attention on parent compounds and as a consequence, little attention is paid to their metabolites and their transformation products (TPs) [3, 4].

Water Analysis

The extraction of metformin and guanylurea from waters seems to conclude in low recoveries when applying conventional methods due to their physicochemical properties (high polarity, low molecular weight, high pKa). So, an SPE procedure was applied and in order to achieve better recoveries for these two compounds, aqueous solution of anionic surfactant SDS (Sodium Dodecyl Sulfate) was used.

A LC-UV/Vis-ESI-MS system was used for the determination of metformin and guanylurea in wastewaters. The samples were analyzed using the ESI interface in positive (PI) ionization mode. Next, for further confirmation of positive findings of metformin and guanylurea, liquid chromatography coupled to a high resolution Orbitrap mass spectrometer was used.

Results and Discussion

The extensive work demonstrated the occurrence of these two compounds in the influents and the effluents of all

WWTPs. Concentrations ranged between bql and 1166.7 ng/L in the influents and between bql and 627 ng/L, in the effluents. High concentrations of metformin in the influents resulted in high concentrations of guanylurea in the effluents. Removal efficiencies of metformin were up to 98.5 %, indicating that its elimination might be related to its biological formation to guanylurea. In all effluents RQ < 0.001 for metformin, which means that low risk is suspected for the three trophic levels in the receiving water bodies of the eight WWTPs.

Conclusions

Application of an analytical method based on SPE and followed by LC-MS, provided wider knowledge about the occurrence and fate of metformin and its transformation product guanylurea, in the influents and effluents eight WWTPs of Greece. Further confirmation of positive findings took place by means of LTQ Orbitrap MS. To the authors knowledge this was the first time that ecotoxicology of metformin in the effluents of WWTPs was assessed in Greek aquatic ecosystem and therefore a further research is required in order to identify its environmental risk. Moreover, further studies are strongly recommended in order to estimate acute and chronic toxicity data of guanylurea.

Acknowledgments

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OP09
ACT1081

OCCURRENCE AND ENVIRONMENTAL RISK ASSESSMENT OF PHARMACEUTICALS IN SURFACE WATERS IN NW GREECE

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Summary

This study considers the occurrence and potential risk of pharmaceuticals in aquatic systems, with the view to prioritize some drugs which are likely to persist in the environment and hence be considered for environmental screening programmes. To be more specific, the aim of this work was to develop a simple, sensitive, selective and reliable method, based on off-line SPE followed by LC-ESI-MS to identify pharmaceuticals belonging to various therapeutical classes in the region of Epirus and especially in the River Kalamas and Lake Pamvotis. A list of 23 pharmaceutically active compounds (PhACs) was selected based on their consumption, persistency and likelihood of occurrence in surface waters and supported by observational studies where possible. The compounds investigated covered various classes, such as non-steroidal anti-inflammatory drugs (NSAIDs), analgesics-antipyretics, antibiotics, antihyperlipidaemics, psychoactive stimulants, antipsychotics, antiepileptics, antibacterials, estrogens, H2-antagonist, glucocorticoid steroids and b-blockers. The method developed was used to conduct a monitoring study in the sampling area, whilst the results acquainted were used to estimate the environmental risk assessment for the target compounds in terms of both acute and chronic toxicity.

Introduction

There is growing concern about the presence of pharmaceuticals in the wider aquatic environment. Common "over the counter" and prescription medicines are increasingly reported in waste and surface waters in the scientific literature [1].

Routinely used drugs enter wastewater and consequently ground and surface water through the excretion of drugs following administration to patients and via the drain. Some of these drugs are not fully metabolised or insufficiently biodegradable and therefore can resist biological as well as physical removal processes during wastewater treatment.

The potentially adverse effects they pose in the environment induct the study of data concerning their concentration, fate and behaviour in the environment. To fulfil this requirement analytical methods for a rapid, sensitive and selective determination of a broad range of compounds in complex environmental matrices are required [2].

Sample preparation

Water samples were collected in May, July, November and February, in order to cover all the seasons, from 8 sampling stations along the river Kalamas and Lake Pamvotis and were transported to the laboratory within 48 h. Upon reception, samples were filtered in order to eliminate particulate matter prior to SPE application. SDB-RPS disks were used to isolate the target analytes, while methanol was selected as elution solvent.

Results and Discussion

Concentrations ranged between below quantification limit and 3505,7 ng/L, for all compounds. Caffeine showed 100 % detection, indicating widespread presence of this compound in water in the study area, and its potential persistence, being hence the most abundant compound. In general, Lake Pamvotis presented the highest and the maximum mean concentrations, for the compounds analyzed, in all sampling months. According to the results obtained, salicylic acid, erythromycin and triclosan showed in many cases RQ>1, indicating the risk of environmental threat due to their presence.

Conclusions

The emerging demands to improve monitoring strategies require the screening of more and more pharmaceutical compounds. This comprehensive study identified - for the first time in this aquatic ecosystem - the significance of investigating pharmaceutical residue mixtures and their associated toxicity in surface waters. The analytical method described, based on SPE/LC-ESI-MS was successfully applied to monitor various PhACs in a target analysis approach, providing good sensitivity and selectivity for the simultaneous trace-level detection of 23 multi-class pharmaceuticals. Regarding the Environmental Risk Assessment, the results obtained can be taken into consideration for further study of the compounds involved with both chronic and acute toxicity.

Acknowledgments

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

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OP10

ACT1085

ASSESSING SUBSURFACE LATERAL TRANSFER OF PESTICIDES ABOVE A LOW-PERMEABLE HORIZON, IN A VINEYARD SLOPPY SOIL

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Summary

Surface water contamination by pesticides has been highlighted at both national and international levels. In order to reduce this contamination, it is necessary to better understand how pesticides are transferred by runoff and through the soil towards surface water. In the vineyard region of Beaujolais, eastern France, these transfers into the soil can be redirected towards surface water by a subsurface lateral flow triggered by a shallow impeding soil layer. The aim of this study is to better understand the way pesticides are transferred by this lateral subsurface flow and to compare its characteristics with runoff. Dissolved-phase pesticides fluxes are monitored on an instrumented farmed hill slope during natural rainfall events. Part of subsurface lateral flow is collected and measured by an experimental trench and runoff by a Venturi flume. Pesticides concentrations are monitored thanks to automatic samplers programmed to achieve flow dependant fractionated samples. Results from year 2013 and 2014 farming seasons illustrate the differences between runoff and lateral subsurface transfer (reactivity, flowing concentrations and duration ranges). Collected lateral subsurface fluxes for five pesticides are calculated and compared qualitatively according to their physico-chemical properties to highlight processes involved in their transfer.

Introduction

The European Water Framework Directive established regulatory requirements in order to improve surface water quality, including pesticide contaminations. Runoff has been proved to be the cause of high and short river contamination pics [1]. However, another process has been identified to contribute to their diffuse contamination: lateral transfers in shallow soil [1]. Little is known about the potential of field-infiltrated pesticides to reach rivers at mid or long term by this subsurface lateral way of transfer [2]. In Beaujolais, pesticide transfers are favoured by very permeable soils, steep slopes, and storms during pesticide application periods. A shallow low-permeable layer is often observed (clay horizon or shallow bedrock), possibly leading to lateral flows and pesticide transfers atop this layer. Here, we focus on lateral fluxes occurring atop a clayey impeding horizon less than one meter deep.

Hill slope instrumentation and sampling strategy

Water fluxes are collected and measured along the slope, on a 6000 m^2 plot. Discharge of runoff collected from half the plot is monitored by a Venturi flume with a water level sensor. Across the slope, part of subsurface lateral fluxes is intercepted by a three meter long trench, and measured with a tipping bucket. Rainfall induced shallow water table levels are also monitored by twenty-three piezometers (Figure 1).

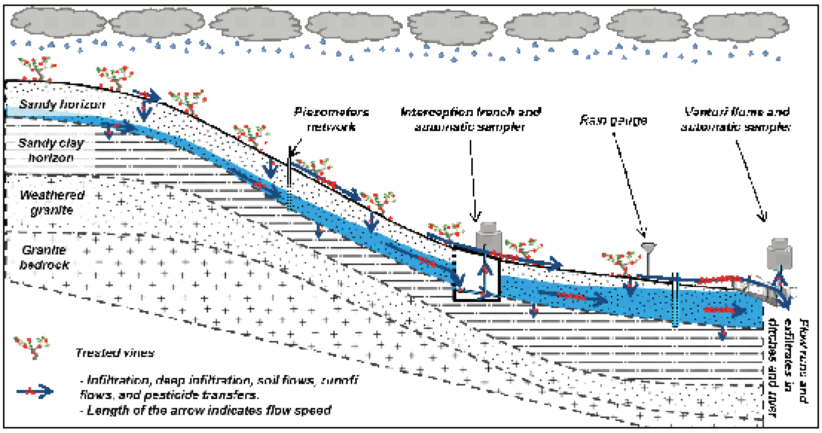

Figure 1: detailed diagram of the instrumented hill slope

Results and Discussion

Two main first results are highlighted by year 2013 survey. Subsurface lateral flow events last far longer than runoff events (Figure 2). Dissolved pesticide concentrations in subsurface flows can be high (3.3 µg/L maximum) and were always detected in our samples, although they are lower than in runoff (15.9 µg/L maximum). Higher concentrations in piezometers are observed when the water level is rising.

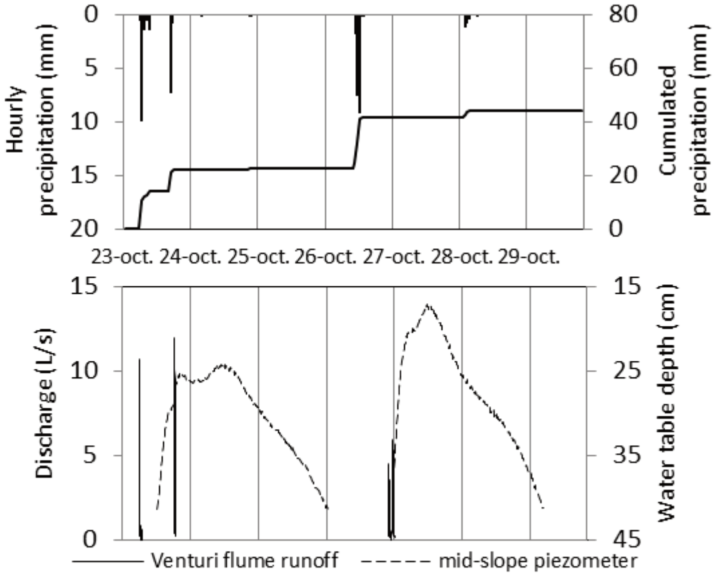

Figure 2: runoff, water table level and precipitation evolutions, 23rd-30th of October 2013.

Conclusions

This study provides first results showing subsurface lateral transfers can be significant processes in the transport of pesticides from plots to rivers, involving multiple and complex mechanisms. This survey must be carried on over several years to confirm the observed concentration dynamics and to estimate pesticide fluxes in subsurface flow.

Acknowledgments

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OP11
ACT1115

IMPROVE VINIFICATION OF MUSTS WITH RESIDUES OF PESTICIDES AND EFFECT OF RESIDUES IN THE QUALITY OF WINES

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Summary

Although the average concentrations of pesticide residues found in wines are mostly below the legal limits, the fact remains that some of them can affect the alcoholic fermentation. In this study, the ability of detoxification of several oenological products has been tested on a wine supplemented with different pesticides used in vine protection against mildew. When the must was treated with oenological products, the alcoholic fermentation was affected and it appears that the latency period was reduced. Oenological treatments enhance yeast activity limiting volatile acidity production and sometimes accelerating the alcoholic fermentation. The release of aromatic compounds appears to be modified, as noticed in the tasting of wines produced.

Introduction

In general, there is a widespread presence of pesticides in our environment. Several studies have identified and analyzed the links between exposure to pesticides and certain cardiovascular diseases, certain cancers, as well as the effects of pesticides on mental function and the central nervous system, or the effects on male fertility.[1] Each year, more than 220 000 tons of pesticides are spread annually in the European environment. The annual results show that the EU would use 108,000 tons of fungicides, herbicides 84,000 tons, 21,000 tons of insecticides and 7000 tons of growth regulators - which is about half a kilo of active substances for every man, woman and child living in the European Union.[2,3,4] The use of pesticides in viticulture promotes the production of healthy grapes and winemaking quality, but can also result in residues in grapes and wines. Technical routes must be found in order to limit the levels of pesticide residues in wines. In the vineyard, it is optimal to register vine protection inputs and implement a comprehensive program to protect the vine effective, resulting in a minimum of residues in wines.[5]

Residues of pesticides in musts and wines

In 20 years we went from about 900 active substances listed in 250 (European unfavorable revaluation, the active substance was not supported). Wine has a little more than 100 active sustances.[6] According to the European surveillance program of 24 food products analyzed, the grape is in 4th position for the greatest number of MRL.[3]To ensure food safety, the European Regulation sets Maximum Residual Levels (MRLs) of pesticides for many food products, including wine grapes. No MRLs have been set on wine by the European community, it is well accepted that the MRLs applied to wine grapes are defined on. Indeed they integrate the proceeds of winemaking and transfer of residues from grape to wine to ensure consumer safety. However, some countries have already set MRLs for certain pesticide residues.[7] MRLs is a legislative limit rather than toxicological that legalize the presence of residues to an acceptable level for the consummator. Although no toxicological real risk has been associated with the presence of pesticide residues in wines, this issue is a major concern of consumers and producers.[8,9] However, certain pesticides authorized in Europe are banned in Japan, the United States or Canada. This way the exportation of wine becomes a complicate case.[5]

All molecules produced in the vineyard are not necessarily found in the wine. Some degrade completely on the vine or in the wine and leave no trace. Other more or less degraded partially and may provide secondary metabolites.[9,10,11]

The analysis of pesticide residues has evolved significantly in recent years. The tandem mass spectrometry coupled with either a gas chromatography (GC-MS-MS) or with a liquid chromatography (LC-MS-MS) is used because it allows the isolation of the pesticide the matrix and provides a mass spectrum of the molecule to a comparable fingerprint. It thus achieves LOQ (limit of quantification) very sensitive to the order of micrograms per liter of wine. The analysis becomes more efficient detecting levels which were not previously measurable.[12] Analytically speaking in wine should be measured over 400 molecules associated with pesticide residues.[13,14,15] The analyzes, however, require time and are expensive, not allowing to take the right decisions at the right time during the short period winemaking and therefore the selling price of wine is increased.

In the winery, the interest of different operations (addition of oenological products, filtration) has been evaluated and compared several types of wine.[16,17,18]Different techniques such as the thermovinification, the cold settling replaced by filtration and the carbon filtration or nanofiltration can reduce these compounds in wines.[19] The aim is to identify routes that reduce overall wine or eliminate the risk of residues in wines without affecting the organoleptic qualities.[20,21]The concentrations of plant protection molecules in wine vary according to the winemaking, and a reduction or elimination of a large number of active ingredients is observed after fermentation. [22,23,24] Residues levels in wines are strongly related to positioning and choice of molecules.[25,26] Thus, choosing preferentially treatment with molecules that do not "pass in wines" from the flowering stage and positioning those known to transfer in wines early before flower, the goal of "reducing residues in wine "can be achieved. The winemaking process allowed decreasing residues of the initial musts.[27,28] Residues present high affinity to be adsorbed on the suspended solid matter. Results of in vitro assays showed that the presence of residues can either affect or not affect alcoholic nor malolactic fermentations. [29]

Study the activity of yeast

If oenological products can reduce pesticide residues in wine, can also eliminate them in the must? The interest would be multiple: limiting concentrations of active molecules in the wine and improve winemaking concerning the fermentation process and the production of aromatic compounds. In this study, by measuring the fall of the must concentration during the alcoholic fermentation we showed that the addition of enological products helps accelerate the fermentation relative to the control mode and to reduce the latency period. Analyzes in wine on the end of alcoholic fermentation, show differences in the production of volatile acidity (less volatile acidity than the control mode) and this results in a higher quality wine.

Conclusions

Determining residues of pesticides in order to verify and ensure good viticulture practices on vine protection treatments, optimize the management of treatment programs, ensure food security of the finished wine and reassure consumers. The goal is to have plant protection technical routes to minimize the residues while maintaining effective and consistent with good practice strategies.

The addition of oenological products in musts before alcoholic fermentation can remove a portion of pesticide residues. However, the incidence of oenological products could vary according to the active substance of the residues. The vilification's conditions can also influence their decrease. Furthermore, several yeast hulls also have very interesting detoxifying properties; they help to reduce wine's volatile acidity by accelerating the progress of the alcoholic fermentation. By acting preventively, the winemaking of potentially contaminated musts can be improved as well the quality of produced wines.

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OP12
ACT1019

EFFECT OF DIFFERENT FACTORS ON THE MOBILITY OF CYMOXANIL IN A VINEYARD SOIL

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Summary

The mobility of cymoxanil in a vineyard soil from La Rioja (Spain) unamended and amended with spent mushroom substrate (SMS) at two different doses 5 and 50% was studied using packed soil columns. Breakthrough curves of the fungicide were obtained as well as the pesticide distribution in the different sections of the column for different washing conditions (saturated flow and saturated-unsaturated flow) and different fungicide treatments within the column (non-incubated or incubated in soil for one month). The results indicated the total leaching of cymoxanil in unamended and amended soil under saturated flow conditions. However, the fungicide washed in the same conditions decreased drastically in the amended soil under saturated-unsaturated flow and similar results were found for the amended and unamended soil after fungicide incubation for both washing flows.

Introduction

The addition of organic matter (OM) to the soil through the application of organic residues can influence agricultural practices, such as pesticide application [1]. The processes that more strongly affect the behavior of the pesticide in the soil, such as adsorption, mobility and degradation depend heavily on the soil OM content. The influence of OM on the mobility of pesticides is of special concern because the movement of pesticides through the soil can reach the groundwater as has been indicated in the last years. The aim of this work was to study the mobility of cymoxanil in a vineyard soil unamended and amended with an organic residue, generated in the cultivation of mushrooms (SMS), by using packed soil columns. The effect of factors such as the dose of amendment, washing flow and aging time of the pesticide in the soil were studied in order to assess their influence on the behavior of cymoxanil, which is a fungicide widely applied in different types of crops. Of special interest is its application in vineyard crops in La Rioja (Spain) where the SMS is also frequently used as a soil organic amendment.

Materials and Methods

Leaching experiments were performed in glass columns of 3 cm (i.d) x 25 cm (length) packed with soil (100 g) with 67.0% sand, 11.9% silt, 21.1% clay and 51% carbonate content. The soil was amended with SMS at 0%, 5% and 50% on a dry weight basis. The pH and OM content were 7.52 and 0.67% (unamended soil, S), 7.26 y 1.76% (amended soil with 5%, S + SMS 5%) and 7.19 and 16.3% (amended soil with 50%, S + SMS 50%). Each column was oversaturated with water and the different pore volume (PV) of the packed columns was estimated by the difference in weight between water saturated columns and oven dry columns. Cymoxanil was applied in the top part of the columns by adding 1 mL of a solution of 1 mg mL^{-1} in methanol with a specific activity of 500000 dpm mL^{-1}. Two different washing flows were applied, saturated and saturated-non saturated. $CaCl_2$ solution was continuously pumped under saturated flow conditions or under a saturated-non saturated flow conditions (25 mL for 20 days) up to 500 mL. The flow rate was maintained constant at 1 mL min^{-1} by a peristaltic pump. Fractions of leaching solution (15 mL) were taken by an automated fraction collector. The leaching was carried out 24 h after the fungicide application in soil column (non-incubated) and after one month (incubated at 20ºC). The ^{14}C-cymoxanil concentration in the leached fractions was determined on a Beckman LS6500 Liquid Scintillation Counter (Beckman Instruments Inc., Fullerton, CA, USA) and the non-leached ^{14}C-cymoxanil in the soil was determined after leaching by soil combustion in an oven, Biological Oxidizer OX500 (R.J Harvey Instrument Corporation, Tappan, NY, USA).

Results and Discussion

The cymoxanil cumulative curves under saturated flow were similar in unamended and amended soil with 5% and 50% doses of SMS. These curves show the total leaching of the fungicide, although the maximum peak decreased in amended soils 4 times compared to the unamended soil. However, the total amount of fungicide leached under saturated-non saturated conditions decreased in the unamended soil, and especially in the one with 50% of SMS (<40%) (Table 1). After fungicide incubation the leached amounts decreased for all soils, although this decrease was much higher for amended soils (<30%) than for unamended soils (<70%). Differences in the maximum peak were also observed according to the soil and the flow conditions studied. The total balance of fungicide in the soil columns indicated the retention of fungicide in a similar amount for the two different flows in the incubated columns packed with unamended and amended soil. However, there was a higher fungicide mineralization in the unamended soil than in the amended ones for these incubated soils.

Table 1. Total amounts of leached, retained and mineralized fungicide for columns of unamended and amended soil, non-incubated and incubated, and under two washing flows

	Non Incubated soil column		Incubated soil column	
Soil	Saturated	Sat-Unsat	Saturated	Sat-Unsat
Total retained (%)	0.00	0.00	20.79±0.46	18.25±0.07
Total leached (%)	109.89±3.13	91.97±1.32	60.52±1.11	68.21±2.62
Mineralized	0.21±0.03	2.00±0.03	18.76±1.93	18.59±0.43
Soil + SMS 5%				
Total retained (%)	0.00	25.89±2.24	47.48±6.62	39.02±1.59
Total leached (%)	96.67±3.39	72.49±0.94	17.20±6.24	27.19±2.04
Mineralized	0.57±0.16	5.80±0.39	11.59±1.35	12.80±2.35
Soil + SMS 50%				
Total retained (%)	0.00	40.96±1.00	51.04±1.78	44.41±0.61
Total leached (%)	98.12±2.98	36.84±1.19	23.42±0.90	27.19±2.04
Mineralized	0.16±0.00	9.38±3.29	7.63±1.1	10.51±1.52

Conclusions

This study highlighted that organic residue reduced the leaching of cymoxanil under some of the experimental conditions studied, which is important in order to reduce the pollution of groundwater by pesticide drainage.

Acknowledgments

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OP13

ACT1025

ASSESSMENT OF TRANSFORMATION PROCESSES OF EMERGING POLLUTANTS OCCURRING IN SURFACE WATERS

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Summary

This study was aimed to enlighten the fate of selected emerging pollutants (EPs) in river water, focusing on their degradation and on the identification of their transformation products.

In order to investigate the biotic and abiotic transformations of selected micropollutants, we designed two experiments. Firstly, laboratory experiments in the dark and under illumination were performed on river water spiked with drugs to simulate all possible transformation processes occurring in the aquatic system. Under illumination, these EPs were degraded and transformed into numerous intermediate compounds.

Secondly, all the possible main and secondary transformation products (TPs) were searched for in several samples collected from the Po river water (Northern Italy) and in wastewater plants. The selected pollutants and several TPs were detected in all samples.

Introduction

The occurrence of a wide variety of so called "emerging pollutants" worldwide is well documented and the focus of research has now shifted toward the fate of these compounds once they have entered the water bodies. A major class of EP comprises pharmaceuticals; in particular, antibiotic and anticancer drugs were identified as an important topic as, at present, the first ones are among major sources of bioactive molecules in water and, the second ones need to be overlooked owing to their cytotoxicity. In recent years, increasing evidence has pointed toward the importance of investigating the presence of both human metabolites and photo-transformation products of EPs in surface waters. Assessing the presence of transformation products can help in understanding the fate of parent compounds in environments influenced by multiple mixing and degradation processes. Human metabolites of pharmaceuticals are of rising concern as may potentially be as toxic and/or persistent as their parent compound. On the other hand, photoinduced processes including, direct photolysis processes and reactions mediated by photosensitizers, are known to play a key role among the abiotic transformation following their discharge in the environment.

Materials and methods

A sunlight simulator apparatus (SOLARBOX CO.FO.MEGRA) was used to irradiate different aqueous solution of the selected pollutants. Analyses were performed by liquid chromatography-LTQ-FT-Orbitrap mass spectrometry. Unknown compound were characterized by analyzing MS and MS^n spectra, whereas HRMS with MS/MS fragmentation was used as a confirmatory step for proper identification of compounds in natural water.

Results and Discussion

Several drugs (i.e antibiotics and anticancer drugs) and some personal care products were investigated. Laboratory experiments were performed on river water spiked with EPs to simulate potential transformation processes occurring in the aquatic system. Then, all the identified TPs were searched for in natural samples. They were detected in all samples. The presence of selected EPs and several TPs, corresponding to human metabolites and abiotic transformation products were assessed in river water samples taken at various locations in the Po river tract and in a wastewater treatment plant. It was possible to find key TPs that could be considered as markers for EPs photochemical environmental transformation in the aquatic environment.

Conclusions

This approach permitted not only to assess the selected EPs presence in natural waters, but also to identify which of the transformation routes recognized in simulation experiments also occurred in the aquatic environment.

OP14
ACT1065

FATE AND REMOVAL OF PHARMECEUTICALS IN WATERS FROM AQUACULTURE: THE CASE OF CIPROFLOXACIN

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Summary

Agrochemicals such as antibiotics and pesticides are heavily used in fish farming plants. This investigation focuses on i) the fate of antibiotics used in aquaculture and, ii) the development of photocatalytic water treatments for their removal. The results deal with the ciprofloxacin (CPF) dissolved in artificial freshwater. In a first step, CPF was exposed to simulated sunlight and showed half-lives ranging from 20 to 70 min for irradiation intensities of 750 and 250 Wm^{-2}, respectively. Although CPF undergoes a relatively fast photolysis, no mineralisation was observed and the formation of toxic by-products was confirmed by LC-MS/MS analysis. In a second step, CPF was exposed to UV-A light and/or to TiO_2 thin films in a circular photoreactor. The presence of the photocatalyst resulted in up to 9-fold enhancement of the removal of CPF. The complete elimination of CPF in less than 30 min and the abatement of 95% of initial TOC in 3h were achieved. The elimination of the initial and residual toxicity was observed by applying the *Vibrio fischeri* test. Photocatalysis with immobilized nano-TiO_2 provides a fast and efficient methodology for the removal of CPF and its toxic by-products in waters from aquaculture.

Introduction

Aquaculture production has rapidly developed over the past decade to address the shortages in capture fisheries. It is the fastest growing food-producing sector, accounting for about 50% of the food fish worldwide [1]. For the first time in 2013, the production of farmed fish has outstripped the one of farmed beef. However, intensive culture practices, with heavy input of formulated feeds and/or agrochemicals as well as deficient waste management, have negative impacts on local environments [2]. The use of herbicides may cause irreversible damage in the environment. The administrated antibiotics, lost in the surroundings of the plants, may affect non-target species and lead to the development of drug-resistance issues [3]. It is currently recognized that the improvement of the practices to protect water is essential for the sustainability of the aquaculture activities.

The present study is carried out in the frame of the project INNOVAQUA for the development of technological innovations in aquaculture in Italy and Slovenia.

Photolysis and Photocatalysis experiments

CPF solutions (50 mgL^{-1}) were prepared in artificial freshwater (pH 7.3). CPF was exposed to simulated sunlight in a Suntest at different intensities (250-750 Wm^{-2}) and to UV-A light (λ_{max}=355nm) in the presence of TiO_2 thin films in a circular photoreactor. The CPF concentrations were followed on a spectrophotometer and by HPLC-DAD/FLD. Samples were taken at different times of exposure and analysed for TOC and F^- contents. The toxicity of CPF and its reaction intermediates was investigated applying *Vibrio fischeri* test. The main organic by-products were identified by LC-MS/MS analysis.

Results and Discussion

CPF is efficiently photodegraded by simulated sunlight with half-lives ranging from 20 to 70 min for intensities of 750 and 250 Wm^{-2}, respectively. However, no mineralisation is observed and the photolysis leads to the formation of stable and toxic organic by-products. The presence of the photocatalyst resulted in up to 9-fold enhancement of the removal of CPF (Figure 1). The results give promise for i) the complete elimination of CPF in 30 min, ii) the abatement of 95% of TOC in 3h and ii) the elimination of the initial and residual toxicity.

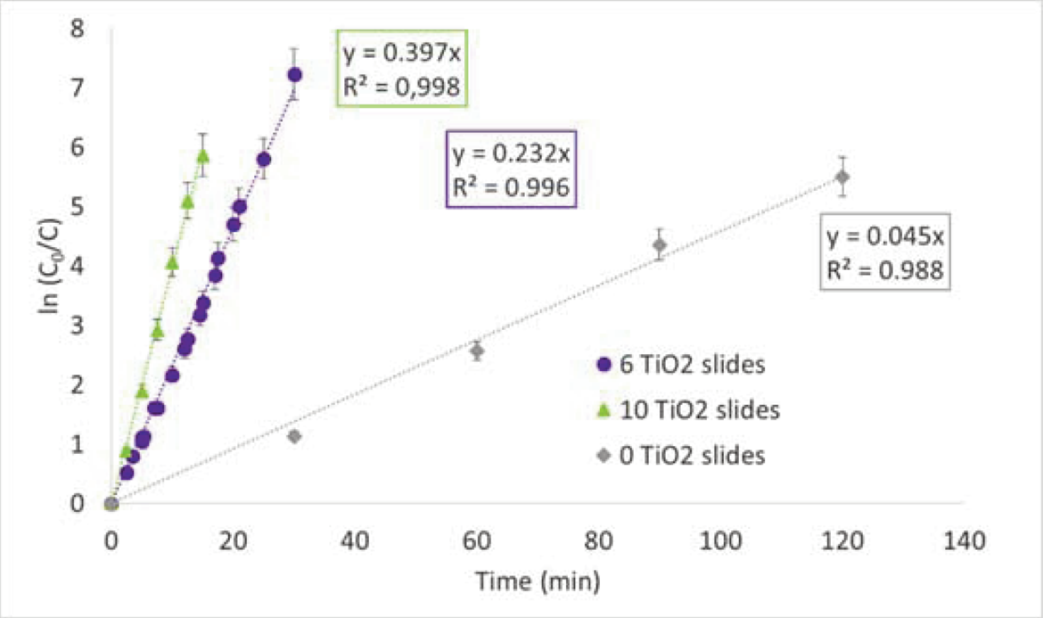

Figure 1: Photocatalytic kinetics of CPF in the presence of i) 0, ii) 6 and iii) 10 TiO_2 slides and UV-A light.

Conclusions

For the sustainability of aquaculture activities, reliable water treatment processes should be implemented. Photocatalysis methods based on TiO_2 films are promising alternatives and can be adapted to the needs of the fish farms.

Acknowledgments

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OP15
ACT1113

OCCURRENCE, REMOVAL AND ENVIRONMENTAL RISK ASSESSMENT OF PPCPs IN WASTEWATER TREATMENT PLANT (WWTP) OF GREECE (VOLOS)

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Summary

In this study, the occurrence, removal and environmental risk assessment of fifty seven pharmaceuticals (various therapeutic classes) and personal care products (PPCPs) in waste water treatment plant (WWTP) of Volos, Greece, has been investigated. Influent and effluent samples from four sampling campaigns, in 2013, were extracted through Oasis HLB cartridges, and quantified through liquid chromatography with diode array detection and electrospray ionisation mass spectrometry (LC-DAD-ESI/MS). The method was validated and good results were obtained to determine 57 analytes. The most commonly detected pharmaceuticals were furosemide, atenolol and metoprolol, paracetamol, nimesulide, salicylic acid, diclofenac and caffeine with mean concentration ranged between 112 and 4819 ng/L for influents, and between 109 and 1507 ng/L for effluents. The removal efficiencies ranged between10 and 100%, which demonstrates that WWTP is not able to efficiently remove the complex mixture of PPCPs. Negative removals were also obtained for compound such as carbamazepine, diclofenac, cetirizine and venlafaxine Finally, the potential ecotoxicological risk posed by PPCPs to different trophic levels of aquatic organisms, exposed to the effluent wastewaters studied was evaluated by means of risk quotients (RQ).

Water Sampling and Analysis

24-h composite samples collected from the influent and effluent of Volos WWTP, which treats water from an area of 125,248 inhabitants and receives urban wastewaters (including domestic, industrial and hospital effluents). Sampling campaigns, carried out in 2013, were performed during a one year follow-up study, embracing four sampling periods.

Results and Discussion

Results demonstrated that furosemide, atenolol and metoprolol, paracetamol, nimesulide, salicylic acid, diclofenac and caffeine were the most frequently detected compounds. The highest values corresponded to simvastatin, caffeine, paracetamol, nimesulide, salicylic acid, diclofenac, ciprofloxacin, cetirizine, atenolol, furosemide, metoprolol and salbutamol (Figure 1).

Removal efficiencies ranged at three different levels: low- medium and high level, whereas negative removals were observed in some cases. Regarding the risk assessment diclofenac proved to be the most critical compound (Figure 2).

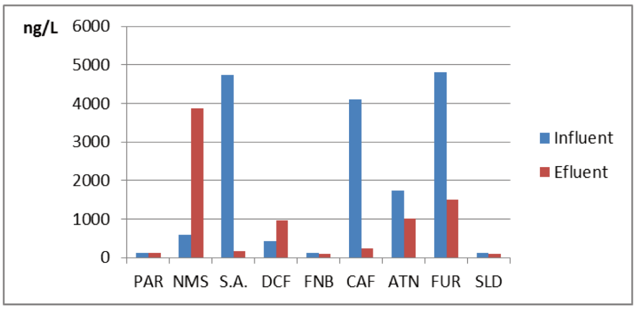

Figure 1: Mean concentrations of PPCPs in influents- effluents of Volos WWTP

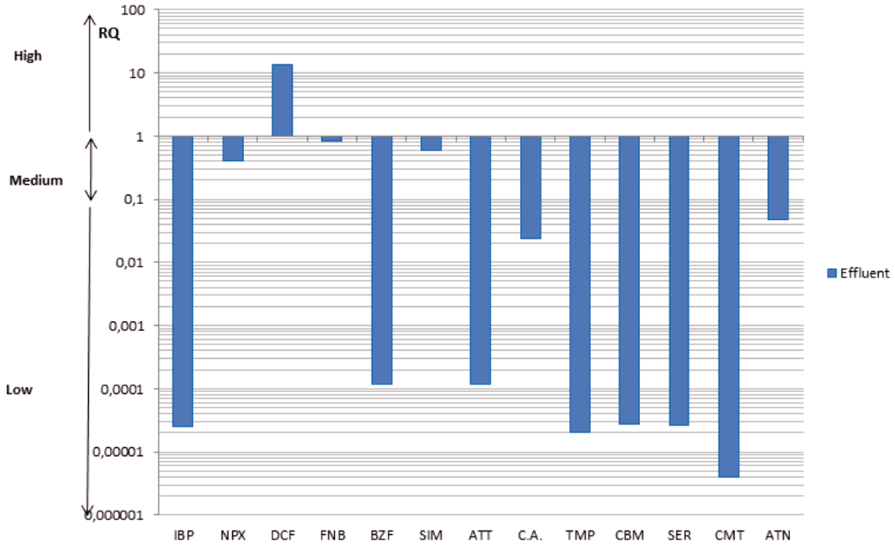

Figure 2: Risk quotients of selected pharmaceuticals in effluents of Volos WWTP for fish

Conclusions

A multiresidue analytical method was developed for screening of 57 PPCPs in influents and effluents of Volos WWTP. Furosemide and nimesulide presented the highest concentration in effluent samples. Regarding the risk assessment diclofenac proved to be the most critical compound.

Acknowledgements

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OP16
ACT1010

PHOTOLYTIC DEGRADATION OF ALLOXYDIM ON MODEL SOIL SURFACES AND PHYTOTOXICITY STUDY OF ITS MAIN PHOTOPRODUCT

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Summary

Photolysis of herbicide alloxydim has been investigated on different soil model systems. In addition, the degradation products formed in this process have been separated and identified by HPLC coupled to Qtof mass spectrometer. The main photoproduct, alloxydim-desethoxy, was isolated to compare its phytotoxicity with the active substance on the germination of succeeding crops and grass weeds.

Introduction

Alloxydim belongs to the herbicide family of cyclohexanedione oximes (CHDs) and it is used for the post-emergence control of gramineous weeds in sugar beet, vegetables and other broadleaf crops. Because the herbicide is sprayed at early growth stages of grass weeds, a large fraction of alloxydim is directly deposited on soil surfaces. Once on soil surfaces, the herbicide can degraded due to biotic and abiotic factors being photochemical process an important route which affect its environmental fate and persistence. Due to the complexity of soil matrices, a frequently approach to evaluate the soil photolysis of pesticides is to use different solid surfaces such as glass disk or silica gel plates as simple models of soils.

As consequence of the photodegradation of alloxydim, a number of photoproducts can appear that could be more toxic and/or persistent than the parent herbicide. Therefore, it is of utmost importance to study the photoproducts formed to ascertain if these compounds represent any environmental risk.

The aims of this work were to identify the photoproducts of alloxydim herbicide on soil model systems and to evaluate the phytotoxicity of the photoproducts generated.

Experimental

Methanolic solutions of alloxydim were deposited on glass disks and silica gel plates and exposed to simulated solar light ($\lambda \geq 290$ nm) for different irradiation times in a Suntest CPS+ apparatus.

Extracts of irradiated samples were analysed using HPLC-DAD to determine the remaining concentration of alloxydim. Kinetic parameters of the herbicide were calculated assuming a first-order kinetic. Moreover, transformation products formed during the photolysis of alloxydim were identified using HPLC-ESI-Qtof. The main photoproduct of alloxydim was isolated by SPE using Isolute ENV+ cartridges and eluted with 3x2 mL of methanol.

For the germination bioassays, different species were selected: two winter wheats (*Triticum Turgidum* L., *Triticum aestivum* L.), one grass weed (*Bromus diandrus* L.) and two dycotiledonous crops (sugar beet and tomato). Seeds were treated with different doses of alloxydim and alloxydim-deallyoxylated and place in a growth chamber. Root and coleoptile lengths of seeds were recorded after 5 days for wheat and 9 days for grass weed and dycotiledonous crops to determine EC50 values from the doses-response curves.

Results and Discussion

Alloxydim was rapidly photodegraded in systems that simulate soil surfaces with half-lives of 8.1 min and 1.2 min on glass disk and silica gel plates, respectively. Therefore, it can be expected that alloxydim suffers a rapid photolysis on real soils. Moreover, some interaction between molecules of alloxydim and silica gel particles was suspected because it was observed a higher degradation rate of the herbicide on the silica gel plates compared to the glass disks.

Two different photoproducts were detected and identify by Qtof mass spectrometry; *Z*-isomer of alloxydim and alloxydim-deallyoxylated. The latter was formed in a much higher concentration than the *Z*-isomer and resulted more stable than the active substance.

The EC50 values calculated from the root lengths of wheat species and grass weed ranged from 0.38 to 0.50 mg L^{-1} for alloxydim. In contrast, the EC50 values for deallyoxylated alloxydim ranged from 94 to 600 mg L^{-1} in the same species and in crops where the herbicide was applied.

Conclusions

Alloxydim on different soil model systems undergoes a rapid photolysis, indicating that this degradation process could be an efficient route of dissipation for the herbicide on soil surfaces. On both surface models, alloxydim-deallyoxylated compound was identified as the main photodegradation by-product of alloxydim while *Z*-isomer was formed in small amounts.

Bioassays show that deallyoxylated alloxydim presents low phytotoxicity to the species tested, while alloxydim can cause injury on succeeding crops and non-target plants.

OP17
ACT1083

ANALYSIS OF FUNGICIDE RESIDUES IN WHITE GRAPE MARC BY PRESSURIZED LIQUID EXTRACTION. METHOD PERFORMANCE COMPARISON OF GAS AND LIQUID CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Summary

Ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE) followed by gas and liquid chromatography-triple quadrupole-mass spectrometry (LC- and GC TQ-MS) were used for the determination of 11 fungicides (metalaxyl, cyprodinil, procymidone, iprovalicarb, myclobutanyl, kresoxim-methyl, benalaxyl, fenhexamide, tebuconazole, iprodione and dimethomorph) in white grape marc. The PLE procedure showed much higher efficiency than UAE for the target fungicides. Recoveries for the majority of studied fungicides were higher than 80%.

GC- and LC- ESI-TQ MS were compared for the quantitative analysis of several fungicides commonly used in viticulture. The performance of both techniques was evaluated in terms of detection limits (LODs), precision and linear working range.

For most target fungicides, higher sensitivity was obtained using LC-TQ MS (about one order of magnitude), with LODs at the low pg mL^{-1}, (sub ng g^{-1} for the majority of the target fungicides). These values were well below the European maximum residue limits (MRLs) for wine and table grapes, and vine leaves. However, some compounds such as procymidone and cyprodinil could not be adequately determined using LC-ESI-TQ MS. Iprodione showed an enhanced response by using LC-APCI-TQ-MS.

Eighteen white grape marc samples were analyzed and nine out of eleven targets were detected in the samples. Seven of them were detected in more than 50% of the samples and most samples content at least 4 of the target analytes. The most frequently found compounds were tebuconazole and dimethomorph with concentrations between 1.6-130 and 2.0-1788 ng g^{-1} respectively. Some samples showed very high levels of many of the studied fungicides (µg g^{-1} for cyprodinil, fenhexamide, iprodione and dimethomorph), but all of them below the European maximum residue limits (MRLs) for wine grapes.

Introduction

Fungicides are a class of pesticides widespread used in viticulture to avoid fungi infection of *Vitis* plants. Their use has brought many benefits with respect to enhanced quality of produced crops, but there are concerns about the presence of their residues in crops, which may pose a health hazard to the consumers. In addition, several studies have shown that some fungicides and their degradation products can be transferred from grapes to wine during the winemaking process, depending on the type and concentration of fungicides applied to grapes and other operational conditions. Consequently, fungicide residues are frequently found at low concentration levels in the final commercial wine.

Marc is the residue left behind after the juice has been removed from bunch of grapes during winemaking. There is an increasing interest, supported by environmental and economic reasons, to recover and exploit these wastes from the food industry, because such residues can be used as a source of natural bioactive compounds, which could in turn be used in pharmaceutical, cosmetics or back in the food industry. Therefore, the levels of fungicides in grape marc must be controlled in order to avoid environmental pollution and human exposure to these compounds.

The European Union (EU) has regulated the Maximum Residue Limits (MRLs) for fungicides in grapes. Although pesticide MRLs have been suggested for wine in order to guarantee as much as possible the safety of the beverage, the presence of fungicides in wine is not still regulated, which is a matter of concern for both consumers and producers.

Nevertheless, a look at the scientific literature evidences the lack of studies devoted to the development of methodology for the determination of fungicides in marc samples whereas in grapes, other fruits and vegetables, environmentally friendly procedures including microwave assisted extraction (MAE), ultra sound assisted extraction (UAE), QuEChERS, solid phase micro-extraction (SPME) or matrix-solid phase dispersion (MSPD) are substituting traditional methodologies like Soxhlet extraction. Pressurized liquid extraction (PLE) was also employed to determine various chemical classes of fungicides in different matrices such as mushroom compost, vineyard and agricultural soils, green leafy vegetables or green tea.

Considering that the contents in wines are significantly lower than in grapes, sensitive and selective analytical methods are required to detect pesticide residues in wine. These analytical methods are based on liquid chromatography (LC) or gas chromatography (GC). Liquid or gas chromatography in combination with triple quadrupole mass spectrometry (MS-MS) is a valuable approach that improves selectivity and analyte sensitivity.

Our recent previous study addresses the validation of a new method to analyze 11 fungicides from different chemical classes (metalaxyl, cyprodinil, procimidone, iprovalicarb, myclobutanyl, kresoxim-methyl, benalaxyl, fenhexamide, tebuconazole, iprodione and dimethomorph) in white grape marc, based on pressurized liquid extraction-gas chromatography- -mass spectrometry [1].

The present study aims at comparing the method performance achieved by GC-TQ-MS and LC-TQ-MS for the determination of the same fungicides. Quantitative results in 18 real white marc samples are also presented.

Materials and methods

2.1. Marc sample

Marc samples were selected among five different varieties of white grape of Galicia (Albariño, Caiño, Loureira, Treixadura and Godello).

2.2. UAE and PLE procedures

Ultrasound-assisted extraction (UAE) was carried out using an ultrasonic cleaning bath with a working frequency of 50 kHz and 110 W of power (Ultrasound Med-II, J.P. Selecta, Barcelona, Spain).

PLE extractions were performed on an ASE 150 (Dionex, Co., Sunyvale, CA, USA), equipped with 10 mL stainless steel cells and 60 mL collection vials.

2.3. GC TQ-MS and LC TQ-MS analyses

GC TQ-MS analyses were performed using a Thermo Trace 1310-Triple Quadrupole 8000 with autosampler IL 1310 from Thermo Scientific (San Jose, CA, USA). The mass spectra detector (MSD) operated in selected reaction monitoring mode (SRM), monitoring two transitions per compound. A Thermo TG-5SILMS capillary column (30 m x 0.25mm i.d., 0.25mm film) was used.

LC-MS/MS analyses were carried out using a Thermo Fisher Scientific (San Jose, CA, USA) instrument consisting of an Accela 1250 HPLC pump/autosampler coupled to a Quantum Ultra triple quadrupole mass spectrometer, with an heated electrospray ionization (HESI) source or with an APCI source. 10 µl of standard or extract solution were injected into the Hypersil GOLD aQ C18 column (100 x 2.1 mm, 3µm) (Thermo Fisher Scientific, San Jose, CA, USA). The analytical separation was performed using a gradient elution of water/ammonium formate (3 mM) containing 0.15% formic acid (mobile phase A) and methanol/ammonium formate (3 mM) also containing formic acid (0.15%) (mobile phase B).

3. RESULTS AND DISCUSSION

3.1 Ultrasounds assisted extraction

First efforts were focused on the development of an *"easily to implement"* low cost methodology based on the use of ultrasound energy. Ultrasound extraction employing an ultrasonic bath is a strategy affordable for any laboratory due to its low cost and simplicity of use. Most studies for extraction optimization are carried out on spiked samples, implying that the real interaction of the sample with the analytes is not assessed. In the present study, a real non-spiked marc sample containing most target compounds was employed. The multifactor ANOVA study showed that the solvent was the most relevant factor being statistically significant for all analytes. The other factors, temperature and salt addition, were not significant.

3.2 Pressurized liquid extraction

Under the optimal conditions, UAE was compared with PLE for the same real sample. PLE extractions were performed at 80ºC for 15 minutes. Unexpectedly, the responses were clearly lower for UAE extraction and thus, we decided to continue the study using PLE. Temperature (A) and time (B) parameters were studied at three levels: 80, 100 and 120 ºC and 5, 10 and 15 min respectively, and optimized by means of an experimental design 2^3. Once again, the study was performed using a real non-spiked marc sample.

Temperature (A) was significant for four of the ten target analytes present in the sample. On the other hand, the time (B) was not significant for any of the compounds. Therefore, 5 min and 120 ºC were the experimental conditions selected.

The optimization of the extraction procedure by PLE is described in a recent published paper [1]. Recovery studies were carried out by applying the optimized method to the extraction of a real sample, spiked at 100 ng g^{-1} and 1000 ng g^{-1}. Recoveries were between 81-120 % in all cases.

3.3. Method performance comparison

For GC TQ-MS, precision values (% rsd) ranged from 0.6 to 9% (intra-day), and from 3.3 to 13% (inter-day). Instrumental detection limits (IDLs), calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3), were below 0.05 ng mL^{-1} (1 ng g^{-1}).

Regarding LC TQ-MS, precision values (% rsd) ranged from 1 to 10% (intra-day) and from 2.1 to 12.3% (inter-day). Higher sensitivity was obtained than with GC-TQ-MS, about one magnitude order.

Out of the 11 target fungicides, only iprodione gave much better ionization yield (one magnitude order) by APCI than by HESI.

In any case, detection and quantification limits were several orders of magnitude lower than the European MRLs laid down for wine and table grapes and vine leaves.

It is important to emphasize that the PLE extract (20 mL) was directly analyzed without concentration and then these limits could be even improved by concentrating the PLE extract. However, this concentration step could hamper the analysis by LC-ESI-MS/MS owing to the likely happening of matrix effects.

3.4. Application to real samples

The validated method was applied to the analysis of 18 real white grape bagasse samples including five Galician varieties: Albariño (Alb), Caiño (Cai), Loureira (Lou), Treixadura (Tre) and Godello (God). The target fungicides were detected in all samples. Tebuconazole and dimethomorph were the most abundant (found in 17 and 16 samples, respectively). Fenhexamide and myclobutanyl were found in 72 and 67% of the samples, respectively. Metalaxyl, cyprodinil and iprodione were also detected in 9 of the 18 studied samples. Iprodione levels were quite high (6021 and 8800 ng g^{-1}) but they were below the European maximum residue limits (MRLs) for wine grapes (the highest MRL among our target compounds). Iprovalicarb and benalaxyl were detected in 4 and 13 samples, respectively. Procymidone and kresoxim-methyl were not found.

4. CONCLUSIONS

PLE has been successfully applied to the determination of 11 fungicides in white grape marc. Under the optimized conditions, fungicides were extracted with a mixture of hexane: acetone (1:1 v/v) for 5 min at 120ºC. Method accuracy and precision were satisfactory, showing mean recovery values higher than 80% and precision generally below 12%.

From the comparison of LC-TQ-MS and GC TQ-MS techniques, it can be concluded that the former provides better IDLs (low pg mL^{-1}) and LODs than the latter. In most cases, this difference was about one order of magnitude, which undoubtedly provides an advantage for the detection of fungicides in real marc samples when they are present at trace levels.

However, procymidone could not be analyzed by LC and cyprodinil gave better response by GC.

ACKNOWLEDGEMENTS

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OP18
ACT1034

DEVELOPMENT OF NEW CERTIFIED REFERENCE MATERIALS AS KEY TOOL FOR QUALITY ANALYSIS OF PESTICIDE RESIDUES IN VEGETABLES

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Food safety is a general human health issue that is a concern for all consumers. In the European Union the uses of pesticides as well as the maximum residue limits in food are therefore regulated. This involves regular pesticide residue monitoring, which requires comparability of analytical results regardless sample treatment and/or instrumental approach. Harmonisation of reliable analytical methods is of utmost importance. Control laboratories apply validated methods to ensure accurate, repeatable and reproducible results. According to ISO 17025 the use of reference materials and the participation in proficiency testing are essential tools for assuring and controlling the quality of analytical data. Certified reference materials (CRMs) are used for verification of accuracy, estimation of uncertainty and to establish the traceability of analytical results to a common reference. The CRM themselves must be homogeneous and stable materials, ensuring the validity of the certified values.

In the field of pesticides the number of available CRMs is rather limited. One of the challenges for the analytical laboratories is the wide range of compounds and matrices to be covered for method development and validation. The role of the reference material producer involves providing CRMs to both facilitate the duty of the analytical control laboratories and underpin their results. For the CRM producer the challenges are then to identify representative combinations pesticide-matrix, and to identify the optimal conditions for the material preparation and storage, taking into consideration the characteristics of fresh vegetable matrices and the low stability of the pesticides. The task requires parallel in house development of accurate analytical methodology for the selected pesticides-matrix combinations. All these aspects are discussed here using a case study of a recent testing material preparation for pesticide residue analysis in vegetables as an example.

Acknowledgments: The IRMM-SID processing team is acknowledged for the preparation of the cucumber material employed in the study. The EURL FVs is acknowledged for their contribution during the selection of relevant pesticides target of the study.

0P19
ACT1055

ANALYSIS OF MULTIPLE PESTICIDE RESIDUES IN MELON USING LIQUID CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Summary

A rapid, simple and efficient method for the determination of seven insecticides and three fungicides in melon using quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method coupled with liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) was developed and validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 82% to 106% with RSDr ≤ 6% in four fortification levels of 0.01, 0.05, 0.1 & 0.2 mg/Kg. The linearity was ≥0.997 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.001 to 0.003 mg/Kg and the LOQ was 0.01 mg/Kg, which was lower than the Maximum Residue Levels set by European Union (EU-MRLs) for all of the selected pesticides. This method was applied for the residue analysis of 83 fresh melon samples collected from different market places in Thessaloniki, Greece. Among the 83 analyzed samples 20 (23% of the total no. of samples) were found with pesticide residues. None of the samples contained residue levels above the EU-MRLs.

Introduction

Insect pest and diseases are the major constraints for the commercial production of melon. To overcome these situations farmers are using pesticides as it is the most convenient and economical way to control the insect pests and diseases and to increase the production. In this study, 10 of the most commonly used and also registered pesticides for the control of insect pests and diseases of melon in Greece were selected, of which seven insecticides such as chlorpyrifos, dimethoate, deltamethrin, thiamethoxam, thiacloprid, pirimicarb, and indoxacarb and three fungicides such as azoxystrobin, fluopicolide and propamocarb hydrocloride. Registered pesticides were used in this study in order to find out the actual scenario of pesticide residues in melons produced and marketed in Greece. Keeping this view, the present study was initiated to develop and validate a multiresidue analytical method for melon using LC-MS/MS and also to monitor the pesticide residues in melons in Greece.

Materials and Methods

A Liquid Chromatography Triple Quadrupole Mass Spectrometry (Thermo Electron Corporation, Waltham, MA, USA) system equipped with an electrospray ionization (ESI) source operated at positive ion mode was used for the identification and quantification of the selected pesticides. Melon samples were collected from different market places in Thessaloniki, Greece such as supermarkets, open markets and retail vegetable shops. Collected samples were prepared using the QuEChERS method [1] with little modification of cleanup materials.

Results and Discussion

Optimization of cleanup: A series of trials were conducted to determine the appropriate amount of anhydrous $MgSO_4$ and Primary Secondary Amine (PSA) sorbent for the proper cleanup of the samples. At the end of these trials 600 mg anhydrous $MgSO_4$ and 75 mg PSA for 3 mL extract were selected since for all pesticides recoveries were in the range of 91-105% with RSDr ≤ 4%.

Method validation: A very good accuracy and precision was found for all of the analytes at four fortification levels of 0.01, 0.05, 0.10 and 0.20 mg/Kg. The average recoveries ranged from 82% to 106% with relative standard deviations (RSD_r) ≤ 6% for all of the analytes. Reproducibility (Interday accuracy and precision) was evaluated during a period of two months by analyzing fortified samples at two fortification levels of 0.01 and 0.10 mg/Kg. Reproducibility was found to be very good. Average recoveries ranged from 88 to 106% and RSD_R ≤9%for all of the analytes. Relative Percent Difference (RPD) was also calculated at two fortification levels of 0.01 and 0.10 mg/Kg. RPD were ≤ 6 for all of the analytes.

Linearity was evaluated by calculating the correlation coefficient, intercept and slope of the regression line. Linearity was very good and coefficients of determination were ≥ 0.997 for all of the selected pesticides with matrix matched calibration standards. The LODs ranged from 0.001 to 0.003 mg/Kg. The LOQ for all of the selected pesticides was set to 0.01 mg/Kg as at this level the acceptable accuracy (mean recoveries for individual pesticides were in the range of 82% to 104%) and precision (RSDr ≤6%) were achieved. The proposed method was applied for the residue analysis of 83 melon samples. Among the analyzed samples 20 (23% of the total no. of samples) had pesticides residues. The levels of the detected pesticide residues were 0.010-0.029 mg/Kg. None of the samples was found contaminated at a level above the EU-MRLs [2].

Conclusions

The proposed method is an efficient multiresidue analytical method for melon using LC/ESI-MS/MS. In this method, a very good accuracy (82 to 106%) and precision ($RSD_r \leq 6\%$ and $RSD_R \leq 9\%$) was found for all of the analytes, thus fulfilling the requirement set by SANCO document no. SANCO/12495/2011 for accuracy and precision [3].

Acknowledgments

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OP20
ACT1084

DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETERMINATION OF THIABENDAZOLE IN WHITE WINES

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Summary

A competitive indirect ELISA for the determination of thiabendazole in spiked white wines has been developed. Microtitration wells were coated with a thiabendazole-bovine serum albumin conjugate and blocked. After washing, 50 µL of calibrators or wines diluted 30X in assay buffer, and 50 µL of a monoclonal anti-thiabendazole antibody solution were added to wells for 1h. Then, anti-mouse IgG-HRP solution was added, followed by addition of chromogenic substrate (ABTS/H_2O_2) and measurement of the O.D. at 405 nm. The assay had: LOD 0.005 ng/mL; dynamic range 0.01-5 ng/mL; intra- and inter-assay CVs less than 5 and 8%, respectively. It was found, that filtration and 30X dilution of the samples with assay buffer eliminated matrix interferences, resulting in accurate determination of the spiked samples (recovery 95-112%). The results obtained were in agreement with those received by LC-MS/MS analysis of the same samples. Thus, the proposed method can be used for thiabendazole determination in white wines.

Introduction

Thiabendazole is a systemic fungicide commonly applied for post-harvest protection of fruits and vegetables [1], thus maximum residue limits (MRLs) are set for a number of plant products including table and wine grapes, i.e. 0.05 mg/kg [2]. Enzyme-linked immunosorbent assays (ELISA) have emerged as alternative methods to chromatographic ones for the determination of environmental contaminants since they are simple, cost-effective and permit fast analysis of large samples number [3]. A number of methods based on monoclonal or polyclonal antibodies have been developed for thiabendazole determination in fresh produce, fruits and fruit juices [4-5]. However, to our knowledge, there is not an ELISA method for determination of thiabendazole in wines. Therefore, the aim of our work was to develop a simple, fast, sensitive and accurate ELISA for the determination of this fungicide in white wines.

Materials & Methods

ELISA plates were coated overnight with 100 µL per well of thiabendazole-bovine serum albumin conjugate solution in 50 mM carbonate buffer, pH 9.25. After 3X washing with 10 mM PBS, pH 7.4, 0.9% NaCl, 0.05% Tween 20 (PBST), wells were blocked with PBST, 2% BSA, for 1 h, and washed as previously. 50 µL of calibrators or 50 µL of wines diluted 30X in PBS, 0.2% BSA and 50 µL of a monoclonal anti-thiabendazole antibody solution were added to wells and incubated for 1 h. Then, 100 µL of a goat anti-mouse IgG-horse radish peroxidase solution were added in each well and incubated for 45 min. After washing, 100 µL of chromogenic substrate solution (ABTS/H_2O_2) were added to wells for 30 min and the optical density was measured at 405 nm using the Victor^3, 1420 Multilabel Counter (Perkin-Elmer).

For the LC-MS/MS method, 15 g of sample were mixed with 3 mL of acetonitrile and 12 mL of milliQ water, shaken and centrifuged. The upper organic phase was filtered through 0.45 µm PTFE filter and 10 µl were injected into the system. For separation, a reversed-phase C8 analytical column was used (4.6 mm x 150 mm, 5 µm).

Results and Discussion

The most sensitive calibration curves with adequate signal were obtained when 200 ng/mL of thiabendazole-bovine serum albumin conjugate was coated in the microtitration wells combined with 100 ng/mL of monoclonal anti-thiabendazole antibody (Fig. 1). The assay had a detection limit of 0.005 ng/mL, IC_{50} of 0.33 ng/mL, dynamic range 0.01-5 ng/mL, and was precise (intra- and inter-assay coefficients of variation less than 5 and 8%, respectively). White wines from organic and conventional vineyards and bottled wines from local market were analyzed by LC-MS/MS and found thiabendazole-free. These wines were then spiked with known amounts of thiabendazole and analyzed by both LC-MS/MS and the developed ELISA. Different dilutions of spiked and filtered (0.45 µm) white wines were tested and the results showed that a 30X dilution eliminated matrix interferences and allowed detection of thiabendazole in undiluted wines at concentrations down to 0.9 ng/mL (Figure 1). The recoveries obtained with the developed ELISA ranged between 95 and 112%, and the concentrations determined for thiabendazole were in agreement with those received by LC-MS/MS analysis of the same samples.

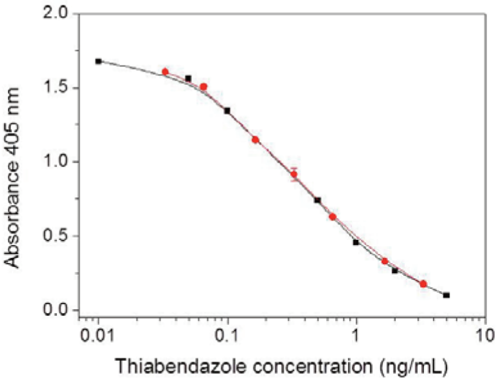

Figure 1. Typical calibration curves obtained in assay buffer (black line) and white wine filtered and diluted 30X (red line).

Conclusions

In this work, a simple, fast, accurate and precise indirect competitive ELISA was developed, and successfully employed to determine thiabendazole in spiked white wines.

Acknowledgements

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OP21
ACT1107

DETERMINATION OF ACRYLAMIDE IN LOW AND HIGH FAT CONTENT FOOD BY DIFFERENT VARIATIONS OF THE QUECHERS METHOD

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Summary

A method for the extraction and mass spectrometric analysis of acrylamide in various food types was applied. Samples were extracted using the European version of the QuEChERS method with citrate buffering, without the hexane defatting step applied by Mastovska and Lehotay[1]. In this way, acrylamide was extracted in the same extract with pesticides. Determination was performed by a LC-MS/MS method that separated acrylamide from interfering compounds having the same transitions with it. Samples of roasted coffee, bakery products and potato crisps were analyzed for acrylamide according to the method and the concentrations found ranged from<0.05μg/g to 3.3μg/g.

Introduction

Acrylamide is produced by roasting or frying food. It is highly toxic and potentially carcinogenic[2] and its levels should be monitored. No MRL has been established in the EU, but the EC published a recommendation with indicative values in a number of food commodities[3].Potato crisps and coffee usually contain acrylamide, at indicative values of 0.1 and 0.045mg/kg respectively, due to the fast heat processing necessary for their production. Flour on the other hand is one of the main ingredients in bread, biscuits and other bakery products found to contain high levels of acrylamide and therefore a method suitable for these matrices is needed.

Materials and methods

Both pesticides and acrylamide were extracted according to EN 15668, the European version of the QuEChERS method. The effect of hexane to minimize the amount of matrix components coeluting was evaluated by adding volumes from 1ml to 5ml, however the major interfering components remained present in the extract. An Agilent 1200 Liquid Chromatograph combined with a Waters Quattro Premier triple quadrupole Mass Spectrometer with an Eclipse XDB C-18, 2.1x150mm column were used. Isocratic elution with 0.1% HCOOH, 5mM $HCOONH_4$ and 5% methanol in water as mobile phase was applied. Column temperature was set at 30°C. Acrylamide eluted at 4.4 min with a flow rate of 0.1mL/min and then methanol passed through the column for 10min at 0.3mL/min to elute the matrix components.

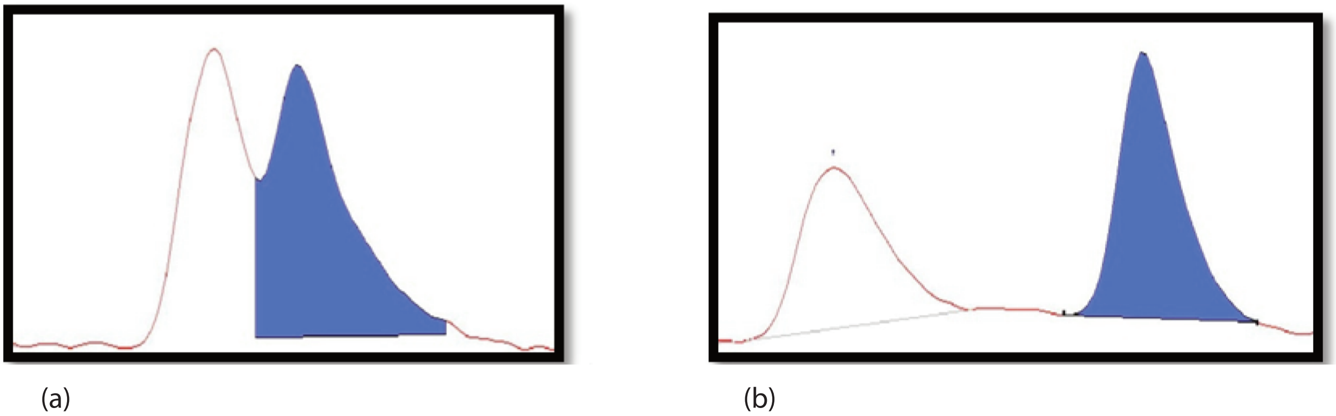
MS/MS was operated at the MRM mode. The precursor ion was 72 and the two fragment ions selected were 71.95 (pseudo-fragment) and 55.2; the second corresponding to the MH^+-(NH_3) adduct. The ion ratio of the two transitions was 3±0.3.

Results and discussion

In order to check the performance of the method, accuracy, LOD, linearity and matrix effects were assessed. Participation in a proficiency test (SCHEMA®) gave a z score of 0.5, showing acceptable trueness of the method. The performance data are shown in the next table.

Matrix	**% Recovery**	**%RSD**	**LOD,** mg/kg
Flour	92.4	10.4	0.015
Coffee	57.3	8.7	0.15
Potato crisps	103.7	15.7	0.15

The analyses of spiked samples at two levels for each commodity provided the performance data of the method. The RSD values are well below the corresponding predicted RSD calculated from the Horwitz equation, showing acceptable precision of the method. In the case of potato chips, an interfering peak occurred in both transitions. Literature studies have shown that the peak may correspond to valine[4], an amino acid present in potatoes with product ions 72 and 55, which are similar to the masses used for the determination of acrylamide. For this reason, the mass measurement accuracy cannot be further improved, making the chromatographic separation of this compound necessary. This was achieved using a weak elution solvent and lowering the flow rate (Fig 1).


(a)

(b)

Fig1: Acrylamide (RT=4.4min) in crisps with: (a) elution solvent containing 20%methanolatflow rate 0.150mL/min (b)elution solvent containing 5% methanol at flow rate 0.1mL/min.

The linearity of the response was tested for all three matrices. In all cases r^2 was >0.98 showing linear regression. The calibration lines for acrylamide in each matrix extract were compared to the respective lines in solvent. In all three cases, statistical analysis using t-test and the Matrix Factor values showed a significant difference in the slope of the calibration lines in the absence and in the presence of the matrix. The addition of citrate buffering salts was assessed in quantitation of acrylamide by extracting samples with and without buffering and no significant difference was observed.

29 samples from the retail market, wholesalers and producers were analyzed to determine acrylamide. The results showed that potato crisps contained acrylamide from0.23 to 3.3μg/g depending on the cooking method. In bakery products the concentrations ranged from <0.05 to 0.22μg/g and in coffee they from<0.15 to 0.51μg/g.

Conclusions

The same extraction procedure (QuEChERS)used for pesticide residues was also applied to the extraction of acrylamide from commonly analyzed food types with LC-MS/MS. Various factors were assessed in order to choose the optimum parametric values for reliable determination. The method was proved to produce accurate results, demonstrated linearity and the attained LODs are sufficiently low compared to the EU indicative concentrations of acrylamide in food.

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OP22
ACT1104

NEW MITIGATION MEASURES TO REDUCE THE RUNOFF AND EROSION IN EXPERIMENTAL FIELD IN POLAND ACCORDING TO TOPPS PROWADIS RECOMMENDATIONS

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Summary

The land use and crop practices examination in experimental fields and catchment in Żelazna nearby Skierniewice, Poland, was carried out in Spring 2012. The runoff and erosion assessment in audited fields was the base for mitigation measures selection. Depending on the runoff and erosion reason the different approach and mitigation measures are proposed.

The positive effect of implemented mitigation measures was observed during the project duration and will serve to protect surface water in the catchment.

Introduction

Surface water protection is one of the main tasks in sustainable use of plant protection products developed by the EU. TOPPS-Prowadis project, started in 2011 is focused on diffuse sources of PPPs, it concerns the water pollution reduction by mitigation measures implementation in the fields. Plant protection products and fertilizers are leached after rain. The *RUN-OFF. Best Management Practices to reduce water pollution with plant protection products from run-off and erosion* [1,2] (run-off & erosion manual) developed by TOPPS-Prowadis experts and partners (7 Member States, 8 institutes and universities and companies, PPP manufacturers, ECPA) include different mitigation measures for runoff and erosion reduction from fields and catchment. In partners' MS the experimental fields and catchments were selected for farmers and advisors trainings to improve different activities to water pollution reduction.

itigation measures in experimental field

In the experimental fields in Żelazna, Poland, during the fields' audit, the runoff direction and potential erosion were examined. Understanding the water pathways in the fields and catchment in order to determine runoff/erosion risk levels is necessary to select the best suited mitigation measures (BMPs). The audit was focused on the qualitative (occurrence) and quantitative (intensity) identification of the problem of run-off and soil erosion related to it, and the transport of agrochemicals (fertilizers, pesticides) within the examined catchment [3].

A consistent mitigation strategy needs to select the measures according to the risks identified during the diagnosis process. Based on the local conditions, the assessment of the concentrated runoff presence, erosion and potential runoff direction, (fig 1), a proposal of new mitigation measures was prepared.

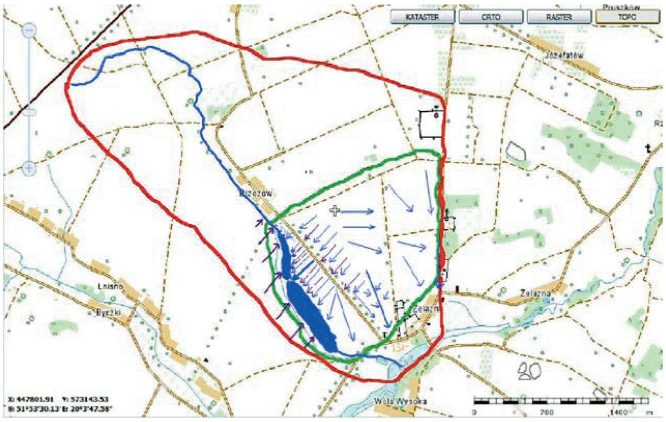

Fig.1 The directions of water flow in the fields

Based on the audit and BMPs manual, implementation of different activities (changes in existing buffer zones and new ones, soil management, cropping practices and retention structures) has been suggested:

- new buffer strips across the field
- enlargement of existing buffer zones
- double sewing
- proper tillage
- reduction of concentrated runoff phenomenon
- cover crop, if not existed

After two years of new mitigation measures implementation, the runoff and erosion reduction have been observed.

Acknowledgments

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OP23
ACT1007

PHOTOLYSIS OF PESTICIDES ON LEAVES CUTICLE MODELS: DIRECT vs SENSITIZED REACTIONS

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Summary

Pesticides deposited on the surface of leaves cuticles after foliar application are exposed to solar light and can undergo photolysis. Two types of reactions may occur: photolysis through solar light absorption in the case of light absorbing pesticides and photosensitized reactions involving plants chromophores. Here, we give examples of both types of reactions from kinetic and analytical points of view considering several acses. The pesticide (sulcotrione, cycloxydim or chlorothalonil) is at the surface of the cuticle, irradiated alone or in the presence of a sensitizer. The sensitizer that can be can be a plant secondary metabolite model (phenalenone) but also another pesticide (chlorothalonil) is at the surface of the cuticle or inside the cuticle.

Introduction

Early dissipation of pesticides just after the spraying is a drawback generally overcame by treatments repetition or applied doses increases. Volatilization, washing off and photochemical degradation contribute to pesticides losses. It is important to get information on these phenomena to find solutions for preventing or at least limiting them. In the laboratory we investigate the photochemical reactivity of pesticides with the goal of understanding the reaction mechanisms and getting insight into the parameters and factors affecting these reactions. Several cases are reported here: 1) the direct photolysis of the herbicides sulcotrione and cycloxydim and of the fungicide chlorothalonil deposited on pure paraffinic wax films, 2) the photosensitized transformation of cycloxydim deposited on pure paraffinic wax films in mixture with chlorothalonil, a potent sensitizer, and 3) the photosensitized transformation of cycloxydim deposited on paraffinic wax films in which phenalenone, a sensitizing phototoxin model has been included.

Experimental part

Films of long-chain alkanes or esters were used to mimic the epicuticular wax films. Pesticides were deposited on wax films as drops and irradiated using simulated solar light after the solvent evaporation. Pesticides residues and photoproducts were recovered by rinsing films with an appropriate solvent and analyzed by HPLC. Phenalenone was included in the paraffinic wax films by heating the different ingredients until melting. Films of around 0.2 nm thickness were then made. Absorption spectra were recorded using a Cary 3 UV-Visible spectrometer (Varian). Solid state spectra were recorded using a DRA-CA-30I integrating sphere accessory (Varian) and a BaSO4 reflectance standard (Spectralon). For the laser flash photolysis experiments, the apparatus consisted of a laser flash photolysis spectrometer from Applied Photophysics (LKS.60) equipped with a Quanta Ray GCR 130-01 Nd:YAG laser.

Results and Discussion

Sulcotrione is a tricetonic herbicide, photolysis of which in natural water is very slow (half-life of 100 days). Yet when it was irradiated on wax after having been deposited in pure water, the half-life was only 4 hours. This drastic difference is due to a protolytic equilibrium (pKa = 3.2) and to the higher photoreactivity of the protonated form of sulcotrione compared the anionic form. Photocyclisation occurred subsequently to HCl elimination, leading to the formation of a xanthene derivative [1]. To check that this photoreactivity also took place in the field, leave samplings were made in a corn field treated with Mikado (formulated sulcotrione) 1 hour after the treatment, the day after and two days after. One hour after the treatment, the xanthene was already detected. Its maximal formation was measured the day after the treatment confirming results obtained in the laboratory experiments.

Cycloxydim is a cyclohexanedione oxime herbicide in the biologicaly active E isomeric form. It was fastly photolyzed on was films with a first order rate constant of 0.11 h^{-1}. The two main pathways were cleavage of the N-O bond with formation of an imine and oxidation of the methylene carbons on the aliphatic ring. Interestingly, the photoisomerization of the oxime observed in water did not take place on wax due to a strong intramolecular hydrogen bond [2].

When phenalenone was added in the wax film, the phototransformation of Cycloxydim was significantly accelerated. This acceleration was attributed to the reaction of Cycloxydim with the singlet oxygen photogenerated by phenalenone. In a good agreement, the oxidation photoproducts were observed. This shows that singlet oxygen produced in the film can migrate to the pesticide at the surface of the film.

Chlorothalonil, a widely used fungicide, was shown to undergo photoreductive dechloration on was films [3]. Interestingly we also demonstrated that Chlorothalonil can photosensitize the dissipation of other pesticides on leaves through its ability to generate oxidant excited states and singlet oxygen upon exposure to solar light. When Chlorothalonil was added at 20 -150 g ha^{-1}, levels representing only 1.3 to 10% of the normal application rate, it could significantly promote the photooxidation of Cycloxydim. Moreover, Chlorothalonil had also a degrading effect on the long-chain alkanes of the wax films. It means that Chlorothalonil is also capable of oxidizing the leaves constituents.

Conclusions

This work highlights that the pesticides photoreactivity may be an important reaction pathway on leaves cuticle. It can occur through direct or sensitized processes. It is affected by the pesticide environment and possibly previous treatments. For instance, chlorothalonil, a fungicide often added in mixture with other pesticide, is itself a strong oxidant and can promote the phototransformation of other pesticides. Altogether, this means that pesticide photodegradation on leaves is a complex phenomenon potentially involving plant constituents.

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OP24
ACT1051

ECOSUSTAINABLE PEST CONTROL: ALDEHYDES OF KNOWN NEMATICIDAL ACTIVITY EMBEDDED IN POROUS MATRICES FOR A TUNED ACTION

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Summary

The growing interest for a more sustainable agriculture renders the development of environmentally friendly strategies of pest control mandatory. Furfural and salicylaldehyde from the fruit of *Melia azedarach*, a plant spread in all warm countries, have been proved of high nematicidal activity against *Meloidogyne* spp but they might easily be leached and degraded in the soil. In this study, a possible innovative solution to limit the aldehyde depletion in soil was aimed through their encapsulation into environment-friendly microporous alumino-silicates (zeolites). The pore dimension and SiO_2/Al_2O_3 ratio of commercially available zeolites were tuned in order to allow the adsorption and the gradual release of the host molecules, thus optimizing the lifetime under field conditions. The release of the encapsulated nematicides was tested in extracts of soils with different texture, pH, and organic matter content. These insights will help to produce the most effective, home-made formulates by contacting the selected zeolite directly with the water extracts of *Melia* fruit.

Introduction

There is an increasing interest towards the nematicidal use of plant secondary metabolites despite the difficulty of the bioactive molecules selection and their variability among extract batches severely limits their use [1]. Furfural and salicylaldehyde from *Melia azedarach* fruit have been proved for their nematicidal activity [2]. In the light of these results, the aldehyde encapsulation in zeolites of controlled release capacity seems a promising tool to preserve them from soil depletion, hence prolonging their bioactivity. Zeolites are extensively used in agriculture as slow-release fertilizers, pesticide carriers, soil conditioners and remediation agents [3]. This preliminary study is aimed at the definition of procedures to be able to tailor sustainable and self-assembling bioformulates for nematode control.

Zeolite	SiO_2/Al_2O_3	Cation	Pore window opening (Å)	Adsorption (% of $C_{initial}$)	
				Furfural	*Salicylaldehyde*
Faujasite Y (Y)	30	H^+	7.0 x 7.1	6 (± 5.0)	49 (± 0.5)
	60	H^+		6 (± 4.9)	57 (± 0.8)
	200	H^+		7 (± 1.0)	69 (± 3.0)
Mordenite (MOR)	200	H^+	6.5 x.7.0 2.6 x 5.7	29 (± 3.2)	71 (± 1.8)
ZSM-5	30	NH_4^+	5.1 × 5.5 5.3 × 5.6	34 (± 5.3)	32 (± 3.7)
	30	H^+		75 (± 2.0)	94 (± 0.0)
	280	NH_4^+		78 (± 2.0)	95 (± 1.0)
	500	NH_4^+		77 (± 4.5)	95 (± 0.0)
	> 1000	NH_4^+		66 (± 4.1)	91 (± 0.7)
Ferrierite (FER)	30	NH_4^+	5.4 × 4.2	61 (± 1.0)	19 (± 2.0)

Table 1. Main characteristics of zeolites and their aldehydes adsorption from water

Materials and Methods

Furfural and salicylaldehyde were purchased as pure standards. The main characteristics of selected zeolites and their adsorption of the aldehydes from water are reported in Table 1. The characteristics of soils, whose water extracts were tested for the embedded aldehyde desorption, are listed in Table 2. The adsorption was assessed on equilibrium solutions and loaded zeolite samples by HPLC-Diodarray and FT-IR analysis, respectively. The desorption of loaded aldehydes in water and soil extracts was also evaluated.

Soil	Texture ($g\ kg^{-1}$)			pH (H_2O)	TOC ($g\ kg^{-1}$)	Water field Capacity (% DW)	Soil extract	pH	Conductivity ($\mu S\ cm^{-1}$)	C_{org} ($mg\ L^{-1}$)	N_{tot} ($mg\ L^{-1}$)
	sand	*silt*	*clay*								
S1	400	440	160	5.3	22	37	S1E	4.9	349.9	82.1	3.19
S2	200	702	98	5.9	11	31	S2E	6.8	209.3	22.2	5.02
S3	790	100	110	7.3	14	27	S3E	7.7	492.1	38.5	7.91

Table 2. Physico-chemical characteristics of selected soils and related water extracts

Results and Discussion

In Table 1, only zeolites able to retain more than 35% of aldehyde initial concentration from water were reported. Clearly, salicylaldehyde adsorption into zeolite Y was positively related to the SiO_2/Al_2O_3 ratio increase. As shown in Table 3, some zeolites were able to release part of loaded aldehydes in the soil extracts within 12 h. Further study has to be done on the persistence of the two embedded aldehydes in the soil extracts in comparison with the behaviour of the bare molecules under the same environmental conditions.

Zeolite (SiO_2/Al_2O_3)	Desorption of the aldehydes (% of embedded amount)							
	Furfural				Salicylaldehyde			
	water	*S1E*	*S2E*	*S3E*	*water*	*S1E*	*S2E*	*S3E*
Y (30)	-	-	-	-	64	0	34	0
Y (60)	-	-	-	-	68	0	30	0
MOR (200)	22	0	38	20	5	0	5	0
ZSM-5 (30)	6	0	34	23	14	0	0	0
ZSM-5 (500)	13	0	5	1	3	0	0	0
ZSM-5 (>1000)	17	0	0	0	4	0	0	0

Table 3. Desorption in water or soil extracts of zeolites loaded with aldehydes

Conclusions

The ability of environmentally friendly zeolites to encapsulate/ release furfural and salicylaldehyde in soil solution paves the way for defining procedures able to tailor smart and ready-to-use bio-formulates for nematode control by direct contact of commercially available zeolites with natural nematicidal water extract.

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OP25
ACT1023

BIOFILTER MATERIAL FOR DECONTAMINATION OF POINT SOURCE POLLUTION OF PESTICIDES

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Summary

To overcome the problem of point sources pollutions on farms stemming from improper handling, spillages and leakages during filling and cleaning of spraying equipment, an environmental friendly and low-cost technology filter system is under development. A suitable ratio of biomixtures (soil, biochar and digestate) will be used in the final technical setup of biofilter. Therefore different fundamental processes like microbial respiration, sorption-desorption, degradation, and transport /retardation behavior of three radiolabelled pesticides (Bentazone, Boscalid and Pyrimethanil) will be investigated. In this presentation, I will focus on the impact of the above mentioned organic amendments on degradation of pesticides.Introduction.

Introduction

Unsustainable management of hydrophobic organic contaminants such as pesticides, PAH compounds like naphthalene, pyrene, benzo pyrene etc. can cause high concentrations in soils, ground and surface waters. This is perceived as a major environmental issue in Europe (1- 3). In general, sources of pollution are categorized into diffuse and point sources. Diffuse contamination via leaching, runoff, surface drainage, and drift usually contributes only to a smaller part of pollution of ground and surface water. Many observations at the catchment scale have demonstrated that 40 to 90% of surface water contamination by pollutants is due to point source pollution (4-9), which is caused by spillages during storage and filling, as well as imprudent management of remnants, stemming from leftover spray solution and sprayer washing water.

Hypotheses

a) When digestate and chars will be used for as a novel filter material they will be able to sorb and degrade studied pollutants effectively that may lead to reduced leaching and less chances of ground water pollution.

b) Chars being highly microporous in structure and having high surface areas might act as "super sorbents". On the other hand, digestate with more easily degradable C and lower C/N ratio will lead to enhanced microbial growth and enzymatic activities that may relate to degradation of pollutants in later stages.

Results and Discussion

Application of the organic residues to the soil resulted in a different behavior of soil respiration. In general, cumulative fluxes increase over time for all soil types and mixtures, whereby the increase flattens out over time. As expected, highest CO_2–C release was measured for the application of 30 % digestate due to the large amount of fresh carbon added to the system for both the Kaldenkirchen and the Merzenhausen soil. Nevertheless, total cumulated CO_2-C is slightly larger for the Merzenhausen soil mixture (in case of 30 % digestate) with 16.38 mg compared to the Kaldenkirchen soil mixture with 14.67 mg, whereby the difference is 11 %. For the 5:1 and 5:5 digestate and high temperature biochar mixture the percentage difference is 10% and 19 %, And even the native soil without any addition of amendments showed a difference in CO_2-C evolved of 51 percent (0.44 mg for the Merzenhausen and 0.29 mg for the Kaldenkirchen soil), irrespectively of the fact that the total carbon content of both soils is nearly the same with a TOC content of roughly 1%. Surprisingly, the difference in 5:5 low temperature biochar and digestate mixture is higher with 30 %. Additionally, the 5 % digestate and 5:1 low temperature biochar digestate mixture shows differences between the two soils with 29 and 27 % flux differences for the Kaldenkirchen and Merzenhausen soil, respectively.

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OP26
ACT1049

CLEAN-UP OF WATER POLLUTED WITH VETERINARY SULFONAMIDE ANTIBIOTICS BY HIGH SILICA ZEOLITE Y: FROM MODEL STUDIES TO REAL APPLICATIONS AND REGENERATION TECHNIQUES

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Summary

The anionic nature of veterinary sulfonamide antibiotics (sulfa drugs) makes them highly mobile along soil profile and is responsible for their accumulation into water bodies. Owing to their environmental diffusion and persistence, sulfonamides are responsible to induce high level of resistance in bacteria through by-pass mechanism. To limit the diffusion of resistance determinants, it is of utmost importance to identify sustainable strategies against this antibiotic class to be adopted for water cleanup purpose. Recently, a high silica zeolite Y ($SiO_2/Al_2O_3 = 200$) revealed an excellent ability to extract sulfonamides from pure water in terms of kinetics, capacity and irreversibility of the adsorption. In this study, the favorable adsorption kinetics and properties of the zeolite was confirmed in the presence of both acidic and neutral models of humic monomers (namely, caffeic acid and vanillin) in the 5-8 pH range. Sulfa drugs were quickly removed also when the sorbent was applied to fresh and sea waters. A displacement of adsorbed sulfa drugs from zeolite pores was observed for longer contact time and the mechanism was identified. Finally, among several regeneration techniques applied to exhausted zeolite samples, thermal treatment and solvent extraction showed the best performance.

Introduction

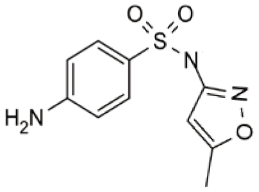
High silica zeolites have been recently tested for their ability to quickly extract sulfonamides from water: the host-guest interactions, adsorption capacity and sulfonamide arrangement into their porosities have been elucidated [1-4]. Here, sulfonamides adsorption into zeolite Y was investigated in the presence of two humic monomers as a function of water pH. The zeolite was also applied to natural waters to evaluate its adsorption selectivity in the presence of dissolved organic matter (DOM). Finally, the best regeneration techniques of the exhausted zeolite were identified.

Materials and Methods

Zeolite Y (SiO_2/Al_2O_3 200 mol/mol), sulfadiazine (SD), sulfamethazine (SM), sulfachloropyridazine (SC), and sulfamethoxazole (SMX) sulfonamides were selected. SMX adsorption into zeolite was investigated in the presence of homomolar concentration of caffeic acid or vanillin in the 5-8 pH range. The maximal adsorption for a SD/SM/SC/SMX homomolar mixture was evaluated in fresh and sea waters. The adsorption was assessed on equilibrium solutions and loaded zeolite samples by HPLC-Diodarray and FT-IR analysis, respectively. Exhausted samples were exposed to regeneration/reuse cycles and their structural stability evaluated by XRD analysis.

Results and Discussion

In the presence of caffeic acid, SMX preferentially adsorbed into zeolite Y within the entire pH range in accordance with its higher pK_a (5.7) with respect to that of caffeic acid (4.5). Similar results were obtained with vanillin despite its neutral nature. Likely, the entrapment into the cage strongly depends upon the sorbate structure with preference for SMX "V" shape with respect to vanillin "flat" shape.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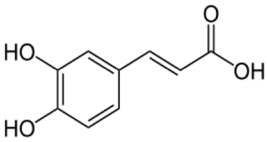
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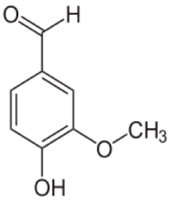
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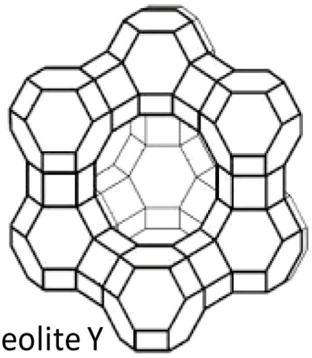
zeolite Y

Sulfonamides mixture was extracted from both fresh and sea waters within 30 min. Interestingly, after longer contact times, antibiotic molecules were desorbed with a mechanism that does not involve their displacement by DOM as shown by IR/TGA analysis. Both solvent extraction and thermal treatment completely removed sulfonamides, thus allowing sorbent recycle.

Conclusions

The extraction of sulfonamides in the presence of naturally occurring DOM exerted by zeolite Y along with its recyclability paves the way for its applications to real waters.

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OP27
ACT1053

REMOVAL OF SELECTED ENDOCRINE DISRUPTORS FROM SECONDARY EFFLUENT BY RESIN ADSORPTION AND PHOTOCATALYSIS

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Summary

This work examined the removal of selected EDCs from secondary effluent by a hydrophobic synthetic resin and subsequent heterogeneous photocatalysis. After 4 cycles of adsorption-regeneration, the resin's performance was very satisfactory. Heterogeneous photocatalysis oxidized the selected EDCs to over 90% for most of the compounds, without significant interference of the effluent organic matter.

Introduction

Endocrine disrupting compounds (EDCs) are chemicals, natural or synthetic, which can affect the endocrine system of human, wildlife and aquatic organisms [1]. Due to their continuous entry in the environment, their toxicity and the unknown side effects, EDCs are called pseudo-persistent and classified in the group of emerging micro-pollutants. EDCs enter municipal wastewater mostly through human use. Elimination of the majority of the endocrine disrupting compounds during wastewater treatment is often insufficient resulting in contamination of natural water bodies. Conventional treatment methods cannot efficiently remove most of EDCs, while new advanced methods are necessary.

The objectives of this work were: (i) To evaluate the removal of selected EDCs by adsorption on a synthetic resin, (ii) To study the effect of resin regeneration on resin efficiency, and (iii)To study the degradation of the selected EDCs by photocatalysis.

In all experiments, the water medium was treated municipal wastewater (secondary effluent) collected from the Chania Municipal Wastewater Treatment Plant.

Sampling and analysis

For the adsorption experiments the secondary effluent samples were spiked with 13 EDCs. The non-ionic synthetic highly porous brominated styrenic polymer resin Sepabeads SP207 (Mitsubishi Chemical Corporation) was used. Both kinetic (up to 30h) and equilibrium tests were conducted. At the end of the appropriate adsorption time, the resin particles were filtered out and regenerated, while the filtrate was analyzed for its EDCs and Dissolved Organic Carbon (DOC) concentrations. Regeneration of the resin took place by washing the particles with NaOH 1M for 24 h. The resin was separated from the base solution and reused for 4 additional cycles of adsorption – regeneration using the spiked effluent.

The heterogeneous photocatalysis of the EDCs was conducted in a cylindrical lab-scale reactor using a 9W UVA lamp. The concentration of Degussa P25 TiO_2 photocatalyst was 0.5 g/L and 0.1 g/L. Analysis of EDCs was performed by Solid Phase Micro-Extraction coupled with Gas Chromatography–Mass Spectroscopy [2].

Results and Discussion

The adsorption capacity of the resin SP207 was very high. The removal rates were higher than 90% for most of the EDCs, while for carbamazepine, Bisphenol A, norethindrone and ethinyl estradiol the removal rates were about 80%. Regeneration of the resin indicated that after four cycle of adsorption-regeneration the removal rates for most of the compounds was greater than 80% except for carbamazepine and iminostilbene (40%) (Figure 1)..

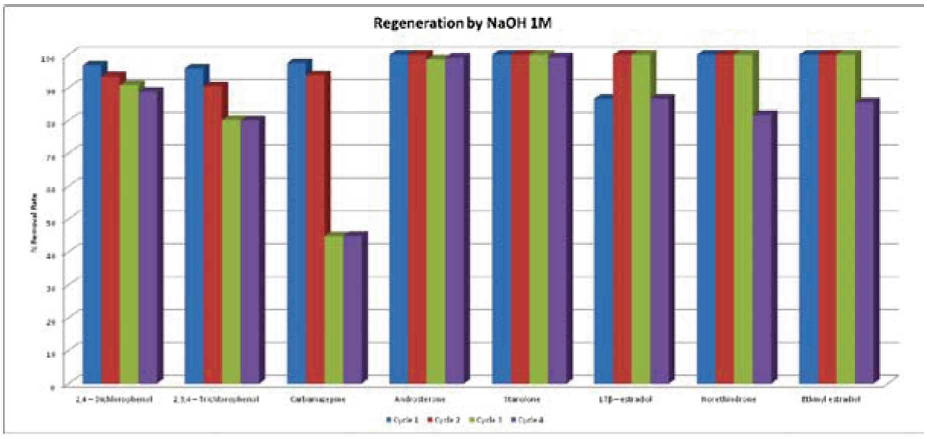

Figure 1. Removal rates of EDCs during adsorption-regeneration cycles

The heterogeneous photocatalysis demonstrated high efficiency in the oxidation of most EDCs. The removal rates for the compounds were greater than 90% for both levels of TiO_2 except for norethindrone (70% and 30% for 0.5 g/L and 0.1 g/L respectively). On the other hand, the removal of DOC (effluent organic matter) was much lower ranging from 3% for the low TiO_2 dose to 27% for the high TiO_2 dose. Figure 2 shows a representative example of the removal of EDCs by photocatalysis.

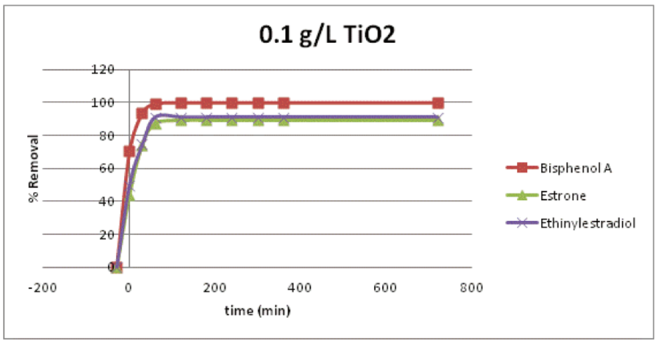

Figure 2. Removal of selected EDCs by heterogeneous photocatalysis

Conclusion

Removal of selected EDCs from secondary effluent by a hydrophobic synthetic resin was very efficient. After 4 cycles of adsorption-regeneration, the resin's performance was very satisfactory. On the other hand, heterogeneous photocatalysis oxidized the selected EDCs to over 90% for most of the compounds, without significant interference of the effluent organic matter. These experiments indicate that there are good prospects of combining these two processes for concentration and eventual oxidation of EDCs present in secondary effluent

Acknowledgements

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OP28
ACT1096

EFFICIENCY OF SOME PHARMACEUTICALS' REMOVAL FROM AQUEOUS ENVIRONMENTS BY USING PHOTODEGRADATION COUPLED WITH TiO2 POWDER DISPERSION AND TiO2 IMMOBILIZED ON BLUE GLASS SLABS

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Summary

Photo-catalysis using titanium dioxide (TiO_2) is currently being considered for many environmental applications such as water decontamination. This is due to its ability to degrade organic pollutants to CO_2, water and mineral acids. Despite the usage of this semiconductor as catalyst for environmental applications has proved very effective, one main problem of using TiO_2 is the separation of the powder in the aqueous media after the photo-catalytic process. To avoid this problem, TiO_2 and other metal oxides used as photo-catalysts have been supported in a variety of materials, keeping out an enough active surface, and obtaining at the same time an easier separation of the catalyst at the end of the process. The aim of our research was to compare the effectiveness as photo-catalyst of glass supported-TiO_2 and suspension-TiO_2 in the degradation process of some pharmaceutical compounds.

Introduction

Pharmaceuticals compounds (PhCs) have been identified and detected in wastewater, river water and even sewage sludge and soil at ng L^{-1} levels, which may cause a potential hazard for the aquatic environment [1]. Since traditional wastewater treatment plants are not often able to eliminate PhCs, due to their high chemical stability and low biodegradability [2], enhanced technologies such as Advanced Oxidation Processes (AOPs) have been employed to reduce the presence of these compounds in the aquatic environment. Among all technologies of AOPs employed in wastewater treatment field, heterogeneous photo-catalysis with semiconductors is the most popular and effective one. In the heterogeneous photo-catalysis, dispersed solid particles of semiconductor absorb efficaciously large fractions of the UV spectrum generating chemical oxidants from dissolved oxygen [3]. Due to its high resistance to chemical and photo-corrosion, safety and low cost, TiO_2 is considered the most active semi-conducting material [4]. However, a separation of the fine TiO_2 catalysts after treatment can be energy intensive and time consuming, resulting in a significant reduction in the benefits of this technique in water treatment industries. This technical constraint has encouraged researchers to the development of different inert support-immobilized photocatalyst aiming at the removal of costly separation processes after treatment [5].

Our work deals with the efficiency evaluation of glass supported-TiO_2 and suspension-TiO_2 as suitable photocatalyst for the degradation of some pharmaceuticals (Diclofenac, Ibuprofen and Naproxen) most frequently found as recalcitrant compounds in surface waters.

Materials and Methods

Kinetics of photo-degradation and photo-products identification of the different pharmaceutical compounds in water phase were determined in laboratory-scale experiments using light irradiation by a solar simulator in presence either of Titanium dioxide (TiO_2) in aqueous suspension or the metal oxide immobilized on a glass surface. Photochemical reactions were carried out by using a cylindrical 250 mL reactor. The identification of the photoproducts was performed by liquid chromatography system coupled to a hybrid linear quadrupole ion trap (LTQ) – Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer. Similar systems kept in the dark were used as control during the whole experimental time.

Results and Discussion

In presence either of titanium dioxide (TiO_2) in aqueous suspension or the metal oxide immobilized on the glass surface, all the investigated compounds kept in the darkness did not show any significant degradation during the experimental time. While, the irradiated samples of all drugs followed apparent first order kinetics, which are confirmed by the linear behaviour of ln(C_t) as a function of irradiation time. Al the kinetic parameters obtained are listed in the table below.

Pharmaceuticals	Kinetic parameters	Photocatalysis/ TiO_2 powder dispersion	Photocatalysis/ TiO_2 immobilized on glass
Diclofenac (DCF)	k (min^{-1})	0.0127	0.0087
	$t_{1/2}$ (min)	54.6	79.7
	R^2	0.994	0.996
Ibuprofen (IBP)	k (min^{-1})	0.0598	0.0168
	$t_{1/2}$ (min)	11.6	41.2
	R^2	0.988	0.984
Naproxen (NPX)	k (min^{-1})	0.0700	0.0100
	$t_{1/2}$ (min)	9.9	69.3
	R^2	0.995	0.986

As expected, the semiconductor immobilized on the glass surface was less effective for the degradation of all pharmaceuticals than TiO_2 powder dispersed in the aqueous phase. Nevertheless, our final results showed that the photocatalysts tested in the experiments are able to degrade the pharmaceuticals used as model molecules and may find application in the remediation of water contaminated with recalcitrant residues.

All intermediate photoproducts have been successfully identified using LC/FTICR-ESI-MS

No.	Accurate m/z $[M-H]^-$	Molecular formula $[M-H]^-$	Rt (min)	Error |ppm|
1-DCF mother molecule	294.00841	$C_{14}H_{11}NO_2Cl_2$	25.31	0.94
2-DCF	310.00355	$C_{14}H_{10}O_3NCl_2$	9.06	1.05
3-DCF	274.02704	$C_{14}H_9O_3NCl$	5.56	1.79
4-DCF	258.03204	$C_{14}H_9NO_2Cl$	15.71	1.59
1-IBP mother molecule	205.12343	$C_{13}H_{17}O_2^-$	4.1	0.14
2-IBP	221.11835	$C_{13}H_{17}O_3^-$	6.2	0.13
3-IBP	191.10765	$C_{12}H_{15}O_2^-$	4.6	0.50
1-NPX mother molecule	230.25922	$C_{14}H_{14}O_3$	2.3	1.01
2- NPX	203.10566	$C_{13}H_{14}O_2$	2.0	1.11
3- NPX	201.09102	$C_{13}H_{12}O_2$	1.8	1.34
4- NPX	185.09609	$C_{13}H_{12}O$	2.5	0,67
5- NPX	191.06927	$C_{11}H_{10}O_3$	1.9	0.99

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OP29
ACT1111

TRAMADOL DEGRADATION BY MEANS OF HETEROGENEOUS PHOTOCATALYSIS: KINETICS, IDENTIFICATION OF TRANSFORMATION PRODUCTS AND MECHANISM INVOLVED

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Summary

The present study describes for the first time the photocatalytic degradation of a widely used pharmaceutical, Tramadol (TRA) in TiO_2 suspensions under simulated solar irradiation. Complete degradation of TRA (C=10 mg L^{-1}, C_{TiO2}=100 mg L^{-1} and I=500 W m^{-2}) takes place in 20 min, whereas 82% of mineralization was achieved within 180 min irradiation. The degradation for both TRA and total organic carbon (TOC) followed pseudo-first order kinetics according to the Langmuir-Hinshelwood model. NO_3^- and NH_4^+ reach about the 80% of the expected stoichiometric nitrogen amount at 180 min of irradiation. High resolution accurate mass liquid chromatography (HR-LC-MS) was used to identify the degradation products. The main TRA transformation pathways were observed to be hydroxylation, oxidation and dealkylation followed by ring cleavage. Scavenging experiments indicated that HO•, h^+ and $O_2^{-\cdot}$ radicals were principally responsible for TRA degradation.

Introduction

Tramadol (TRA), is a centrally acting analgesic mainly used for the treatment of moderate to severe pains and has a wide range of applications [1]. Like many other illicit and licit drugs, this substance has been usually found in environmental waters, due to its low elimination efficiency in wastewater treatment plants [2]. Heterogeneous photocatalysis with TiO_2, has been demonstrated as a good alternative to the conventional methods for the removal of a variety of pharmaceutical compounds [2,3]. Since the literature concerning the heterogeneous photocatalytic treatment of TRA is scarce, the present work focuses on the detailed kinetic and mechanistic investigation of TRA degradation in the presence of TiO_2 under simulated solar light.

Material and methods

Photocatalytic experiments were carried out in a solar simulator Atlas Suntest XLS+ (Heraeus, Germany), equipped with a xenon lamp (2.2 kW) and special filters restricting the transmission of wavelengths below 290 nm. TRA concentration was determined by a Dionex P680 HPLC equipped with a Dionex PDA -100 Photodiode Array Detector and a mobile phase of LC-grade water pH 3 (30%) and acetonitrile (70%) with a flow rate of 1 ml/min. TOC of filtered samples were measured via a Shimadzu, TOC V-csh Analyzer. NO_3^- ions released during the process, were determined by a Dionex ICS-1500 equipped with ASRS Ultra II self-regenerating suppressor. NH_4^+ ions were analyzed by the colometric method based on indophenol blue formation. The intermediates products were identified by high accurate LIT-Orbitrap MS/MS experiments.

Results and Discussion

The degradation kinetics for both TRA and TOC (Fig. 1a) follow apparent first-order degradation curve which is consistent to the Langmuir-Hinshelwood. Rate constants of $k_{app}=15.3\times10^{-2}$ min^{-1} and $k_{app}=9.7\times10^{-3}$ min^{-1} and half lives ($t_{1/2}$) of 4.5 min and 71.4 min were recorded for TRA and TOC respectively.The much slower TOC removal rate, implies the formation of more stable intermediates, which need long irradiation time to achieve complete removal. After 180 min of irradiation, nitrogen was released in stoichiometric amounts as nitrates (almost 30%) and ammonium ions (50%). The investigation of the role of the reactive species suggests that HO•, h^+ and $O_2^{\bullet -}$ play a major role in the degradation mechanism of TRA, as shown in Fig. 1b. The major transformation products of TiO_2 photocatalysis of TRA have been characterized by accurate mass measurements, obtained by the use of high resolution liquid chromatography coupled to an Orbitrap mass spectrometer. The photoinduced transformation of TRA proceeded through the formation of numerous intermediate products and involved mainly hydroxylation, followed by oxidation and demethylation.

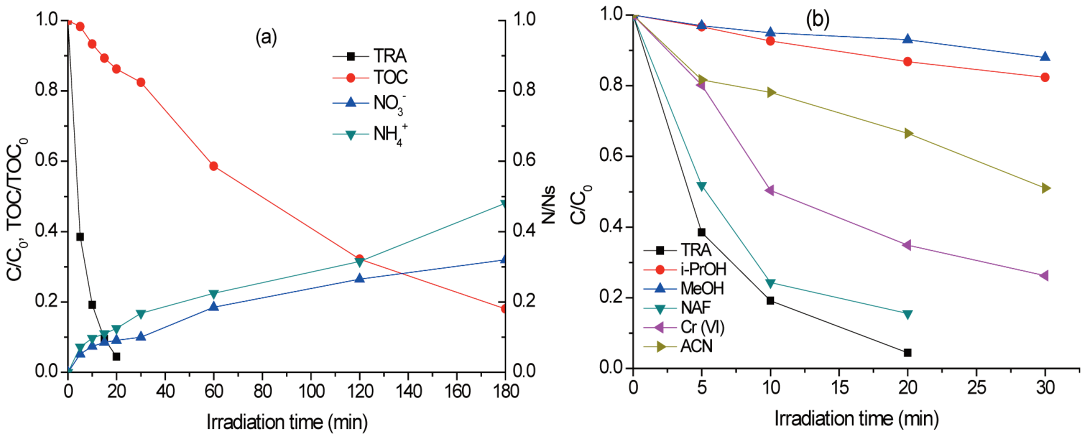

Figure 1: (a) Kinetics of degradation of TRA, TOC and evolution of nitrogen ions vs. irradiation time, (b) Degradation kinetics of TRA in the presence of scavengers.

4. Conclusions

The photocatalytic degradation of TRA in the presence of TiO_2 was investigated in detail focusing on the kinetic and mechanistic study. The results of the present study demonstrated that heterogeneous TiO_2, is a suitable treatment method for the elimination of TRA from the aqueous phase. The number of identified TPs proposed the existence of various degradation routes resulting in multi-step and interconnected pathways.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALIS. Investing in knowledge society through the European Social Fund.

The authors would like to thank also the Unit of Environmental, Organic and Biochemical high resolution analysis-orbitrap-LC-MS analysis of the University of Ioannina for providing access to the facilities.

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OP30
ACT1112

REMOVAL AND PHOTO-DEGRADATION OF AQUEOUS SOLUTIONS OF FOUR MEDICINES

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Summary

In this work we investigated the effect of sunlight and its combination with TiO_2 and sea-salt for the removal of four medicines, i.e. zinadol, zirtec, pricefil and pariet. Direct photolysis caused some degradation of the medicines examined here, while TiO_2 seemed to play a more complex role, depending on the employed medicine.

Introduction

The traditional biological wastewater treatment methods cannot remove or degrade water pollutants such as medicines, pesticides, herbicides and other organic compounds used as drastic ingredients employed for the treatment of disease. Hundreds of tons of pharmaceuticals are released annually into the environment unchanged or as metabolites. It is noteworthy that the majority of pharmaceuticals have been detected at concentrations ranging from ng L^{-1} up to g L^{-1}. The inability of known biological processes for treating municipal wastewater for complete degradation of pharmaceuticals, has created the need to upgrade them further comprising alternative methods of treatment such as the advanced oxidation processes (AOPs). The basic function of AOPs is based on the production of free radicals that act as powerful oxidisers which degrade organic pollutants transforming them into inorganic products (mineralization).

In recent years, there have been many studies on the pharmaceutical sector using some AOPs, such as heterogeneous photocatalysis, photolysis, ozonation, oxidation with Fenton, photolysis in the presence of H_2O_2 and to a lesser extent sonolysis, electrolysis and wet oxidation (Baran et al., 2006, Klavarioti et al., 2009).

In this work we investigate the effect of sunlight and its combination with TiO_2 for the removal of four medicines, i.e. zinadol (antibiotic), zirtec (allergy medication), pricefil (antibiotic) and pariet (treatment of stomach ulsers). The scope of this research is two-fold. First, to get some guidance on the potential efficiency of applied photo-treatment processes for the removal of such compounds from sewage treatment plants and next to assess the effect of natural sunlight on their removal from fresh and sea-waters. For this purpose, tablets of the aforementioned four medicines, as supplied by the producer pharmaceutical company were photo-treated as explained next.

Materials and methods

Tablets of the aforementioned four medicines, as supplied by the producer pharmaceutical company (Zinnat 500 mg, GlaxoSmithKline, Zyrtek film-coated tablets 10 mg UCB, Pariet 20 mg JANSSEN-CILAG and PRICEFIL 500 mg VIANEX S.A.) were dissolved in 1 L of double-distilled water each. The solution was then filtered and used as it was for photolysis measurements or mixed with TiO_2 Degussa P-25 for photocatalytic experiments. Four series of experiments were performed for each solution, one for the study of the effects of direct photolysis and one for the study of photocatalysis in fress water (use of distilled water and the medicine with or without catalyst) and surrogate sea water by with addition of sea-salt to form a 3.5% w/w NaCl solution. A number of 10 ml transparent glass vials were half-filled with 5 ml of solution each and they were sealed and placed under direct sunlight. The vials employed in the photocatalytic measurements were shaken regularly and they were opened for aeration twice in the early morning and late evening when the outside temperature was low. The samples remained in the sun for 45-91 days.

They were subsequently analysed using a Hitachi U-2000 UV Spectrophotometer 1212301-10 with cuvettes made of quartz of 3.5 ml capacity. The total organic carbon (TOC) content of each solution was also measured by means of combustion catalytic oxidation / NDIR method on a Shimadzu TOC-V CSH analyser.

Results and Discussion

Although differences were observed between different medicines, it was broadly found that in all cases UV absorption in the photocatalytic measurements at lower wavelengths was reduced as a function of treatment time as Figure 1a shows. However, UV absorption at longer wavelengths or at other but the photocatalytic experiments was unaffected, although the absorption values were too small to derive any firm conclusions.

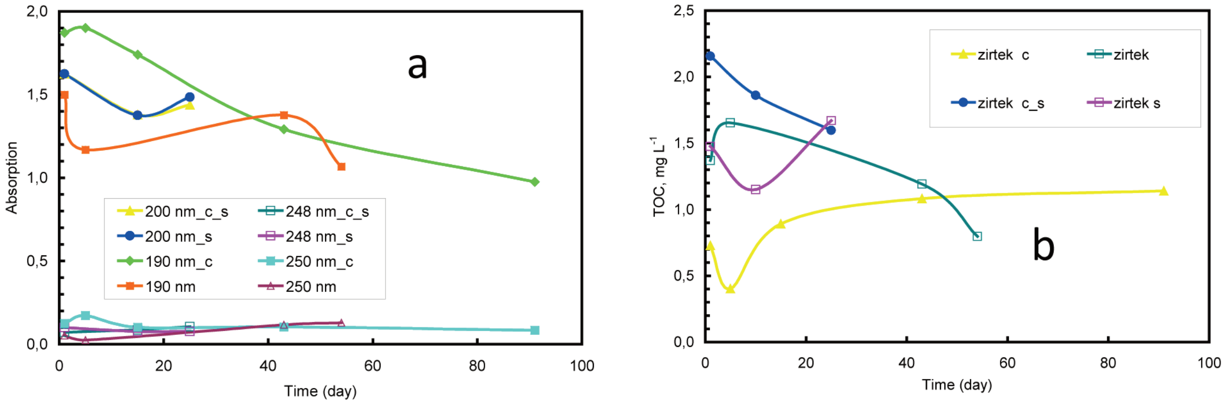

Figure 1 (a) UV absorption of aqueous solution of Zyrtek at two different wavelengths as a function of time with TiO2 catalyst (index c), without any catalyst, with TiO_2 and sea-salt (index c_s) and with sea-salt only (index s). (b) TOC content of the same solutions as a function of time.

TOC on the other hand was constantly reduced in photolytically treated solutions, but TOC was usually increased in solutions with TiO_2 without sea-salt as can be seen in Figure 1b. This indicated that the catalyst, which had initially adsorbed a large quantity of the medicine, was gradually liberating organic products into the solution. This was not always the case when sea-salt was present.

Conclusions

Direct photolysis of the medicines examined here seems to cause some degradation of the medicines examined here, while TiO2 seemed to play a more complex role, depending on the employed medicine.

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OP31
ACT1046

EFFECT OF THE INSECTICIDES CHLORPYRIFOS, SPINOSAD AND SPINETORAM ON NATURAL POPULATIONS OF EPIGEAL ARTHROPODS

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Summary

Ten different treatments of spinosad, spinetoram, and chlorpyrifos were used as toxic references and were allocated at random to experimental plots in order to evaluate the effects on ground-dwelling arthropod diversity and abundance. The insecticides were applied on a herbaceous field in two different doses for one and two spraying applications. A total of 18 Orders and Suborders of epigeal arthropods were captured in pitfall traps that were placed in the insecticide-treated and untreated (control). The most abundant taxa were Hymenoptera, Collembola, Araneae, Acari, Coleoptera, Isopoda, Julida and Opiliones, which were investigated further. The total number of arthropod individuals was notably decreased after the insecticidal applications, with significant differences in biodiversity among treated plots, but not between spinetoram-treated plots and control. Significant arthropod population density reduction in chlorpyrifos-treated plots was observed for Coleoptera, Collembola, Opiliones and Hymenoptera. The population density of Collembola was reduced after spinosad application, while population of Opiliones was reduced after the application of both spinosad and spinetoram. Our findings show that spinosad and spinetoram disrupt the structure and the abundance of surface-active arthropod fauna causing considerable changes in the ground dwelling biodiversity of this ecosystem.

Introduction

Spinosad is classified as a low risk insecticide and has been approved for use in organic farming by many regulatory agencies [1]. In the last decade, many semi-synthetic spinosyn analogues have been designed for more selectivity on target pests, with spinetoram being the first semi-synthetic spinosyn registered [2]. However, spinosyns are considered hazardous on beneficial arthropods on 19 out of 40 field and semi-field studies, raising the question of their actual compatibility in IPM programmes and in organic farming [3]. In this context, the present study aims to evaluate the impact of spinosad and spinetoram on ground-dwelling arthropod diversity and abundance in a non crop habitat.

Materials and methods

Ten different treatments of spinosad, spinetoram and chlorpyrifos in two different rates for one and two spraying applications were allocated at random to the plots of a 1 ha non crop habitat. Three pitfall traps [4] filled by one-third in volume with ethylene-glycol, were randomly deployed on each plot and checked every 14 days. The selected adults were identified to the taxonomic level of Order or Suborder using appropriate taxonomic keys.

Results and Discussion

Our findings stand in accordance with previous field studies which showed that chlorpyrifos spraying applications reduces the densities of Coleoptera [5], Collembola [6] and Hymenoptera [7]. Collembola and Hymenoptera presence was reduced after spinosad application, while Opiliones were reduced after the application of both spinosyns and chlorpyrifos. As far as plots that were sprayed twice are concerned, no significant differences in diversity were found between spinetoram-treated plots and control, but those differ significantly from the rest of the treatments. In addition, the biodiversity in chlorpyrifos-treated plots was significantly different from the other treatments. Collembola were distributed randomly prior to spraying applications, but they were aggregated after that.

Conclusions

Our results show that the application of insecticides, in many cases, notably affected the densities and the diversity of several epigeal arthropod taxa, regardless of the insecticide and the number of applications. Moreover, despite the fact that EU legislation clearly indicates the arthropod taxa groups that are considered as reliable bioindicators, there are some additional taxa groups, such as Opiliones, that could be further evaluated for this purpose.

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OP32
ACT1063

ISOLATION AND CHARACTERIZATION OF ALLELOPATHIC COMPOUND FROM AMBROSIA ARTEMISIIFOLIA

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Summary

Concerns about environmental involvement and insurgence of resistant weeds are moving the attention from synthetic herbicides to new solutions. In such context, allelopathic plants could provide natural molecules useful in agricultural weed management. *Ambrosia artemisiifolia* L. (common ragweed) is an invasive plant which allelopathic behavior has been demonstrated, but the nature of the phytotoxic compounds produced by this plant has not been investigated.

In this work, the phytotoxicity of *Ambrosia artemisiifolia* L. was confirmed by testing the capacity of a crude methanol extract to inhibit the germination of cress and radish seeds. It was demonstrated that the extract contained a single phytotoxic molecule, mainly present in the leaves. Such molecule was isolated and identified by LC-UV, LC-MS/MS and LC-HRMS.

Introduction

In last decades the consciousness about the environmental impact of chemicals, used in agriculture for the control of pests and weeds, has resulted in a worldwide interest in more eco-friendly technologies and less-persistent compounds, for the protection of soils and waters. Allelopathic plants may be considered a potential source of new molecules with herbicidal action to be used in biological agriculture.

Ambrosia artemisiifolia or common ragweed, is an annual weed indigenous of North America now widespread in Europe and Asia, note for the invasiveness in urban areas and crop fields [1]. Many studies revealed the allelopathic potential of this weed in experiments with crops and weeds, in field and greenhouse conditions [2]. Nevertheless the nature of the phytotoxic molecules present in A. *artemisiifolia* has not been fully investigated.

The aim of this work was the identification and isolation of allelochemicals deriving from *A. artemisiifolia*, to explore the applications of these bioactive molecules as natural herbicides.

Materials and Methods.

Ragweed plants collected on the campus of the Department (Grugliasco, Italy) have been extracted with different solvents. The phytotoxicity of the crude extracts was assessed by germination tests conducted on cress and radish.

Purification of the crude extracts aimed to separate the active compounds was performed by liquid/liquid extraction and liquid chromatography.

The identification and quantification of the active molecule was performed by LC-MS/MS, GC-MS and on LC-HRMS.

Results and Discussion

Methanol crude extracts of *A. artemisiifolia* have shown to inhibit the germination of radish and cress. Further separation of the methanol extracts into different polarity fractions has allowed isolating a phytotoxic fraction containing a single molecule able, even at low concentration, to completely inhibit cress and radish germination. The active molecule had a molecular weight of 260.10 Da and formula $C_{15}H_{16}O_4$. Its concentration in the plant was in the order of magnitude of 100 mg Kg^{-1}(on a fresh weight basis) and it was not strongly affected by the seasonal variation. The active molecule was mainly present in the leaves (> 80 %) while less than 10 and 5 % was found in the stem and in the roots respectively.

Conclusions

The active molecule extracted from *A. artemisiifolia* has given promising results as potential natural herbicide at laboratory scale. Further studies are being conducted in order to assess its field behavior, mainly as far as its persistence is concerned.

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OP33
ACT1067

MULTIPLIER EFFECTS OF PENDIMETHALIN HERBICIDE USE: EVIDENCE FROM GREECE

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Summary

The main aim of this paper is to identify the effects of a hypothetical pendimethalin withdrawal from the market and, consequently, no use as a basic weed control herbicide in selective crops (cotton, processing tomato & onion) in Greece. Additionally, this paper aims to explore the benefit/cost ratio of the several alternative weed control scenarios and to estimate the probable income impacts at regional level.

Introduction

Undoubtedly, the selective crops are poor competitors against many of the weeds that infest fields in Greece. Thus, a Greek farmer hardly can harvest cotton, processing tomato & onion (Nalayini et al., 2013) without chemical weed control. However, during the last decade, the number of approved herbicides is drastically decreasing, leaving farmers with less choices and higher herbicide prices. On the other hand, weed control could be based not only on solely chemical methods but also on integrated approaches including cultural, mechanical and biological techniques (Ali et al., 2013). However, the cost of a complete hand weeding practice elevates very much labor cost (Giannopolitis, 2012; ABAF, 2013). Therefore, solely hand weeding practice is not included in the cost effectiveness analysis.

Methodological framework

In this paper a comparative analysis performed, building several scenarios which can be followed by farmers to manage weed control. Available data were used and also data for crucial variables were gathered. The most commonly used technique of weed management control serves as the baseline scenario. Furthermore, an attempt was made to measure the probable economic impacts at regional and country level due to a change in weed control management (pendimethalin withdrawal). The required data collected, updated to year 2012, from several sources. In particular: a) for weed control costs used the data from the briefing document of Giannopolitis (2012) while b) for other costs used survey data from the accounting monitoring of several Greek cotton farms, gathered over time by our own Department of Agricultural Economics (ABAF, 2013). For the purposes of this analysis representative farms from all geographical regions were selected.

Results and Discussion

According to the results, in some crops (processing tomato and cotton) herbicides offer the only practical, cost-effective and selective method of managing weeds although pendimethalin can provide good and acceptable benefit/cost ratios in weed management for all three crops. On the other hand, a hypothetical withdrawal of the use of pendimethalin in these three crops could bear significant income losses for both farmers and regional economy.

Conclusions

From the analysis it is clear that the withdrawal effects of pendimethalin per hectare are more important for the growers of domestic dry bulb onion and subsequently for the growers of domestic processing tomato than for the growers of domestic cotton. However, considering the extensive cultivation of cotton and the use of pendimethalin, the multiplier's effect in cotton cultivation, is much higher for the economy of the whole Greece and the regional economies. Thus, any change in the status of herbicides use in cotton and especially in pendimethalin will cause huge income loss in Greece and particularly in the region of Thessaly.

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OP34
ACT1037

A HAZARD-RANKING SYSTEM FOR PESTICIDES USED IN GREEK AGRICULTURE BASED ON THEIR CARCINOGENIC POTENTIAL

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Summary

We have used a hazard-ranking system to classify pesticides widely used in Greek agriculture, based on their carcinogenic potential. Latest data from Eurostat were employed concerning the amount of pesticides used for the period 1992-2003. Cancer "weight" classification was based on IARC and EU documentation and "potency" was based on Integrated Risk Information System (IRIS). "Volatilization flux" and "soil persistence" were used to assess exposure potential for each pesticide. This way a hazard-adjusted index for carcinogenic effects was computed. *Atrazine*, *Alachlor*, *1,3 Dichloro-propene*, *Chlorotanolil* and *Metoalachlor* were the top 5 hazard-adjusted pesticides used in Greek agriculture, as regards their carcinogenic potential. With the exception of *Chlorotanolil*, all other pesticides are now banned by EU legislation. This ranking system can help improve hazard prioritization in agricultural communities and serve as a preliminary guide for job-exposure matrix development in occupational epidemiology.

Introduction

Pesticide exposure is a major health and safety issue among farmers. Identifying and prioritizing pesticides with a hazard-ranking scheme that considers both pesticide use and exposure is necessary for a more accurate assessment of health risks in the agricultural profession [1]. The present study uses this approach to prioritize pesticide hazards according to their carcinogenic potential.

Materials and Methods

Data on pesticides used in Greece (1992-2003) were retrieved from Eurostat [2]. Cancer risk was calculated according to the equation:

Cancer Hazard Factor = (Class × Potency × Flux × Persistence)/1000

Hazard-adjusted pesticide use was calculated by multiplying the computed cancer hazard factor by the average amount of pesticides applied over the 12-year period:

Hazard-Adjusted Pesticide Use = Cancer Hazard Factor × Amount Used (tons)

IARC and EU documentation was used to attribute a "class" from 1 to 10 to pesticides [3-4]. Cancer slope factors were retrieved from the Integrated Risk Information System (IRIS), in order to assess a cancer "potency" weight [5]. To assess exposure potential for each pesticide, volatilization "flux" and soil "persistence" were both weighted on a scale of 1–10 from low to high [6].

Table I. *Parameters used to calculate the carcinogenic hazard*

NA=Not available

	CLASS		POTENCY	FLUX	PERSISTENCE
WEIGHT	IARC	EU	mg kg^{-1} day^{-1}	Volatilization day^{-1}	$t_{1/2}$ days
10	1	Carc. 1A	>1	$>10^{-1}$	>100
8	2A	Carc. 1B	>0.1-1	$>10^{-3}$-10^{-1}	76-100
5	2B	Carc. 2	>0.01-0.1	$>10^{-5}$-10^{-3}	51-75
3	3	Carc. 3	0.001-0.01	10^{-5}-10^{-7}	25-50
1	4	N/A	<0.001 or N/A	$<10^{-7}$	<25 or N/A

Results and Discussion

The top 5 pesticides by cancer hazard are presented in Table II. The cancer hazard factors ranged from 0.08 to 3.2. The ranking system differed substantially, once pesticide use was adjusted for carcinogenic potential. *Atrazine* and *Alachlor* were 6th and 10th in the overall ranking for total amounts used, but ranked 1st and 2nd respectively, when cancer hazard-adjusted use was considered.

Table II. *Hazard-adjusted use of pesticides based on their carcinogenic potential*

	PESTICIDE	AMOUNT	HAZARD	HAZARD-ADJUSTED
1	ATRAZINE	89.6	3.2	287
2	ALACHLOR	63.1	0.6	38
3	1,3 DICHLORO-PROPENE	101.8	0.25	25
4	CHLOROTANOLIL	18.4	0.36	7
5	METOALACHLOR	77.5	0.08	6

Conclusions

A pesticide hazard-ranking system that considers exposure potential in association with health effects, in addition to pesticide use, can help identify and prioritize pesticides of greatest health risk in agricultural communities. This method can serve as an effective tool to assess community-specific cancer risks with respect to pesticide exposure.

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OP35
ACT1038

A HAZARD-RANKING SYSTEM FOR PESTICIDES USED IN GREEK AGRICULTURE BASED ON THEIR ENDOCRINE DISRUPTING POTENTIAL

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Summary

We have used a hazard-ranking system to classify pesticides widely used in Greek agriculture, based on their endocrine-disrupting potential. Latest data from Eurostat were employed concerning the amount of pesticides used for the period 1992-2003. "Weight" classification for endocrine disruption was based on EU documentation and "potency" was based on Integrated Risk Information System (IRIS). "Volatilization flux" and "soil persistence" were used to assess exposure potential for each pesticide. This way a hazard-adjusted index for endocrine disruption was computed. *Atrazine*, *Alachlor*, *Glyphosate*, *Mancozeb* and *Fenthion* were the top 5 hazard-adjusted pesticides used in Greek agriculture, as regards their endocrine-disrupting potential. *Glyphosate* and *Mancozeb* are still used in Greek agriculture. This ranking system can help prioritize hazards from pesticide-related endocrine disrupting chemicals in agriculture.

Introduction

Pesticide exposure is a major health and safety issue among farmers. Identifying and prioritizing pesticides with a hazard-ranking scheme that considers both pesticide use and exposure is necessary for a more accurate assessment of health risks in the agricultural profession [1]. The present study uses this approach to prioritize pesticide hazards according to their endocrine-disrupting potential.

Materials and Methods

Data on pesticides used in Greece (1992-2003) were retrieved from Eurostat [2]. Hazard for endocrine disruption was calculated according to equation:

Hazard Factor = (Class × Potency × Flux × Persistence)/1000

Hazard-adjusted pesticide use was calculated by multiplying the computed hazard factor by the average amount of pesticides applied over the 12-year period:

Hazard-Adjusted Pesticide Use = Hazard Factor × Amount Used (tons)

EU documentation was used to attribute a "class" from 1 to 10 to pesticides based on endocrine disrupting potential [3]. Reference Doses (RfDs) were retrieved from the Integrated Risk Information System (IRIS), in order to assign a "potency" weight [4]. To assess exposure potential of each pesticide, volatilization "flux" and soil "persistence" were both weighted on a scale of 1–10 from low to high [5].

Table I. *Parameters used to calculate endocrine disrupting hazard*

NA=Not available

	CLASS	POTENCY	FLUX	PERSISTENCE
WEIGHT	**EU**	**mg kg^{-1} day^{-1}**	**Volatilization day^{-1}**	**$t_{1/2}$ days**
10	1	<0.001	>10^{-1}	>100
8	2	0.001-0.01	>10^{-3}-10^{-1}	76-100
5	3a	>0.01-0.1	>10^{-5}-10^{-3}	51-75
3	3b	>0.1-1	10^{-5}-10^{-7}	25-50
1	N/A	>1	<10^{-7}	<25 or N/A

Results and Discussion

The top 5 pesticides as regards the endocrine-disrupting potential are presented in Table II. Although pesticides like *Atrazine*, *Alachlor* and *Fenthion* are now banned by EU legislation, others like *Glyphosate* and *Mancozeb* with "known" or "suspected" endocrine-disrupting potential are still widely used in Greek and European agriculture.

Table II. *Hazard-adjusted use of pesticides based on endocrine disrupting potential*

	PESTICIDE	AMOUNT USED	HAZARD FACTOR	HAZARD-ADJUSTED USE
1	ATRAZINE	89.6	4	358
2	ALACHLOR	63.1	1.92	121
3	GLYPHOSATE	217.3*	0.225	49
4	MANCOZEB	134.7	0.27	36
5	FENTHION	144*	0.192	28

**Estimated Amounts*

Conclusions

A pesticide hazard-ranking system that considers exposure potential in association with health effects, in addition to pesticide use can help identify and prioritize pesticides of greatest health risk in agricultural communities. This method can serve as an effective tool to assess community-specific endocrine-disrupting risks with respect to pesticide exposure.

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OP36
ACT1121

TETRAHYMENA THERMOPHILA**: A MODEL FOR TOXICITY MONITORING THROUGH PROTEOMICS

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Tetrahymena is a protozoan living in aquatic ecosystems. Strain *thermophila* is a teardrop-shaped, non-pathogenic unicellular organism with the ability of thermal adaptation between 15 and 40ºC. It has been utilized as an indicator in toxicological studies, whereas the survival rate has been assessed.

Currently, high throughput analytical approaches can give important information on the overall biological impact of xenobiotics on biological systems, as well as on the discovery of new biomarkers for toxicity monitoring.

The aim of the present work was to investigate whether selected pesticides affect the expression of proteins in *Tetrahymena thermophila,* which has been genetically mapped, and to compare the data of proteomics analysis with data from biological activities of xenobiotic enzymes and the viability of the protozoan.

In this respect, we used the organophosphorus insecticides Diazinon and Chlorpyrifos, that inhibit acetyl-cholinesterase, as well as the herbicide Propanil.

OP37
ACT1057

ARPEGES, A METHOD TO ASSESS THE RISK OF SURFACE WATERS CONTAMINATION BY PESTICIDES AT THE NATIONAL SCALE

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Summary

Arpeges is an innovative and coherent method to assess the potential surface waters contamination by pesticides at the national scale. In France, it was implemented at Water Framework Directive water bodies' scales. The objectives are to identify vulnerable zones and to determinate the main risk factors implied in pollution transfer, according to the considered zones. Actually, this method allows a better understanding and a ranking of pollution's causes considering three essential aspects of the contamination: uses, environmental vulnerability to pesticides pollution and substances properties. The method is mostly based on transfer processes. In order to take into account the seasonal variability, analyses are separately carried on winter and summer, and both acute and chronic components of the risk are considered. 18 relevant variables describing pesticides transfer are chosen (infiltration, pedology, drainage, climate, buffer zones…); they are aggregated according to up to date expert knowledge thanks to a Bayesian network to obtain pesticides contamination. Intermediary results as intrinsic vulnerabilities are relevant to identify the main environmental causes of transfer.

Introduction

Awareness about freshwaters contamination by pesticides is rising and measures to control it have been taken. At the European level, the Water Framework Directive implementation requires a coherent method to assess water bodies' status. In this context, the Arpeges method [1] was created to explore the potential surface waters contamination by pesticides at a national scale. It allows an identification of vulnerable zones and a first determination of main risk factors in chemicals transfers.

Materials and methods

The method proposes better knowledge and a ranking of pollution causes considering three essential aspects of the contamination: uses, environmental vulnerability to pesticides transfer and chemicals properties. It is essentially based on the understanding of pesticides transfer dominant processes. In order to consider temporal variability, analyses are separately carried on winter and summer, both with an acute and a chronic risk approach. The segregation between the two risks is built on the different processes of transfer times to reach surface waters. 18 relevant variables describing pesticides transfer (infiltration, pedology, drainage, climate, buffer zones…) are aggregated according to up to date expert knowledge thanks to a Bayesian network to obtain pesticides contamination. The method is designed for the cocktail of molecules applied on the water body's basin. The following graph summarises the method:

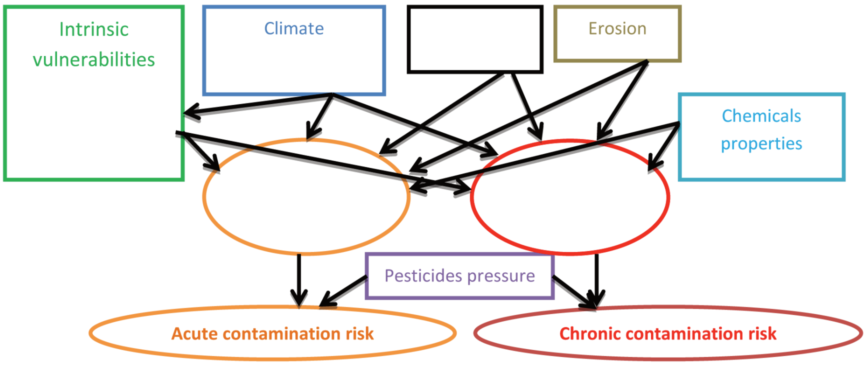

Figure 3 : Scheme of the global aggregation of the Arpeges variables

Results and discussions

The final result discriminate water method considers the different pesticides transfer processes and the relative environmental vulnerability which is an important determinant for assessment but rarely taken into account at the national level. These intermediary results can be mapped and used to understand the main causes of chemicals transfers.

The method was created to be coherent on large scale to discriminate zones. Furthermore, the probabilistic approach allows associating an uncertainty factor to each result and increasing the adaptability of the method. It has not been validated towards contamination observations in water bodies, but some French experts confirm results are consistent with the local knowledge. A current weakness of the final risk maps is the available data used so far to take into account pesticides pressure. Actually, for this variable accurate and spatialized data for large scales are not available yet in France.

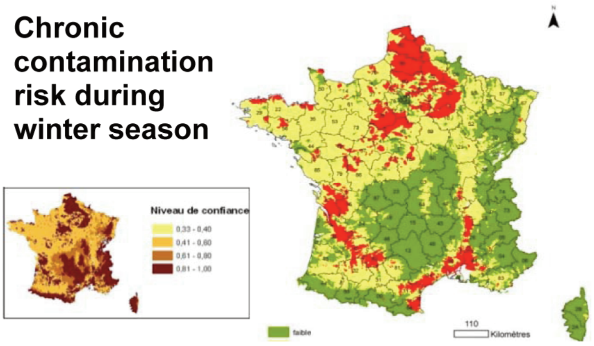

Figure 4 : Chronic contamination risk by pesticides in winter

Conclusion

The Arpeges method is a homogeneous risk assessment of pesticides water contamination by pesticides. In order to validate this method we are currently working on a one by one molecule approach to avoid the cocktail approach uncertainty. We are thus implementing a comparison with monitoring data, recorded since 97.

Acknowledgements

This work benefits from the technical and financial support of Onema, the national research program Ecophyto and ANSES.

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OP38
ACT1056

DEVELOPMENT AND APPLICATION OF A MAGNETIC SOLID-PHASE EXTRACTION FOR THE DETERMINATION OF ENDOCRINE DISRUPTORS IN MILK SAMPLES BY GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Summary

In this study, we describe the chemometric optimization and validation of a simple and rapid method based on magnetic solid- phase extraction (MSPE), which is followed by gas chromatography coupled with mass spectrometry for the determination of endocrine disruptors in milk samples. The synthesized magnetic nanoparticles are characterized by several techniques. The applicability, accuracy, precision, sensitivity of the proposed method have been demonstrated based on SANCO/12571/2013 European Guidelines.

Introduction

Endocrine disruptors (EDCs) are chemicals that interfere with endocrine (or hormone system) and are of an increasing concern because of their potential impacts on the environment, wildlife and human health. The European Commission has published a draft list of chemicals, which are believed to damage health by interfering with the way hormones work. The range of substances reported to cause endocrine disruption is diverse including certain organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), selected because of their potential for human exposure and their high production volumes.

Food is likely to be one of the most important routes of human exposure to EDCs. Cow's milk is considered a nearly complete food since it is a good source of protein, fat and major minerals. Additionally, it is the main constituent of the daily diet, principally for vulnerable groups such as infants, school age children and the elderly. On the other hand, milk is an ideal liquid to dissolve environmental contaminants such as pesticides because most of them are fat-soluble. Due to their lipophilic properties, OCPs and PCBs are primarily stored in fat-rich tissues and subsequently translocated and excreted through milk fat [1].

Magnetic solid-phase extraction (MSPE), which has drawn extensive attention in sample preparation in recent years, is a new mode of SPE based on the adoption of magnetic nanoparticles (MNPs) as sorbents, at micro- or nano-scale and shows great advantages in separation science. The powdery magnetic adsorbent can be reversibly agglomerated and redispersed in solution or suspensions by the application and removal of an appropriate magnetic field, thus realizing the phase separation conveniently.

Results and Discussion

Magnetite octadecysilane nanoparticles (Fe_3O_4@SiO_2@C18) are synthesized by coating magnetite Fe_3O_4 nanoparticles with silica and subsequently functionalizing it by trimethoxy(octadecyl)silane The synthesized MNPs are characterized by several techniques, such as scanning electron microscopy (SEM), X-ray diffraction (XRD),Elemental Analysis (EA), Fourier transform-infrared (FT-IR) and Brunauer–Emmett–Teller (BET). For example, SEM revealed the nearly spherical shape of Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@C18 nanoparticles (*Fig.1*).

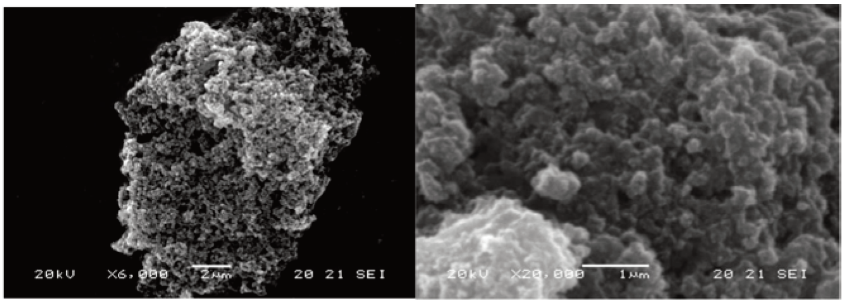

Fig.1: SEM images of (a) Fe_3O_4@$SiO_2$and (b) Fe_3O_4@SiO_2@C18.

The MSPE is performed by dispersion of the Fe_3O_4@SiO_2@C18 nanoparticles in milk samples with sonication, after protein precipitation. Then, the sorbent is collected by applying an external magnetic field and the analytes are desorbed by *n*-hexane. Several parameters affecting the extraction efficiency of target analytes by the magnetic nanoparticles are investigated, including washing and elution solvents, amount of sorbents, time of extraction and elution, sample and elution solvent volume. The proposed method is optimized by means of experimental design and response surface methodology. When coupled with gas chromatography–mass spectrometry detection and under optimum extraction conditions, average recoveries of target analytes are found to be in the range of 79% to 116%. The proposed MSPE–GC–MS analytical method has a linear calibration curve for all target analytes with coefficients of determination to range from 0.9950 to 0.9999. The limits of quantification are found to be between 0.2 and 1 μg/L ensuring compliance with the maximum residue limits established by European Commission and Codex Alimentarius, for OCPs and PCBs residues in milk. The proposed method is applied to the determination of target analytes in milk samples.

Conclusions

Functionalized MNPs were synthesized to serve as solid-phase extraction sorbents for simultaneous enrichment and detection of endocrine disrupting compounds in milk samples with the aid of GC–MS. Analyte extraction and desorption were carried out quickly and the whole pretreatment process could be accomplished by simple vortex and ultrasonication. The applicability, accuracy, precision, sensitivity of the proposed method have been demonstrated based on SANCO/12571/2013 European Guidelines. LODs were below the MRLs set by the European Union for all compounds studied. Considering that Fe_3O_4@SiO_2@C18 nanoparticles inherit the virtues of being easy-to-prepare, cost-effectiveness and ease of separation and dispersion, this protocol is believed to be promising for the screening of the selected compounds in milk matrices.

Acknowledgements

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OP39

ACT1058

DEVELOPMENT AND OPTIMIZATION OF AN SPE, LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF 24 PESTICIDES IN WATER USING EXPERIMENTAL DESIGN

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Summary

A novel method for the simultaneous determination of 24 pesticides in water was optimized using an experimental design procedure. The effect of various experimental SPE factors on the selected response (recovery) was studied. Tested SPE factors were: sample pH, sample volume, two step elution with eluents A (MeOH:MTBE) and B (CH_2Cl_2), % formic acid in eluent A, eluent volumes (A and B) and -volume of MeOH in initial sample. The method optimization procedure included a two-step approach, with a Placket – Burman Design for an initial selection of the main factors affecting recoveries and a subsequent Central Composite Design (CCD) for the mathematical description of the factors' effects and interactions.

Introduction

The latest EU Directive on Environmental Quality Standards (EQS Directive 2013/39/EU) has proposed new strategies against water pollution and novel monitoring methods for priority substances. Among other amendments, it includes 15 additional priority substances, 6 of them designated as priority hazardous substances. The EQS Directive implements revised and stricter environmental quality standards for existing priority substances, rendering imperative the development and validation of novel multi-residue methods for the determination of micropollutants and especially pesticides. Various pesticides are designated as Priority Substances in the 2013/39/EU Directive, including Alachlor, Atrazine, Chlorfenvinfos, Chlorpyrifos, Diuron, Isoproturon, Simazine and Trifluralin, which are respresentative members of various pesticide classes. The objectives of this study were: to develop and optimize an extraction and chromatographic method, implementing experimental design for the simultaneous determination of 24 pesticides in water (including the above mentioned compounds as well as Azinphos-ethyl, Azinphos-methyl, Chlorotoluron, Cyanazine, Diazinon, Dimethoate, Ethion, Fenthion, Linuron, Malathion, Methidathion, Metribuzine, Monuron, Phosalone, Propazine and Triazophos) using SPE, LC-MS/MS.

Materials and methods

For the chromatographic separation of the pesticides, a reversed phase column Atlantis dC18 (2.1 x 100mm, 3 µm) was used. A gradient elution program with mobile phase Acetonitrile (A) 15-100% and (B) Water, at a flow rate of 0.5 $mL.min^{-1}$, APCI ionization, injection volume 20µL were used, resulting in analysis time of 55 min. Detection of target compounds was carried out using multiple reactions monitoring (MRM) mode, with two precursor – product ion transitions for each pesticide. Solid Phase Extraction (SPE) technique was used, utilizing Oasis HLB (6cc, 200 mg) cartridges. The effect of various SPE factors on the recoveries was studied, using an experimental design approach. Factors were: sample pH, sample volume, two step elution with eluents A (MeOH:MTBE) and B (CH_2Cl_2), %formic acid in eluent A, eluent volume (A and B), % of ACN in the reconstitution solution and volume of MeOH in initial sample. The procedure included the selection of the most important factors using a factorial Plackett-Burman design. Pareto charts and other statistical tools were employed in order to determine the factors with the highest significant impact on the selected responses (recoveries). Based on the selected main factors, a Central Composite Design (CCD) was used for the description of the various effects of main factors on recoveries and the interactions between them.

Results and Discussion

Peak resolution and separation performance was adequate for validation purposes. Quantification was based on selected product ions and ratios of two product ions from each compound. SPE was optimized using an initial Placket-Burman Design and a subsequent Central Composite Design. The use of an initial Placket-Burman design, included 8 factors at two levels plus 3 repetitions of the central point and provided the main factors affecting the selected response (recovery) for each analyte. After adequate transformation of the data series for each analyte, various combinations of the most important factors with highest t-values of effect in Pareto and Half-Normal Probability plots were realized and selected as model terms. The final selection was based on factors that can be fitted in models with increased significance (Prob>F -> p: 0.001-0.05) and minimum lack of fit (based on Prob>F -> p>0.05). For most of the compounds, the main SPE factors affecting recovery were: a) % of ACN in the reconstitution solution (contribution 34-59%), b) % of formic acid in eluent A (contribution 23-34%, c) % of MeOH in volume A (contribution 9-14%) and d) volume of eluent B:CH_2Cl_2 (contribution 3-8%). According to the physicochemical properties of each compound, the principal factors may vary among different chemical groups, therefore the selection was based on common effects. Applied models exhibited correlation coefficient varying 0.905-0.991.

Based on the preliminary Design, a Central Composite Design of these four factors at two different levels, 6 central points and alpha values of 2.0, was prepared. The significance of the proposed CCD model for each compound was tested and validated statistically by the F-test (Fisher variation ratio) as far as significance and lack of fit are concerned. Model terms and their mathematical relation were estimated based on probability P-values, with 95% confidence level. The correlation coefficients of the selected models were generally high (R^2>0.900). Optimization of the selected SPE factors using various criteria (optimum recovery, eluent volume minimization, etc) provided the optimal SPE factor values, which included the use of 400mL of sample at pH 7 (including 5mL MeOH), gradual elution using 5mL Eluent A: 57% MeOH : 43% MTBE (including 3% of HCOOH), 4mL of DCM as eluent B and final reconstitution using 0,4mL (40% ACN: 60% Water including 0.1% of HCOOH). Recoveries for 20 out of the 24 pesticides ranged 74.27-103.70% while Fenthion, Chlorpyrifos, Ethion and Trifluralin ranged 31.91 – 69.83%.

Conclusions

Optimum conditions for the quantitative analysis of 24 pesticides in water were determined, using an experimental design for the initial selection of the most important SPE factors and a subsequent CCD design for the mathematical description of their effects and interactions. Main factors were proven to be a) % of ACN in the reconstitution solution, b) % formic acid in eluent A, c)% of MeOH in volume A and d) volume of eluent B:CH_2Cl_2. Optimized conditions for the multi-residue analysis were determined for the validation of the method.

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OP40
ACT1095

DETERMINATION OF PESTICIDE RESIDUES IN WINE BY MONOLITHIC MATERIAL SORPTION EXTRACTION (MMSE)

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Summary

Monolithic Material Sorption Extraction (MMSE) coupled to gas chromatography (GC-μECD and GC-NPD) was optimized by chemometric experiments for the determination of multi-class pesticides from wine samples. Method validation revealed high precision and reproducibility as well as low LOQs values ranged from 1.0 to 500 μg/L.

Main text

A new sample preparation technique was evaluated for the extraction of pesticides, belonging to different chemical classes and largely used in vineyard's cultivation, from wine samples by using Monolithic Material Sorption Extraction (MMSE). Studied monolithic material is available in two types of disks; DSC18 disks contain only C18 groups on the silica structure, while DCC18 disks contain C18 and activated carbon. Monolithic materials without activated carbon are ideal for hydrodrophobic compounds with medium to high boiling point, while these with activated carbon recommended for more polar compounds with low to medium boiling point.

In preliminary experiments two types of disks (Mono Trap^{TM} DSC18 and DCC18), four different sizes of bottle (40mL, 20mL, 12mL and 8.0mL) and different volumes of donor solution were considered. Studied pesticides were better extracted with DSC18 than DCC18 disks and thus DSC18 disks were selected to be used at all experiments in this study. The best combination between bottle size and volume of donor solution was 8.0 mL bottle and 5.0 mL of donor solution.

In order to optimize conditions concerning pesticides adsorption by the DSC18 disk factors such as volume of wine solution, size of glass vial, duration of the extraction (time), agitation rate, water temperature in agitation bath, pH and ionic strength were considered. For the part concerning pesticides desorption from the DSC18 disk by sonication, extraction solvent (dichloromethane, acetone, acetonitrile and hexane) and extraction duration were considered. Factors influencing MMSE were optimized by a multivariable strategy. A Plackett-Burman experimental design (PBD) was initially used to screen significance of selected factors on the extraction, and the significant factors were optimized by the use of a central composite design (CCD).

Pesticide residues were determined in a Hewlett-Packard model 6890 gas chromatography, fitted with a HP-5 (30m x 0.32mm i.d.) column coupled to a Nitrogen-Phosphorous detector (NPD) or a micro Electron Capture detector (μECD). Optimized method validation indicated that the method could be applied with success for the determination of fludioxonil, dimethomorph, metalaxyl-M, iprodione, pyrimethanil, myclobutanil, vinclozolin, chlorpyrifos-ethyl, procymidone, kresoxim-methyl, fenhexamide, bifenthrin, lambda-cyhalothrin, boscalid, deltamethrin, penconazole, chlorothalonil in wine samples with excellent precision and reproducibility and low values of limit of quantification (LOQ) ranged from 1.0 to 500 μg/L. The validated method will be used for the analysis of wine samples available in local markets.

Acknowledgments:

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OP41
ACT1061

COMBINED IMPACTS OF PHYSICAL AND CHEMICAL PARAMETERS ON THE ACCUMULATION RATE OF PESTICIDES IN SBSE STIR BARS USED AS PASSIVE SAMPLERS

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Summary

Passive sampling is a sampling technique for the monitoring of organic micropollutants such as pesticides in the aquatic environment. This technique allows the determination of time-weighted average (TWA) concentrations, providing first, prior determination of the sampling rates (R_S) of all target micropollutants via laboratory calibration, and second, a possible correction for in situ application, depending on the micropollutant and the experimental conditions. Indeed, flow rate, temperature and the concentration of dissolved organic carbon (DOC) in water may have an impact on the accumulation kinetics of micropollutants in passive samplers, and may imply biased TWA concentrations. Recently, we developed the passive stir bar sorptive extraction (SBSE) for the determination of moderately hydrophobic to hydrophobic pesticides in freshwaters. In the present laboratory work, we studied the impact of flow rate, temperature and the concentration of DOC on the accumulation kinetics of 20 pesticides in SBSE stir bars. Eight experiments were conducted simultaneously according to a full factorial experimental design. Results showed, for hydrophobic pesticides, significant effects of the factors on the accumulation kinetics. These experiments allow identifying pesticides whose accumulation rate depends on experimental conditions and which PRC might best correct the laboratory sampling rates.

Introduction

Passive sampling allows the determination of time-weighted average (TWA) concentrations in the aquatic environment. A prior laboratory calibration needs to be performed to determine the sampling rates (R_S) of all target micropollutants [1]. Differences between laboratory calibration and field application conditions regarding flow rate, temperature and the concentration of dissolved organic carbon (DOC) in water may have an impact on the R_S, leading to possible biased TWA concentrations. In field exposition, performance reference compounds (PRC) might be used to correct the laboratory $R_{S.}$ A PRC is an analytically non interfering compound which is spiked in the passive sampler before field application and whose elimination allows obtaining a correction factor to determine field R_S of the target micropollutants. Stir bar sorptive extraction (SBSE) is a solvent free extraction technique for hydrophobic compounds in water samples [2]. Recently, we developed passive SBSE as a passive sampling technique for the determination of moderately hydrophobic to hydrophobic micropollutants in freshwaters [3]. The aim of the present study was to evaluate the effect of flow rate, temperature and the concentration of DOC on the accumulation of 20 selected pesticides in SBSE stir bars and the elimination of 4 PRC, to ultimately identify which PRC to use during field application.

Experimental

We selected 20 pesticides and metabolites with different physical chemical properties. Four deuterated pesticides were also selected as PRC. The combined effects of flow rate, temperature and the concentration of DOC on the accumulation of the pesticides and on the elimination of the PRC were assessed via a full factorial experimental design (3 factors with 2 levels). The 8 experiments were conducted simultaneously for 7 days, at constant pesticide concentrations. The pesticides accumulated in the SBSE stir bars and the remaining PRC were desorbed by liquid desorption and determined by ultrahigh performance liquid chromatography coupled to tandem mass spectrometry (LD-UHPLC-MS/MS) [4]. Statgraphics® software (SigmaPlus) was used for data processing.

Results and discussion

We briefly present the results of flufenoxuron, for which the factors had an effect on the accumulation kinetics (Figure 1).

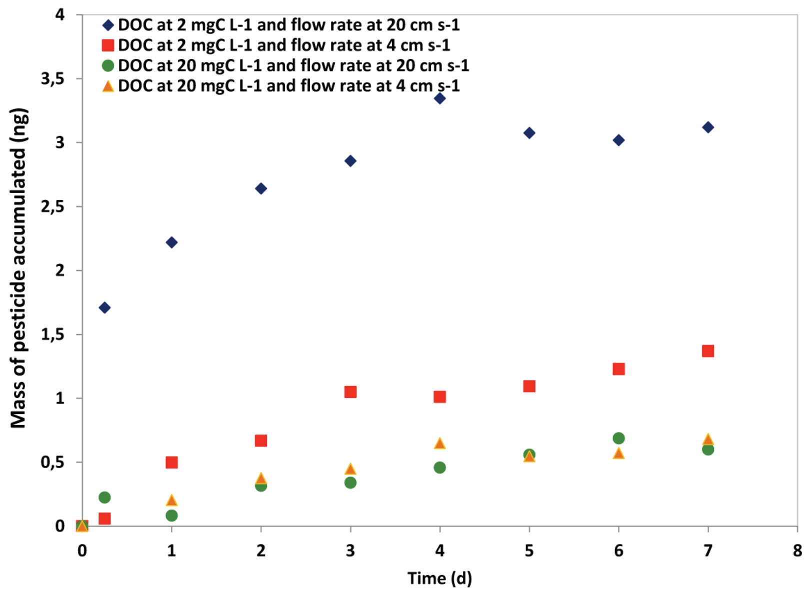

Figure 1. Accumulation kinetics of flufenoxuron in SBSE stir bars during 7 days, at 10 °C, under different concentrations of DOC and flow rates

The flow rate and the concentration of DOC exhibited significant effects on the accumulation kinetics of flufenoxuron. Similar results were observed for the other hydrophobic pesticides and for PRC. This study helps identify the pesticides for which the experimental conditions have an impact on the accumulation kinetics, and ultimately which PRC could best correct the laboratory sampling rates. Further details will be presented in the communication.

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OP42
ACT1087

CHEMOMETRIC ASSESSMENT AND INVESTIGATION OF MECHANISM INVOLVED IN PHOTO-FENTON AND TIO_2 PHOTOCATALYTIC DEGRADATION OF THE ARTIFICIAL SWEETENER SUCRALOSE IN AQUEOUS MEDIA

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Summary

Chemometric optimization tools were employed, such as experimental design and response surface methodology (RSM), to assess the efficiency of two advanced oxidation processes (AOPs): homogeneous Fenton (Fe^{II}/H_2O_2) and heterogeneous (TiO_2) photocatalysis for the degradation of the artificial sweetener sucralose. The aqueous samples were irradiated under a variety of experimental conditions (pH, light intensity) and with different amounts of H_2O_2, Fe^{II} and TiO_2. The use of RSM allowed fitting the optimal values of the parameters leading to the degradation of the contaminant. Also, a single polynomial expression modeling the reaction was obtained for both AOPs. The intermediates formed during the processes were investigated and characterized by means of HPLC/HRMS. The photocatalysed transformation of sucralose proceeds through the formation of eight products, involving four different pathways. All the identified intermediates were easily degraded and complete mineralization was achieved. In addition Microtox bioassay (Vibrio fischeri) evaluated the ecotoxicity of solutions. Results demonstrate the efficiency of the process in the detoxification of the irradiated solutions.

Introduction

Sucralose is an artificial sweetener synthesized by chlorinating sucrose. It is used in more than 80 countries, mostly in the USA, where an average concentration of 17.6 μg/L was found in wastewaters [1]. Screening on European rivers has shown concentrations up to 1 μg/L [2]. It exhibits high stability and persistence with a half-life of several years [3]. It can be found in the aqueous phase and it is rarely bioaccumulated [4].

Materials

Sucralose, sodium hydrogen carbonate and potassium carbonate were purchased by Sigma–Aldrich, acetonitrile by Scharlau and sodium chloride from Carlo Erba. $FeSO_4{\cdot}7H_2O$ and H_2O_2 were obtained by Merck. TiO_2 was Degussa P25 supplied by Degussa AG and water by a MilliQ system. Experiments were carried out in Suntest CPS+ apparatus from Heraeus. Analyses were performed by HPLC-MS. Anions were analyzed using an ion chromatograph. Total organic carbon was measured by Shimadzu TOC-5000 analyzer. The toxicity of intermediates was examined by Microtox Model 500 Toxicity Analyzer.

Results and discussion

The central composite design (CCD) was employed to analyze the effect of variables (H_2O_2, Fe(II), pH, light intensity, TiO_2) in the photocatalytic degradation and to evaluate the interactions between them. The CCD considered low (−1) and high (+1) levels, as well as central points (0) for the variables. Statistica 7.0 (StatSoft, Inc. Tulsa, USA) statistical package was used to generate the experimental matrix. Optimized conditions were: TiO_2 concentration 294 mg/L, light intensity 698 W/m^2 for heterogeneous photocatalysis and Fe^{II}, H_2O_2 concentrations, 6 mg/L and 6 mmol/ L, respectively, solution pH=3 for photo-Fenton treatment. Also, a single polynomial expression modeling the reaction was obtained for both AOPs. The transformation of sucralose proceeds through the formation of eight products, involving four different pathways: hydroxylation of the molecule, oxidation of the alcohol function, dechlorination and the cleavage of glycoside bond. The intermediates were easily degraded and within four hours of irradiation complete mineralization was achieved. In addition Microtox bioassay was employed in evaluating the toxicity of solutions. Observations show that in 10–60 min compounds more toxic than sucralose are formed. After 60 min the toxicity decreased.

Conclusions

The present study points out that both treatments are suitable for the elimination of sucralose from the aqueous phase. The conversion of sucralose depends on the operating conditions employed. The use of RSM allowed fitting the optimal values of the parameters leading to the degradation of the contaminant.

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OP43
ACT1018

ASSESING THE PRESENCE OF PHARMACEUTICAL RESIDUES IN IRRIGATED CROPS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Summary

A rapid, sensitive, and environmental-friendly multi-residue method has been developed for the simultaneous determination of four pharmaceutical (diclofenac, 17a-ethynylestradiol, trimethoprim, sulfamethoxazole) residues in treated wastewater and crops that are irrigated with treated wastewater. The target compounds were identified and quantitatively determined by ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC–MS/MS) operated in multiple reaction monitoring mode. Average recoveries of the four analytes from fortified samples ranged between 70.1% and 110.0%, with relative standard deviations (RSD) lower than 9.4%. The proposed method is fast, sensitive, easy to perform, environmentally acceptable, making it applicable for high-throughput monitoring of pharmaceutical residues in irrigated crops (i.e. tomatoes, roots and leaves).

Introduction

As water scarcity is exacerbated by urbanization and climate change, especially in arid and semi-arid regions around the world like Cyprus, treated wastewater is increasingly an attractive alternative source of water for agricultural irrigation [1-3]. The utilization of treated urban wastewater either for irrigation purposes or groundwater replenishment due to prolonged drought periods constitutes a very important activity on a national level in Cyprus. Currently, there is an increasingly growing momentum towards the reuse of wastewater while at the same time the concern with respect to the existence of xenobiotic compounds including pharmaceutical residues in the treated wastewater effluents follows also an increasing trend.

Over the last decades, several studies show that many chemical compounds, including pharmaceuticals, are present in the treated effluent of wastewater treatment plants (WWTPs) [4-5]. Therefore, when treated wastewater is used for agricultural irrigation, the pharmaceutical's trace has the potential to enter and accumulate in plants. The occurrence of various xenobiotic compounds and especially pharmaceuticals in treated wastewater and their possible transfer into food products causes an unknown human health risk. Multiclass procedures for the determination of pharmaceuticals in crops are more and more required due to their importance in public health and environmental monitoring. As reported by Wu et al., [1] a total of 20 frequently-occurring PPCPs were studied for their accumulation into four types of vegetables (lettuce, spinach, cucumber, and pepper) grown in nutrient solutions containing PPCPs at 0.5 or 5 $\mu g\ L^{-1}$ [1].

The main objective of this study was to develop validated methodologies for the investigation the occurrence of four pharmaceuticals at the irrigated crops of tomato with treated wastewater from two urban wastewater treatment plants in Cyprus, which apply activated sludge and membrane bioreactor treatment, respectively. The general goal of this work was to identify pharmaceuticals that have a comparatively high potential for plant uptake.

Sampling and Analysis

The duration of the growing period for each year (2011-2014) was 4 months (120 days). The irrigation of crops was performed with drips, based upon direct measurements of soil moisture status (15 centibars) by the use of tensiometers. Almost 105 tones of wastewater from each wastewater treatment plant were used to irrigate 100 tomato plants. This means that over the growing season tomato plants were irrigated with 1 tone of wastewater per plant.

For the extraction of TMP, EE2 and DCF the pH of ten grams sample S1 was adjusted to 6-7 with the addition of small amount of solid $NaHCO_3$ while for the extraction of SMX the pH of ten grams sample S2 was left as it is (pH 3-4). Ten mL of EtOH was added to each sample and then extracted in a sonication bath for 1h, followed by centrifugation at 3000 rpm for 30 min. The supernatant was carefully withdrawn and kept, while the residue was resuspended in 10 mL of 1:1 EtOH:H_2O, sonicated for 30 min and centrifuged. The combined extracts were then diluted with H_2O to 400 mL (final %EtOH in the extracted liquid should be less than 5%).

The diluted ethanolic tomato extracts from blanks, samples and spiked blank samples was loaded under gravity onto HLB cartridge (OASIS HBL from Waters) which was preconditioned with 5 mL of methanol followed by 5 mL distilled water. The analytes were then eluted with 8 mL of methanol at 1 mL min^{-1} flow rate. The eluted methanol was evaporated to dry under a gentle nitrogen stream and the residue reconstituted in 1 mL of methanol-water mixture 25:75 v/v.

Results and Discussion

This comprehensive survey of organic micropollunts such as pharmaceutical residues in irrigated crops included four pharmaceuticals. The first step of the study was to develop and optimize the methodology of sample preparation and then the process of solid phase extraction of the determination of pharmaceutical compound in tissue plants.

Average recoveries of the four analytes from fortified samples ranged between 70.1% and 110.0%, with relative standard deviations (RSD) lower than 9.4%. More specifically, the average recoveries for TMP, SMX, DCF and EE2 was 85.9, 70.1, 82.1 and 110.0, respectively. The detailed results of this study will be presented in the full paper.

Conclusions

A multi-residue method for the determination of pharmaceutical residues in irrigated crops like tomatoes plants was developed with satisfactory results using a system based on SPE followed by UPLC-MS/MS.

The method was successfully validated for the simultaneous quantification and identification of four pharmaceuticals belong in different therapeutic groups in tomato plants (fruits, roots, leaves). This study demonstrates the suitability of UPLC-MS/MS for quantitative routine pharmaceutical residue analysis. Results from this study clearly showed that irrigated crops were capable of taking up many pharmaceuticals when exposed to them, but different pharmaceutical residues displayed significant disparities in their potential for root uptake and subsequent translocation. Out of the 4 pharmaceuticals considered in this study, sulfamethoxazole and trimethoprim were identified and quantified in irrigated crops at levels higher than the other pharmaceuticals.

Acknowledgments

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OP44
ACT1119

QUECHERS EXTRACTION METHOD FOR THE ACCURATE AND HIGH RESOLUTION DETECTION OF EMERGING CONTAMINANTS IN DAIRY PRODUCTS BY LC-HYBRID LTQ ORBITRAP MS

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Summary

The present study focuses on the accurate, robust and rapid detection of selected emerging contaminants taking advantage of the innovative hybrid technology and versatility of the U-HPLC coupled to LTQ FT Orbitrap mass spectrometry. The influence of several variables involved in the chromatographic- mass spectrometric process was evaluated in order to increase sensitivity and provide good peak shape for a rapid screening and accurate mass confirmation of 17 compounds (positive ionization) in 15 min. With a view to the further detection of the target analytes in real complex food matrices of animal origin (milk and dairy products), QuEChERS was selected to be optimized and evaluated for its applicability as a multiresidue extraction method. QuEChERS methodology was shown to be linear over a wide range of concentration, exhibited satisfactory repeatability and reached low enough limits of quantification (2.7-25 ng/g). Recoveries were good in the range of 74% to 117%. In general, the sensitivity obtained meets the maximum residue levels (MRLs) established by the European Union regulation for food monitoring.

Introduction

Target emerging contaminants were selected in terms of their diversity, potential endocrine disrupting activity and the possible presence of their residues in food commodities of animal origin that can irreversibly affect the consumer's health. The selected compounds included 13 pesticides (mainly insecticides and fungicides) and 4 pharmaceuticals. Taking into account the complexity of these food matrices and the low concentrations to be analyzed, it is of primary interest the development of analytical methods with quick response, cheap instrument, low consumption of reagents, simplified operation and time-saving [1]. In this sense, QuEChERS procedure (acronym name for Quick, Easy, Cheap, Effective Rugged and Safe) has emerged which involves microscale extraction using small volume of acetonitrile and dispersive solid-phase extraction (d-SPE) with a major reagent and relies on the difference in affinities between the reagents and analytes [2]. This study focuses on the assessment of a UHPLC-LTQ Orbitrap MS analytical methodology of QuEChERS extracted dairy samples for the accurate and sensitive determination of the selected emerging contaminants.

Sample preparation

Full-fat fresh milk (3.5% fat) was obtained from a local market with the purposes both to estimate target analytes recoveries and to evaluate the robustness of the method. In all experiments, 10g of homogenized milk was weighed into a Teflon centrifuge tube (QuEChERS). Then, 10ml of acetonitrile with 1.5% acetic acid was added and the tube was vigorously mixed for 1 min using a vortex mixer. Anhydrous magnesium sulfate (MgSO4) and an amount of sodium acetate were added to the tube and immediately mixed with a vortex mixer for 1 min. The sample was then centrifuged for 5 min at 3000 rpm. A 1ml aliquot of the upper acetonitrile layer was transferred to a centrifuge tube containing $MgSO_4$ and PSA. The tube was vigorously mixed for 1 min and then centrifuged for 5 min at 3000 rpm. An aliquot of the final layer, acidified by 0.1% formic acid, was then subjected to LC Orbitrap LTQ analysis.

Liquid chromatography analysis-LTQ FT Orbitrap mass spectrometry (LC-LTQ FT Orbitrap)

The LC LTQ-FT Orbitrap MS system consisted of an Accela AS autosampler, an Accela quaternary gradient U-HPLC-pump and an LTQ Orbitrap XL 2.5.5 SP1 mass spectrometer (Thermo Fisher Scientific, Inc. GmbH, Bremen, Germany). The linear ion trap (LTQ) part of the hybrid MS system was equipped with an Ion Max Electrospray Ionization (ESI) probe. Full-scan accurate mass spectra were obtained at high resolution (60000 FWHM). Analytes were separated on a 50mm x 2.1mm i.d. Hypersil GOLD PFP column, 1.7 μm, using a gradient elution system consisting of water (A) and methanol (B) both containing 0.1% formic acid at 300 μl/min flow rate.

Results and Discussion

Under positive ion conditions, target emerging contaminants were detected as $[M+H]^+$ ions. Mass accuracy of measured ions (vs. theoretical) was ±5 ppm. The variables involved in the chromatographic process were optimized in instrument auto tune sections. The selected conditions proved to be excellent for the simultaneous separation and detection of 17 analytes in 15 min with the quantification LTQ-Orbitrap LC-MS limits to range from 1 to 25 ppb. Optimization of the extraction conditions was performed using spiked milk samples fortified with all analytes at 100 ng/g. Influence of different experimental factors (screening) on the performance of the QuEChERS method was evaluated using a Plackett – Burman experimental design while final optimum conditions were determined after the application of a central composite design (CCD). The analytical method was shown to be linear over a wide range of concentration, exhibited satisfactory repeatability and reached low enough limits of quantification (2.7-25 ng/g). Recoveries were good in the range of 74% to 117%.

Conclusions

Despite their diversity, an excellent robust and rapid separation and accurate confirmation of all analytes was finally achieved by the U-HPLC-LTQ Orbitrap MS system. QuEChERS extraction proved excellent choice for the routine analysis of dairy products for the selected emerging contaminants residues.

Acknowledgments

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OP45
ACT1048

ASSOCIATION BETWEEN DIABETES AND USE OF PESTICIDES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Summary

Diabetes is a very common metabolic disease and many records have been published on the association between exposure to pesticides and risk of diabetes. In this systematic review we searched for observational epidemiologic studies exploring the association between exposure of any pesticide and diabetes. A meta-analysis of 25 studies exploring the association between exposure to pesticides and risk of diabetes yielded a significant summary odds ratio (OR) even though studies were found to be heterogeneous. We observed similar results for the association of the exposure to pesticides and Type 2 diabetes only, with less heterogeneity. Subgroup analysis by type of pesticides showed an increased risk with less heterogeneity. The findings of our systematic review support the hypothesis that exposure to different types of pesticides increases the risk of diabetes.

Introduction

Diabetes is an epidemic with more than 350 million worldwide considered to be diabetic [1]. There is a steadily increasing body of published reports on the relation of exposure to pesticides and diabetes. Pesticides are considered one of the main factors involved in environmental contamination. We performed a systematic review of observational studies that assess the association between pesticides and diabetes.

Methods

We conducted a comprehensive literature search of peer-reviewed original research pertaining to pesticide exposure and any health outcome. We searched for observational epidemiologic studies published until January 2014. We explored the association between exposure of any pesticide and diabetes. We performed separate analyses for studies that recruited only Type 2 diabetes participants. We summarized OR estimates using fixed and random-effects models [2, 3]. To enable a consistent approach to meta-analysis and interpretation of findings, we transformed risk estimates so that they are comparable across studies [4].

Results and Discussion

We identified 25 studies assessing diabetes. The summary OR for the association of the exposure to any type of pesticide and diabetes was 1.84 (95% CI: 1.54-2.19), with large heterogeneity (I^2=75.5%). Studies evaluating Type 2 diabetes only showed a similar summary effect 1.75 (95% CI 1.40-2.19) with smaller heterogeneity (I^2= 58.9%). Analysis by type of pesticide showed an increased risk with less heterogeneity (0%-74%). The summary effects ranged from 1.34, showing a non-significant risk, up to 2.67 (95% CI: 1.80-3.97). Egger's test was significant suggesting the presence of small-study effects.

Our study has some limitations. First, high heterogeneity was observed, due to the fact that evidence from observational studies was synthesized. Second, for the majority of the studies, the type of diabetes was not well-characterized in the populations, however, in most of the studies over 90% of the participants are expected to have Type 2 diabetes.

Conclusions

The findings of our systematic review support the hypothesis that exposure to different types of pesticides increases the risk of diabetes. Subgroup analyses did not reveal any differences in the risk estimates based on the type of studies or the measurement of the exposure. Analyzing each pesticide separately reduces heterogeneity giving support to the hypothesis that pesticides may increase the risk of diabetes.

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Abstracts of Posters

PP01
ACT1069

A THREE YEAR STUDY OF THE SPATIAL AND TEMPORAL VARIATION OF PESTICIDES RESIDUES IN VIOTIKOS KIFISSOS BASIN AFTER THE APPLICATION OF LOW INPUT CROP MANAGEMENT SYSTEMS

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Summary

A three year study for the spatial and temporal variation of pesticides residues in Viotikos Kifissos basin after the application of Low Input Crop Management systems (LCM) was carried out between 2009 and 2011. 253 water samples were analysed for the presence of 219 pesticides including metabolites, using liquid and gas chromatography coupled to mass spectrometry. Samples were prepared by solid phase extraction using Oasis HLB cartridges. Results showed a significant reduction of pesticides detected and quantified in water samples during 2011 compared to those measured in 2009 and 2010.

Introduction

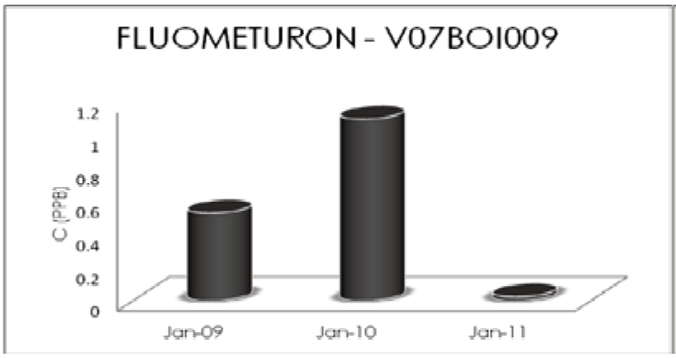
Viotikos Kifissos basin is one of the most productive plains of Greece and an area in which intensive agricultural practises are followed. The basin drains into Lake Yliki, one of the main reservoirs that supply Attiki with drinking water. The area is cultivated mainly with crops such as cotton, maize and plum tomato. The study area covered a pilot area of 900 ha around the towns of Akontio, Agios Vlassios, Cheronia and Thourio. 253 samples were collected from 33 sampling points (boreholes (karstic aquifer), wells (alluvial aquifer), rivers and vadose zone) before and after the application of LCM (2009 and 2010-2011 respectively) [1]. Vadose zone samples provide mean concentration levels of lecheates that can be conceived as the overall footprint of the performed agricultural practises during the sample collection period. Lecheate collection was performed underneath the core root zone development depth of the main cultivars in the studied area.[2]. All samples were analysed for the presence of 219 pesticides using LCMSMS and GCMSMS systems after sample preparation with solid phase extraction using Oasis HLB cartridges. Almost all pesticides registered for use in the crops mentioned above were included in method's scope.

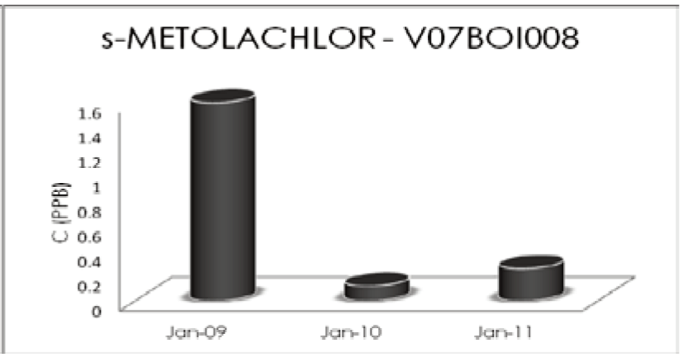
Water Sampling and Analysis

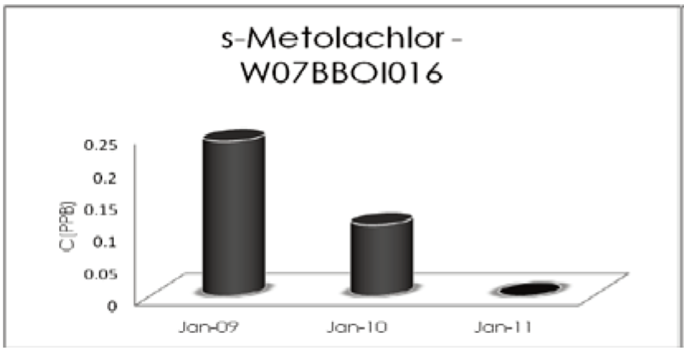
In 2009 samplings took place in April, July, and September but in 2010 and 2011 in February, May, July and September to coincide with key periods: a) prior to sowing and any other agricultural practise, b) post-sowing and after the first applications of plant protection treatments, c) after completion of plant protection treatments and d) after the end of the harvesting period. Samples were collected in amber glass bottles (2.5 L) and stored at 4 ˚C. Within 3 to 4 days from arrival at the laboratory samples were filtered, if necessary, and analysed. The final extract was injected in both LCMSMS and GCMSMS systems.

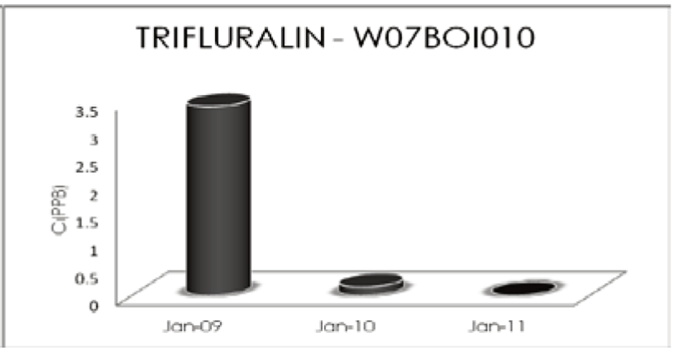
Results and Discussion

70% of the samples were found positive for pesticide residues. Most frequently detected pesticide was fluometuron (55.9% of positive samples), followed by s-metolachlor (50.3%), metalaxyl-M (40.1%), imidacloprid (31.6%), ethalfluralin (19.8%), terbuthylazine (18.1%) and trifluralin (15.8%). Highest concentrations were recorded for fluometuron (322 μgL^{-1}), ehalfluralin (123 μgL^{-1}) and s-metolachlor (36.4 μgL^{-1}). Aside from metalaxyl, all other pesticides detected at high concentrations were herbicides. The high detection frequency and concentrations can be assigned to the wide use of herbicides since one of the main challenges of crop production in the studied area was weed control especially purple nutsedge (*cyperus rotundus*). Regarding the type of samples, 88 % of samples from vadose zone were found positive in pesticides residues, while only 5% of samples from boreholes were found positive. During the three year study, reduction of pesticides concentrations was observed in various sampling points. Results from vadose zone samples though, are directly correlated to rainfall and irrigation thus to concentration of contaminants in the samples. Nonetheless, results showed a reduction in concentrations from 2009 to 2011, as shown in the selected graphs below.









Conclusions

Intensive agricultural practises led to the contamination of aqueous systems in Viotikos Kifissos basin. Elevated concentrations of herbicides were recorded in vadose zone and alluvial aquifer while karstic aquifer was of good quality regarding the presence of pesticides. The application of a Low Input Crop Management system during the 2nd and 3rd year of the study resulted to the reduction of the average value of all pollutants in alluvial as well as in vadose zone.

Acknowledgments

The project was carried out as a part of the Life+ ECOPEST programme which was cofunded by the European Union.

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PP02
ACT1097

RESIDUES OF INSECTICIDES AND HEAVY METHALS CONTENT IN FRUIT SAMPLES

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Summary

In order to investigate presence of organophosphorus, carbamates and pyrethroides insecticides, as well as heavy methals, 40 samples of apple, grape, cherry and sweet cherry were analized. All samples were from local orchards and vineyards. In compliance with ISO 17025 standard for determination of pesticide residues in food of plant origin, for the extraction of pesticide residues method Fillion et al. (2000) was used. Analysis was done by gas chromatography coupled with mass spectrometry (MS). Confirmation of the identity of the compound was set by comparison of characteristic iones from samples and the standard and by comparison with a library of mass spectra. For determination of Cd and Pb Atomic Absorption Spectrophotometry (AAS) Shimadzu AA-6300 was used. In 72% of investigated fruit samples insecticides residues were not detected, or they were below LOD, while in 28% of samples residues were below MRLs. Results of the analysis of heavy metals showed that in all fruit samples content of cadmium and lead were lower than the maximum allowed concentration.

Introduction

Inadequate use of pesticides leads to accumulation of their residues in the environment and in the agricultural products [1-3]. In 2013, on Serbian market were 106 registrated active substances for apple protection, 16 for sweet cherry, 34 for cherry and 89 for grape protection [4].

In terms of agricultural production one of the most important anropogenic sources of pollution are heavy metals, especially lead and cadmium.The maximum residue levels of pesticides and the maximum allowed contents of heavy metals in Serbia are defined in the Regulations [5,6].

Sampling and Analysis

In order to investigate presence of organophosphorus, carbamates and pyrethroides insecticides, as well as heavy metals, 40 fruit samples colected in vicinity of Novi Sad were analized: 10 apple samples (7 from conventional, 2 from integrated and 1 from organic production), 9 grape samples from conventional and one from organic production and 20 cherry and sweet cherry samples from conventional agricultural production.

For the extraction of pesticide residues method Fillion et al. [7] was used. Analysis was done by gas chromatography (Thermo Scientific, Trace GC 1300) coupled with mass spectrometry. Oven temperature program: 70 °C to 200 °C. Column HP-5MS. Carrier gas helium. Inlet splitless. Injection volume 1 μl.

For determination of Cd and Pb, Atomic Absorption Spectrophotometry (Shimadzu AA-6300) was used.

Results and Discussion

In 4 apple, 2 grape, 3 cherry and 2 sweet cherry samples, residues of dimethoat were below MRLs. Residues of carbamate insecticides were not detected or they were below LOD of 0.005 mg/kg in all analyzed samples. Residue of pirimicarb, below the MRLs was detected in one apple sample. Cypermethrine residues were detected in 4 apple, 2 grape and 2 cherry and sweet cherry samples. In analysis provided in 2011, 12% of fruit samples were pesticide free, 75% of samples were with multiple detections of pesticides and the most frequently detected pesticides, with the values over the MRLs were dimethoat, chlorpyrifos and azinphos-methyl.

In all fruit samples content of cadmium and lead were lower than the maximum allowable concentration, and were in the range of 0.00003 to 0.020 mg/kg (Cd) and from 0.0005 to 0.013 mg/kg (Pb).

Conclusions

In all analyzed fruits, investigated insecticides residues were below MRLs. Fruit samples from organic productions were insecticides free. Number of positive findings of insecticides residues is lower than in 2011 year. Content of cadmium and lead in all samples were lower than the maximum allowable concentration.

Acknowledgments

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PP03
ACT1105

UPTAKE AND FRUIT CONTAMINATION OF CHLORDECONE IN ZUCCHINI (*CUCURBITA PEPO*)

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Summary

Chlordecone (CLD) is an organochlorine insecticide used in the French West Indies to struggle against banana weevil. Its use was forbidden since 1993 but twenty years of application led to long-standing contamination of soils by this POP [1] and risks for population by the contamination of food, particularly vegetables. With edible roots, zucchini was a particularly contaminated vegetables. We studied the transfer of 14C radiolabeled CLD in zucchini fruits. The distribution of CLD followed an acropetal gradient in zucchini plant with the higher concentrations in roots and the lowest in apical leaves. In shoot, petioles had the higher concentration followed by leaves then fruits, in accordance with the height in the plant. Gradient was also observed within fruits with higher concentration in zone proximal of peduncle. Total fruit contamination corresponded to alone xylem transfer of CLD. Acropetal gradient was explain by age of organ but also by CLD adsorption on the vascular tissues.

Introduction

Chlordecone was used in the French West Indies from 1973 to 1995 to struggle against banana weevil (*Cosmopolites sordidus*) whose larva causes damages in shoots. CLD replaced hexachlorocyclohexane (HCH), due to HCH resistance of banana weevils. Moreover, CLD was applied at 3 kg/ha whereas HCH was at 300 kg/ha. Now, CLD was classified as a POP since May 2009, the half-life of CLD in soil is evaluated at 30 years (about two fold the one of the DDT) [1]. Indeed, the lipophily (log $K_{OW} = 4.5$) and strong affinity for the organic matter ($K_{OC} = 17500$ L.Kg^{-1}) [3] of this molecule cause its persistence in soils. Moreover, the cubic structure hinders micro-organism degradation. Today around 20 % of agricultural soils are contaminated in Guadeloupe and Martinique. It causes both environmental risk, by bio-amplification in aquatic trophic chain, and human chemical risk [2], by consumption of vegetable cultivated on contaminated soils. Edible roots are contaminated because they grow directly in contact with contaminated soils. However by contrast to tomatoes, zucchini are also contaminated by CLD even in absence of soil contact. Cucurbita are known to have a high potential to transfer organochlorine compounds in shoots, particularly the specie *Cucurbita pepo* [3] such as zucchini. But the uptake in zucchini shoots depends on the varieties.

To manage the risk of fruit contamination of zucchini, we studied the mechanism of transfer of CLD in zucchini from soil to shoot to determine the parameters which control the transfer.

Materials and Methods

In first approach, the uptake was determined by the measure of absorption of CLD by seedlings in some varieties of zucchini.

In second, absorption and translocation of CLD to shoots and fruits was determined on plant grew on hydroponically and controlled conditions. When flowers were fecundated (anthesis stage), a 48 h pulse of [^{14}C]-CLD was applied in hydroponic solution. And distribution was followed by radiometric determination.

Results and Discussion

As already shown with other organochlorines, the uptake of the CLD depended on the zucchini varieties, with weakly accumulating varieties (CV2436) and others accumulating up to 9 times more CLD during the same period, such as Pomme d'Or courgette.

If we compared the characteristics of flow of the CLD in two extreme varieties, CV2436 and Gold Rush, we showed that the transpiration stream concentration factor (TSCF) (0.092 and 0.15, respectively) are inversely proportional to the root concentration (49.4 and 35.4 µg/g, respectively). This suggested that roots of some varieties had a barrier effect on the CLD absorption, consequently reducing the translocation rate to shoots.

Translocation in shoots led to the formation of a concentration acropetal gradient with lower concentrations in the apical leaves of plants. In these leaves, a same type of gradient was observed with higher concentrations in petioles than in lamela. As for leaves, the whole concentration in fruits depended on shoot location. Within fruits, a gradient was also observed with the apical zone less contaminated than tissues close to peduncle. Similarly, the CLD concentration in the fruit periderm (without contact on the contaminated substrate) was higher than in the flesh. For each variety, the total quantity of CLD transferred in fruit corresponded to the contribution of CLD by the transpiration stream current, according to their respective TSCF.

Conclusion

The transfer of the CLD in zucchini plants depended on the transfer by the transpiration stream. However, the various tissues have a strong power of CLD retention resulting in the appearance of a gradient which was found in all organs. This retention mechanism explain a part of the variety difference. Varieties which less adsorbed CLD on roots led an increase of the shoot contamination.

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PP04
ACT1015

COMPARISON OF THE PERFORMANCES OF STIR BAR SORPTIVE EXTRACTION (SBSE) COUPLED TO GC-MS-MS AND UHPLC-MS-MS FOR THE ANALYSIS OF PESTICIDES IN FRESHWATERS

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Summary

The monitoring of freshwaters regarding organic micropollutants such as pesticides requires efficient analytical methods to determine reliable concentrations at low levels in natural waters. In this aim, two complementary methods were developed for the determination of several pesticides in surface waters using Stir Bar Sorptive Extraction (SBSE) followed by liquid chromatography or gas chromatography coupled to tandem mass spectrometry. These two methods were validated and their analytical uncertainties were determined according to French standards. Linearity and limits of quantification (LOQ) were statistically tested and the efficiency, repeatability and expanded uncertainties were evaluated for each compound. The SBSE-TD-MS-MS method achieved lower LOQ although the number of compounds analyzed was lower than for the SBSE-LD-UHPLC-MS-MS method.

Introduction

Monitoring of organic pectidides in freshwaters requires developing robust methods that usually involve an extraction step followed by gas or liquid chromatography. Stir Bar Sorptive Extraction (SBSE) is an innovative solvent free sample preparation technique for moderately hydrophobic organic compounds ($2 < \log K_{OW} < 6$) in aqueous samples [1]. This fast extraction technique can be followed by two possible desorption modes: liquid desorption for liquid chromatography (LD) or thermal desorption for gas chromatography (TD).

The aim of this work was to validate SBSE-TD-GC-MS-MS and SBSE-LD-UHPLC-MS-MS methods for the determination of several pesticides in surface waters and to compare their performances.

Method Validation

The 22 selected pesticides are frequently detected in freshwaters and present various physico-chemical properties. All pesticides were analyzed by SBSE-LD-UHPLC-MS-MS [2] whereas only 8 thermally stable pesticides were targeted by SBSE-TD-GC-MS-MS [3].

Each method was validated according to the French standard NF T90-210 (AFNOR, 2009). The linearity range was statistically tested with 5 calibration curves. Recovery and repeatability were evaluated in EvianÒ mineral water spiked at 3 concentration levels: LOQ, medium and high (5 duplicates for each level) [4].

Analytical uncertainties were determined according to the French standard XP T90-220 (AFNOR, 2003). EvianÒ mineral water and two natural river waters were spiked at 3 concentration levels: LOQ, medium and high (10 triplicates for each level) [5].

Results and discussion

Linearity ranges from 0.005 to 200 µg/L, depending on the compound and the method, were validated. For SBSE-LD-UHPLC-MS-MS, recoveries higher than 60% and relative standard deviations (RSD) below 22% were obtained. LOQ were between 5 and 1000 ng/L, and expanded uncertainties (k=2) varied from 5% to 50% depending on the compound and the concentration level. For SBSE-TD-GC-MS-MS, recoveries were higher than 90% and RSD lower than 17%. The method reached LOQ between 2.5 and 100 ng/L, with expanded uncertainties ranging from 10% to 25%. This method was clearly more sensitive, except for metolachlor and dichloroaniline, partly because the whole amounts of extracted compounds were injected in the GC during the TD.

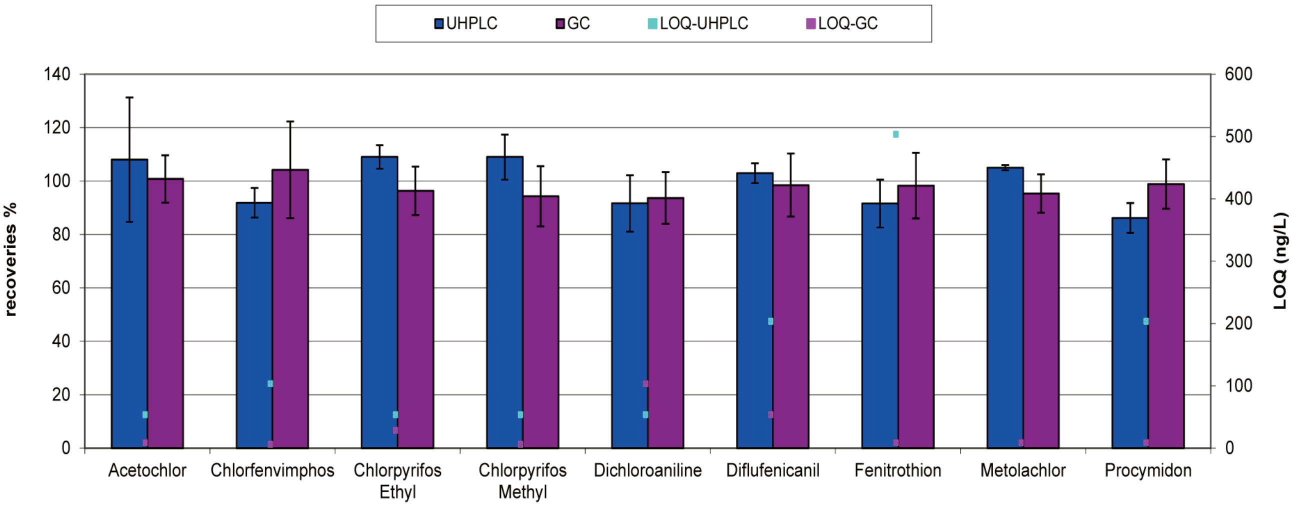

Figure 1. Recoveries at LOQ level for the pesticides targeted by SBSE-LD-UHPLC-MS-MS and SBSE-TD-GC-MS-MS methods

Figure 1. Recoveries at LOQ level for the pesticides targeted by SBSE-LD-UHPLC-MS-MS and SBSE-TD-GC-MS-MS methods

Conclusions

SBSE-LD-UHPLC-MS-MS allows the analysis of the largest number of pesticides. Nonetheless, LOQ and uncertainties were improved using SBSE-TD-GC-MS-MS. Furthermore, SBSE-TD-GC-MS-MS is an automated method and is more environmentally-friendly as it requires less organic solvent.

Acknowledgements

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PP05
ACT1036

MULTI-CLASS PESTICIDE RESIDUE ANALYSIS USING POLY(DIVINYLBENZENE-STYRENE)-COATED OLEIC ACID-MAGNETITE NANOPARTICLES AND GC-MS

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Summary

In this study, the synthesis of poly(divinylbenzene-styrene) coated oleic acid-modified magnetite nanoparticles and application for the enrichment and determination of sub-parts per billion concentrations of several classes of pesticides in water samples was investigated.

Introduction

The world is facing formidable challenges in meeting rising requirement to clean environment. Different nanomaterials have been used in the last few years for the development of analytical methods for the determination of micropollutants in different environmental matrices [1-3]. Magnetically driven separation techniques have received considerable attention in the recent decade because of their potential in analytical systems. In addition nanomaterials are attractive because of their unique chemical and physical properties (size, composition, conductivity, magnetism, mechanical strength, light absorbing and emitting properties).

Materials and methods

To obtain the poly-DVB-styrene nanoparticles, divinyl benzene and styrene were mixed with oleic acid-modified magnetite nanoparticles using 2,2´-azobisisobutyronitrile as initiator, at 70°C for 24h. The synthesized nanoparticles were characterized by BET, IR and SEM

The nanoparticles bearing the target pesticides can easily be separated from the aqueous solution by applying an external magnetic field and the absorbed pesticides are recovered using acetone. The desorbed pesticides are then determined with GC-MS in selective-ion monitoring mode. The effect of pH, temperature, microextraction time, desorption conditions, ionic strength on extraction efficiency of the pesticides of interest are investigated and properly optimized.

Results

The recoveries were in the range 70-110%. Under the optimized conditions the detection limits are in the range 1 – 7 ng/l. The applicability of the nanomaterials was verified using a real sample matrix and the accuracy of the method was evaluated by recovery measurements on spiked samples.

Conclusions

The poly(divinylbenzene-styrene)-coated oleic acid-magnetite nanoparticles are easily synthesized and the developed method is fast, facile and effective for the determination of sub-parts per billion of multi-class pesticides from water samples.

Pesticide	R	Pesticide	R	Pesticide	R
Teflubenzuron	72	Chlormephos	73	spirodiclofen	73
Lufenuron	72	Trifluralin	77	Bitertanol	75
Vernolate	71	Cadusafos	74	Beta-cyfluthrin	73
Chlorpropham	87	hexachlorobenzene	75	Tau-fluvalinate	71
Pencycuron	87	Tefluthrin	75	Difenoconazole	106
BHC	84	Endosulfan ether	77	Dimethomorph	82
Pentachlorobenzene	88	Vinclozolin	77	Alpha cypermethrin	107
Diazinon	109	Parathion methyl	77	Deltamethrin	81
Dinitramine	89	Heptachlor	71	Iprodione	71
Triallate	97	Fenchlorphos	79	Bromopropylate	72
Chlorpyriphos methyl	95	Fenthion	73	Fenpropathrin	108
Pirimiphos methyl	101	Aldrin	77	Phenothrin	86
Chlorpyriphos	98	Parathion ethyl	79	Lambda- cyhalothrin	109
Dicofol	100	Bromophos methyl	78	Cyphenothrin	75
Thiabendazole	99	Penconazole	72	Pyraclostrobin	79
Pendimethalin	110	Tolylfluanid	73	Coumaphos	72
heptachlor epoxide	102	Triadimenol	71	Bifenazate	109
Bioallethrin	91	Alpha endosulphan	82	Phosmet	73
Prallethrin	104	Fenamiphos	81	Bromuconazole	71
Triflumizole	83	Kresoxim methyl	75	Pyriproxyfen	78
Imazalil	92	Dieldrin	76	Benalaxyl	107
Beta endosulfan	73	Fluazinam	72	Tebuconazole	75
Myclobutanil	100	Cyproconazole	83	Spiromesifen	74
Ethion	73	Ofurace	76	Carbosulfan	86

Table 1 Recoveries (R) of extracted pesticides

Acknowledgements

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PP06
ACT1043

OCTYLAMINE-MODIFIED GRAPHENE AS A SORBENT FOR THE EXTRACTION AND SIMULTANEOUS DETERMINATION OF ALLERGEN-FRAGRANCES, MUSKS AND PHTHALATES IN AQUEOUS SAMPLES

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Summary

Allergens, musks and phthalates are three major groups of chemical compounds that apply mostly in commercial cosmetic and household products. Some of them have been banned by regulatory agencies due to their harmful effects and therefore, their determination is deemed critical. Different techniques have been proposed to detect each compound group separately. We have developed a new method, based on the principles of solid-phase extraction using magnetic graphene modified with octylamine (MGR-C_8 NPs) for the extraction followed by analysis with a GC-MS, in order to simultaneously detect seventeen allergen-fragrances, four synthetic phthalates and five synthetic polycyclic musks, in different aqueous samples.

Introduction

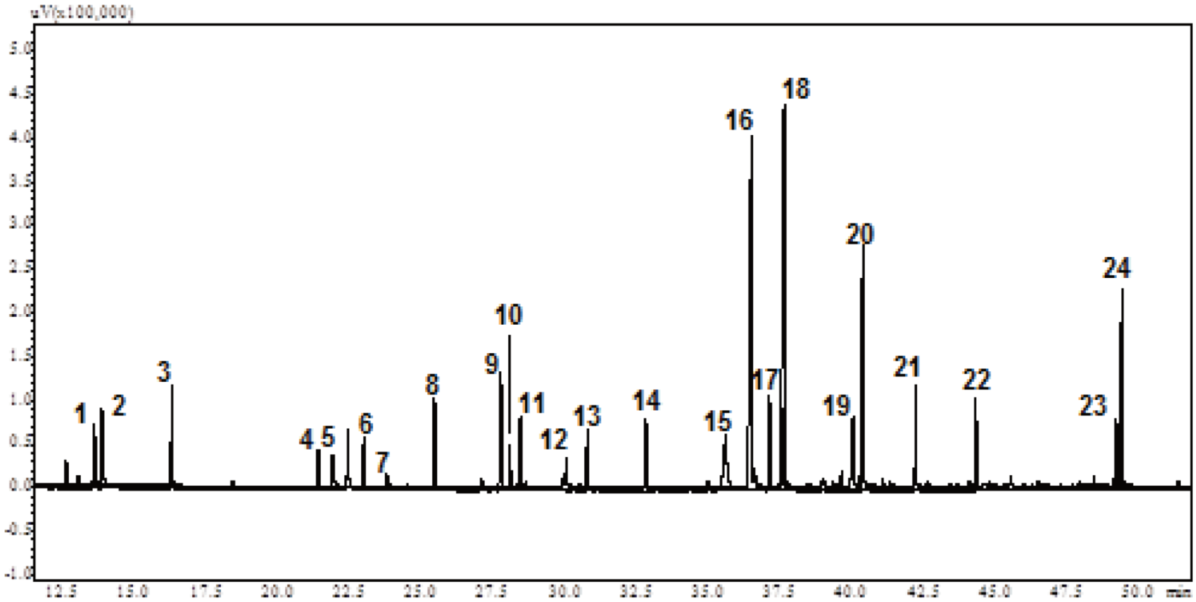
Nowadays, a wide variety of either natural or synthetic odorous compounds are used in commercially available products. Their purpose is to confer pleasant smell in order to enhance the properties of the products or to musk unpleasant odors from raw materials [1]. Different groups of compounds are used for this reason, with the leading groups being fragrances and musks. Synthetic musks through the years have replaced the naturally derived musks because of their lower cost. They have broad uses in industry ranging from common cosmetics to detergent products [2]. Another group of compounds, the fragrances can be found in perfumes, cosmetic and personal care products [3]. Finally, phthalates, the non-halogenated esters of phthalic acid, are mainly used as plasticizers for polymers, food containers and wrappers and cleaning materials [4]. Studies have proven that compounds included in these groups can cause severe allergic reactions to people with a variety of symptoms like coughing, asthma attacks and skin disorders while phthalates may cause tumor genesis to humans after exposure [5]. For these reasons, there is a need for the determination in aquatic samples. Analytical methods have been proposed for the separate detection of these compounds, at sub-μg/L levels, but none for the simultaneous determination of them. Taking advantage of the unique physicochemical properties that NPs possess compared to their bulk analogues, such as their high surface to mass ratio, sorbents with enhanced extraction properties may be synthesized [6]. In this study, we report the synthesis of magnetic graphene, modified with octylamine (MGR-C_8 NPs) as sorbents for the simultaneous determination of the above classes of compounds.

Experimental

The proposed MGR-C_8 NPs were synthesized following the modification of graphene oxide with Fe_3O_4 and subsequent modification-reduction with octylamine. The procedure followed in order to extract and preconcentrate allergen fragrances, musks and phthalates on MGO-C_8 NPs is based on the principles of solid-phase extraction. An amount of MGR-C_8 NPs was weighed out into a glass vial containing the aqueous sample. The mixture was stirred vigorously for 30 minutes using a magnetic stirrer. Subsequently, MGR-C_8 NPs were isolated from the solution with the aid of a magnetic field and the target compounds were back extracted to ethyl acetate and directly injected into a GC-MS system.

Results

Experiments which have been carried out demonstrated the suitability of the proposed method for the simultaneous extraction and determination of the analytes of interest.



Representative, chromatogram of a spiked sample after extraction. Peak assignment: 1) limonene 2) benzyl alcohol 3) linalool 4) citral 5) geraniol 6) hydroxy citronelal 7) cinamyl alcohol 8) eugenol 9) ionone 10) coumarin 11) isoeugenol 12) cashmeran 13) lilial 14) diethyl phthalate 15) amyl cinamyl alcohol 16) celestolide 17) hexyl cinamaldehyde 18) phantolide 19) galaxolide 20) tonalide 21) dibutyl phthalate 22) benzyl cinamate 23) dicyclohexyl phthalate 24) bis-(2-ethylhexyl) phthalate.

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PP07
ACT1062

A NON-DESTRUCTIVE ASSAY OF TOTAL DITHIOCARBAMATE PESTICIDES COMBINING SOLID PHASE EXTRACTION WITH GOLD NANOPARTICLES AS DETECTION PROBES

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Summary

We report on a non-destructive assay of total dithiocarbamate pesticides (DTCs) that combine an extraction step for sample clean-up and preconcentration with a sensitive colorimetric assay based on gold nanoparticles. The target DTCs are isolated from the matrix and preconcentrated by solid phase extraction onto C_{18} cartridges. The extract is then evaporated and re-constituted to methanol and mixed with an aqueous dispersion of citrate-capped gold nanoparticles (AuNPs) The AuNPs aggregate in response to DTCs coordination on AuNPs surface through gold thiolate bonds. This aggregation causes a decrease in the original absorbance of Au nanoparticles at 522 nm and the appearance of a new absorption band above 700 nm. At the same time, the colour of the solution changes from wine-red to purple-blue which can be visualized even by the bare eye at concentrations as low as 50 μg L^{-1}. The method was applied to the determination of DTCs in water samples at environmental realistic concentration levels (low and ultra-low μg L^{-1} levels) with satisfactory recoveries (81.0–94.0%), precision (5.6–8.9%) and reproducibility (9%).

Introduction

To date, only a limited number of reports have utilized nanomaterials as probes for the detection of dithiocarbamate fungicides (DTCs) employing fluorescence microscopy in home-made microfluidic devices [1] or Surface Enhanced Raman Spectrometry (SERS) [2]. Nevertheless, there is still a lack of information regarding (a) the importance of DTC structure on the analytical response since only individual substances [1,2] have been studied, (b) the potential interference effect from co-existing compounds such as other thiol containing pesticides and dithiocarbamate metabolites, and (c) the importance of non-specific interactions of the nanoparticle probes with abundant matrix components such as inorganic salts, metal ions and most importantly natural organic matter, which can modify the surface properties and aggregation of both bare and chemically modified AuNPs [3]. Therefore, simple and cost-effective methods that enable the rapid assessment of total DTC concentration without destructive routes are highly desirable.

Results and Discussion

We recorded the response of DTC pesticides individually by adding appropriate volumes of standard solutions to the AuNP suspension. We chose to assess compounds representing all three sub-classes of DTCs such as dimethyl-dithiocarbamates (Ferbam, Thiram, Ziram), ethylenebisdithiocarbamates (Mancozeb, Maneb, Metiram, Zineb) and propylenebisdithiocarbamates (Propineb) in order to realistically evaluate the possibility of using the method in the analysis of real samples were various DTCs may coexist. We observed that most DTCs, in the presence of AuNPs, exhibited absorption maxima above 700 nm suggesting that the method can be used for a general screening of the total concentration of DTCs. The signal intensity is also related to the structure of DTCs. The absorbance signal decreases according to the order: dimethyldithiocarbamates > ethylenebis(dithiocarbamates) > propylenebis(dithiocarbamates). Accordingly, the colorimetric response of each DTC follows a similar pattern. A typical visual effect with increasing concentrations of Thiram is presented in Fig. 1. The ratio of absorbance at maximum wavelength to the absorbance at the surface plasmon resonance (SPR) of the AuNPs (Amax/Aspr) also exhibits a rectilinear response with increasing DTCs concentration. The only DTC that did not trigger the aggregation of AuNPs was Zineb, possibly due to its fast hydrolysis rate in water.

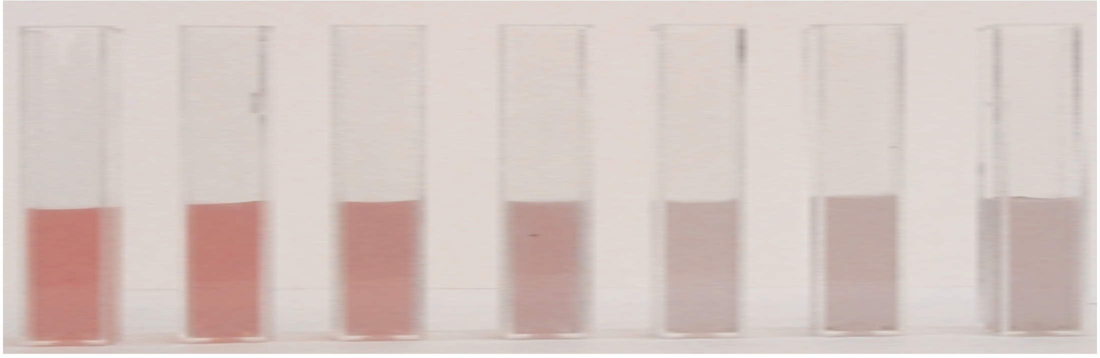

Fig. 1. Colorimetric response of AuNPs to increasing Thiram concentrations. From left to right: 25, 50, 75, 100, 125, 175 and 250 μg L^{-1}.

Conclusions

We present a simple non-destructive method combining solid phase extraction and AuNPs as detection probes for the determination of the total concentration of dithiocarbamate pesticides. Solid phase extraction relieves the sample from matrix components and most interference while affording analyte preconcentration. The utilization of ligand-free AuNP nanoprobes for signal read-out offers amplified sensitivity and satisfactory selectivity due to the high affinity of DTCs for the gold surface. In this manner, the determination of dithiocarbamates is feasible using a small volume of sample and without resorting to destructive techniques, costly instrumentation, derivatization reactions or post-synthetic modification of AuNPs surface. The successful application of the method to real samples at concentrations relevant to the maximum residue limits (MRLs) is promising to the further development of the method for on-site applications.

Acknowledgments

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European Union
European Social Fund
OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
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PP08

ACT1071

A NEW METHOD FOR THE SIMULTANEOUS DETRMINATION OF 219 PESTICIDES AND 13 NON DIOXIN LIKE PCBs IN WATER USING LCMSMS AND GCMS$^{(n)}$ SYSTEMS

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Summary

219 pesticides with distinct physicochemical properties and 13 non dioxin like PCBs were simultaneously extracted from water samples with solid phase extraction using Oasis HLB cartridges and determined using LCMSMS/GCMSMS systems for pesticides and a GCMS system for PCBs. The method was statistically validated at different concentration levels for pesticides (1, 0.1and 0.01 μgL^{-1}) and at 0.1 μgL^{-1} for PCBs (6 replicates per level), by assessing the following parameters: accuracy, precision, limit of quantification. Calibration curves were established for all analytes and regression analysis was performed at 95% confidence level. The method was found suitable for the detection and quantification of the analytes with acceptable values of the assessed parameters in most cases.

Introduction

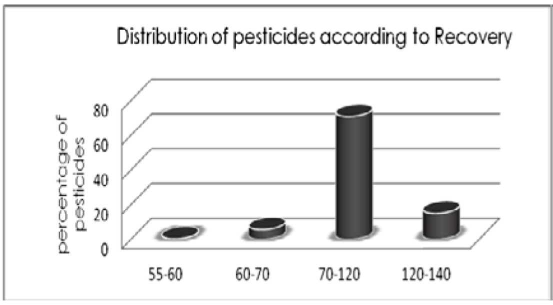
In 1998 the European Union sets limits for a number of parameters, in order to protect human health, from the adverse effects of any contamination of water intended for human consumption [1]. Regarding pesticides, the maximum accepted concentration for each individual pesticide was set at 0.1 μgL^{-1} whereas for the sum of all individuals detected and quantified at 0.5 μgL^{-1} .In 2008 decision 2008/105/EC sets for the first time environmental quality standards for priority substances including 12 pesticides· which was amended in 2013 by 2013/39/EU. According to the latest one, 10 more pesticides are now included in priority substances, whereas in the near future personal care pharmaceutical products and PCBs might be included.

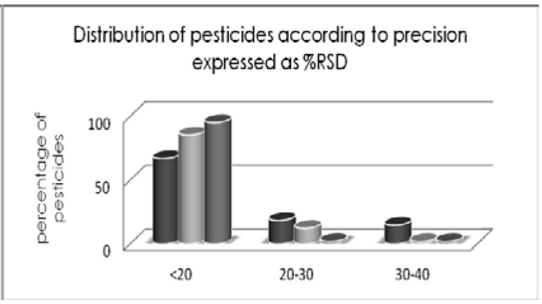
Method Development

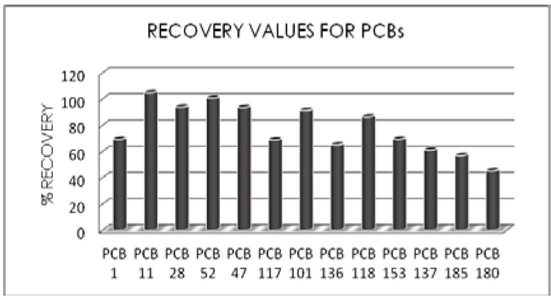
500 mL of fortified water were loaded to a previously conditioned (5 mL ethyl acetate, 5 mL methanol) and equilibrated (10 mL water) Oasis HLB cartridge (200 mg, 6mL), which was attached to a 12-position vacuum manifold, at a flow rate of 15 mL min^{-1}. Air was passed through the cartridges for 30 min to remove residual water. The cartridges were then disconnected and the adsorbed pesticides and PCBs were eluted with 5 mL of methanol and 5 mL of ethyl acetate. The eluate was evaporated to dryness with rotary evaporator, and the residue was reconstituted in 1 mL of acetonitrile and injected to LCMSMS, GCMSMS and GCMS.

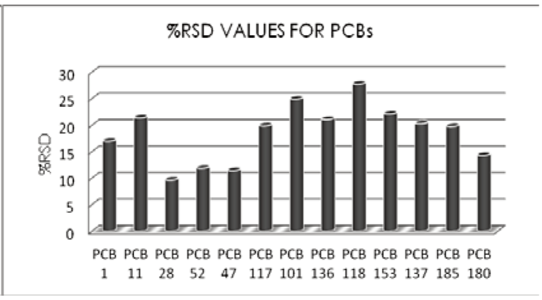
Results and Discussion

The accuracy of the method was evaluated by calculating the attained mean recovery for each analyte at each concentration level. Results from the recovery experiments were used to estimate method's precision expressed in means of relative standard deviation. The results from the recovery experiments are described briefly in the following graphs.









The quantification limit was expressed as the lowest concentration level with acceptable accuracy and precision, thus 0.01 μgL^{-1} for pesticides detected in LCMSMS, and 0,1 μgL^{-1} for PCBs and pesticides detected in GCMSMS. For all analytes (pesticides and PCBs) correlation coefficients were >0.99. The linearity was investigated in the range of 0.0035–0.6 μgL^{-1} (9 levels) for LCMSMS amenable pesticides, 0.01-0.5 μgL^{-1} (6 levels) for GCMSMS amenable pesticides and 0.01-0.05 μgL^{-1} (5 levels) for PCBs. In all cases, correlation coefficient r was >0.98.

Conclusions

A new method for the simultaneous determination of 219 pesticides and 13 non dioxin like PCBs was developed and validated. The method can be applied for monitoring purposes in water intended for human consumption or not, since it includes almost all pesticides included in priority substances with a reporting level equal or minor than 0.1 μgL^{-1}, which is the maximum accepted concentration for each individual pesticide detected and quantified in drinking water.

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PP09
ACT1078

COMPARISON OF THREE MICROEXTRACTION TECHNIQUES FOR THE RAPID AND SENSITIVE DETERMINATION OF FRAGRANCE ALLERGENS IN WATER SAMPLES

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Summary

This work presents a comparison of three extraction techniques -ultrasound-assisted emulsification microextraction (USAEME), single drop microextraction (SDME) and magnetic solid-phase extraction (MSPE)- and evaluates their efficiency in the determination of six selected fragrance allergens in water samples. Extraction parameters for each technique were optimized, and the compounds were detected and quantified using gas chromatography–mass spectrometry.

Introduction

Most personal care products (PCPs) as well as many household products contain fragrances among their ingredients. Fragrances are a group of chemicals incorporated in most cosmetic and other personal care products. The International Fragrance Association (*IFRA*) defines as any basic substance used in the manufacture of fragrance materials for its odorous, odour enhancing or blending properties (*IFRA, accessed June 2010*). The Scientific Committee on Cosmetic products and Non- Food Products (*SCCNFP*) has selected 26 compounds as likely to cause contact allergies (*SCCNFP/0017/98, 1999*). In the European Union (EU) these "suspected allergens" require labelling on cosmetic and detergent products (EU, 2009) [1, 2].

Although the main route of exposition to these cosmetic ingredients is in general, the direct application of cosmetics on the skin, the contact with water containing these fragrances should be also considered. As they are important components of daily use products, allergen fragrances are continuously introduced into the environment at high quantities.

Water Sampling and Analysis

1-methyl-4-prop-1-en-2-yl-cyclohexene 97% ((R)-(+)-limonene), 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one,≥90% (ionone) and 2-(phenylmethylene) octanal, ≥95% (hexylcinnamaldehyde)), were purchased from Aldrich (Sigma–Aldrich, Denmark,Germany). 3,7,11-trimethyldodeca-2,6,10-trien-1-ol, 97% (farnesol, mixture of isomers), 3,7-dimethylocta-2,6-dienal, 95% (citral, cis/trans) and 2-propen-1-ol, 98% (cinnamyl alcohol) were purchased from Merck (Darmstadt, Germany). The solutions were processed individually and in a mixture to optimize the operative conditions.

Water samples were collected in amber glass bottles and stored at 4°C until analysis. Water sample (distilled water) used for optimization purposes was free from target compounds contamination.

Results and Discussion

The MSPE was performed by dispersion of the Fe_3O_4@SiO_2@C18 nanoparticles in water samples with sonication. Then, the sorbent is collected by applying an external magnetic field and the analytes were desorbed by elution solvent.

USAEME is a technique based on ultrasonication which facilitates emusiflication and accelerates mass transfer between two immiscible phases without the need of a disperser solvent which greatly reduces the amount of extraction solvent used.

SDME based on the principle of distribution of analytes between a microdrop of organic solvent, on the tip of microsyringe needle and the aqueous phase (sample). The microdrop is exposed to an aqueous sample in which the analyte is extracted into the drop. After extraction, the microdrop is retracted into the microsyringe and injected into instruments for liquid or gas chromatography for analysis.

Efficiency of USAEME, SDME and MSPE was examined for the target analytes studying different parameters that could affect the performance of each method. Parameters such as elution solvent (carbon tetrachloride, chloroform, toluene, *n*-hexane and chlorobenzene), extraction time, sample and solvent volume, ionic strength and pH effect were tested.

Conclusions

All three developed and validated methods are efficient, non-expensive, simple, rapid and successfully applied for the determination of the target analytes in aqueous samples.

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PP10
ACT1101

GAS CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE-MASS SPECTROMETRY FOR THE ANALYSIS OF UV FILTERS COMMONLY USED IN PERSONAL CARE PRODUCTS

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Summary

In the present work, two sensitive and selective methodologies based on gas chromatography-triple quadrupole tandem mass spectrometry (GC-TQ/MS), liquid chromatography-triple quadrupole tandem mass spectrometry (LC-TQ/MS) are proposed for the simultaneous analysis of different classes of UV filters including benzophenones, salicylates, methoxycinnamates, p-aminobenzoic acid derivatives, and others commonly used in cosmetic products. Sensitive detection of the target analytes could be achieved working in the selected reaction monitoring (SRM) mode. GC analysis required a previous derivatization step. Both techniques were evaluated in terms of chromatographic peak shape, carryover and blank quality, linearity, precision, sensitivity and detection and quantification limits. Matrix effects were also evaluated and compared.

Introduction

To guaranty consumer's health, UV filters are essential ingredients in cosmetic formulations. The use of these additives is extended to almost the total variety of leave-on personal care products, and not only to the solar cosmetics. The high spread use at high levels together with some aspects related to toxicity has made them gaining the consideration as emerging pollutants. Although the levels in cosmetics are high, the complexity of the matrices, the prohibition of some of these substances, and the incessant introduction of amendments in the Regulation makes necessary the analysis of these personal care product (PCP) ingredients at low levels. On the other hand, environmental concerns require their control at very low levels. Therefore, the development of high sensitive and high selective analytical methods is necessary.

Material and methods

PLE extractions were performed on an ASE 150 (Dionex, Co., Sunyvale, CA, USA), equipped with 1 and 10 mL stainless steel cells.

GC TQ-MS analyses were performed using a Thermo Trace 1310-Triple Quadrupole 8000 with autosampler IL 1310 from Thermo Scientific (San Jose, CA, USA). The mass spectra detector (MSD) operated in selected reaction monitoring mode (SRM), monitoring two-three transitions per compound. A Thermo TG-5SILMS capillary column (30 m x 0.25mm i.d., 0.25mm film) was used.

LC-MS/MS analyses were carried out using a Thermo Fisher Scientific (San Jose, CA, USA) instrument consisting of an Accela 1250 HPLC pump/autosampler coupled to a Quantum Ultra triple quadrupole mass spectrometer, equipped with an heated electrospray ionization (HESI) source, working in negative and positive modes in a single chromatographic run. 10 µl of standard or extract solution were injected into the Kinetex C18 column (100 x 2.1 mm, 2.6 µm) (Phenomenex, Micron Analítca, Madrid, Spain). The analytical separation was performed using a gradient elution of water/ammonium formate (3 mM) containing 0.15% formic acid (mobile phase A) and methanol/ammonium formate (3 mM) also containing formic acid (0.15%) (mobile phase B).

Results and Discussion

The performance of both techniques was evaluated in terms of chromatographic peak shape, carryover, linear working range, precision and detection limits (LODs). For many compounds, higher sensitivity was obtained using GC-TQ MS, with LODs at the pg mL^{-1}. Nevertheless, GC analysis required derivatization step for some analytes.

Some compounds such as homosalate and ethylhexyl salicylate could not be adequately determined using LC-ESI-TQ/MS, whereas other compounds like benzophenones underwent significant improvement when using LC-TQ/MS.

Both LC and GC coupled to TQ/MS detectors have demonstrated to be adequate for the analysis of the majority of target compounds.

After chromatographic performance evaluation, both methods were tested in real sample extracts to assess matrix effects. Different experimental conditions were evaluated and different strategies are proposed to mitigate them.

Acknowledgements

This research was supported by FEDER funds and projects CTQ2010-19831 (Ministerio de Ciencia e Innovación, Spain) and CN2012/299 (Xunta de Galicia). M.V. would like to acknowledge the Gil Davila Foundation for her grant.

PP11
ACT1108

LABORATORY CALIBRATION OF 14 PESTICIDES USING POLAR ORGANIC CHEMICAL INTEGRATIVE SAMPLERS

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Summary

Laboratory experiments were conducted in order to determine sampling rates (R_S) of selected pesticides because of a lack of data on sampling efficiency and rates using polar organic chemical integrative samplers (pest-POCIS). The experiments were based on static renewal exposure under stirred conditions for 28 days. The calculated R_S ranged from 0.009 to 0.223 L day^{-1} with RSD<38%. According to the applied method, trace levels (ng L^{-1}) of the target pesticides could be determined in surface waters.

Introduction

Passive sampling is a new emerging tool for monitoring micropollutants in waters. Since the appearance of the first passive sampler for waters [1], these tools have quickly become widely applied in environmental monitoring [2]. This new technique has many advantages compared to grab sampling, such as enables the preconcentration of contaminants, increases the capability for detecting trace concentrations that otherwise requires large volumes of water [3] and enables the determination of time-weighted average (TWA) concentration over extended sampling periods. For the determination of TWA concentration, estimation of the sampling rate (R_S) for the analytes is required. The sampling rate is the volume of water from which the analyte is quantitatively extracted by the sampler per unit time [3]. The aim of this study was to determine the sampling rates of 14 pesticides using pest-POCIS based on laboratory calibration experiments.

Calibration of POCIS

Laboratory calibration experiments were conducted in order to determine the sampling rates of 14 selected pesticides using Pest-POCIS. The samplers containing 200 mg of a triphasic sorbent; Isolute ENV+:Ambersorb 572, 80:20 (w/w), dispersed on S-X3 Bio Beads, enclosed between two polyethersulfone membranes (130 μm x 0.1 μm pore size). The experiments were performed on the basis of static renewal exposure of pest-POCIS under stirred conditions for time periods up to 28 days. The water was renewed every day with freshly water spiked with pesticides. SPE cartridges filled with the Pest-POCIS sorbent were eluted using 30 mL of CH_2Cl_2/ MeOH/toluene (8:1:1 v:v:v). Chromatographic analysis was performed using a QP 2010 GC-MS (Shimadzu). Pesticides recoveries ranged between 71 and 120 % with RSD<18%. The method detection (LODs) and quantification limits (LOQs) were determined using a S/N of 3 and 10, and ranged between 0.5 to 47 ng L^{-1} and 2 to 157.5 ng L^{-1}, respectively.

Results and Discussion

The uptake in POCIS for most of the studied pesticides follows a linear pattern throughout the 28 days exposure, except for fipronil, which uptake follows a linear pattern until 21 days (Figure 1). The correlation coefficients of the linear regressions (R^2) ranged between 0.9294 and 0.9933 indicating the strong linear relationship between the mass of pesticides accumulated in POCIS and time, in every case. The calculated sampling rates ranged from 0.009 L day^{-1} for azoxystrobin to 0.223 L day^{-1} for metribuzin with RSD<38%. Using the described analytical procedure, trace levels (ng L^{-1}) of the target pesticides could be determined in surface waters.

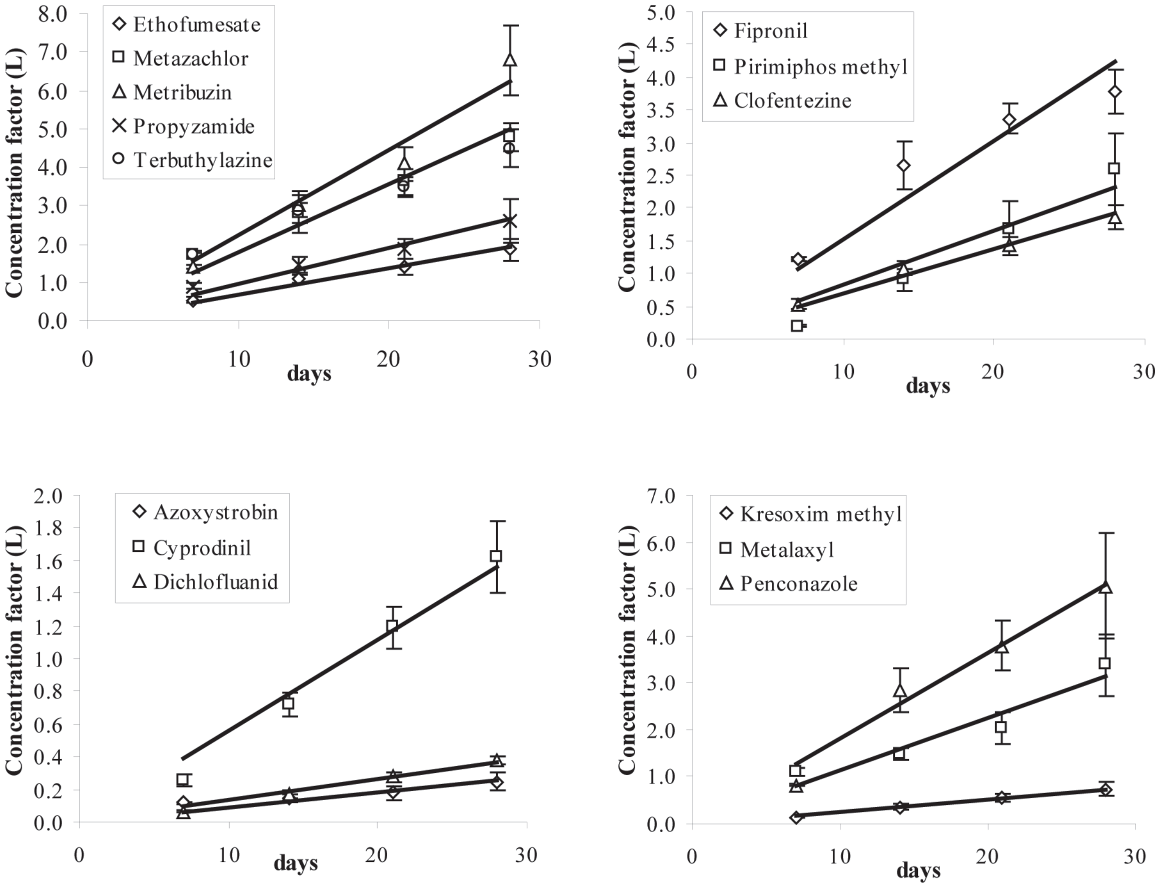

Figure 1. Uptake kinetic curves of target pesticides in pest-POCIS for 28 days period.

Acknowledgments

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "Thessaly- Mainland Greece and Epirus-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

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PP12

ACT1114

UTRASOUND-VORTEX-ASSISTED DISPERSIVE LIQUID–LIQUID MICROEXTRACTION COUPLED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR SIMULTANEOUS ANALYSIS OF HERBICIDES AND FUNGICIDES IN WATER

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Summary

An ultrasound-vortrex-assisted dispersive liquid–liquid microextraction (USVA-DLLME) method combined with liquid chromatography-tandem mass spectrometry was developed for the simultaneous determination of several herbicides and fungicides in water samples. The variable of interest in the USVA-DLLME method, such as the type and the volume of extraction solvent and disperser solvent, pH, extraction time and salt effect, was studied and optimized. First, screening experiments were performed for selecting the variables which significantly affected the extraction procedure. Afterwards, the significant variables were optimized using response surface methodology (RSM) based on central composite design (CCD). Under optimum extraction conditions, recoveries of all target analytes are found to be in the range of 70% to 120%. The proposed USVA-DLLME LC-ESI-MS/MS analytical method was shown to be linear (r^2> 0.99) over the studied range of concentrations, exhibiting satisfactory precision (RSD% < 20%) and reaching limits of detection between <20 ng L^{-1}. The applicability of the method was assessed with the analysis of real river and lake water samples. The validated USVA-DLLME sample treatment in combination with LC–ESI-MS/MS has demonstrated to be a sensitive, selective and efficient method to determine herbicides and fungicides in waters at ultra-trace levels.

Introduction

In recent years, several novel microextraction techniques are being developed in order to reduce the analysis step, increase the sample throughput, improve the quality and the sensitivity of analytical methods and solve the problems associated with the analysis of small size samples [1]. Among these, methods based on ultrasound and vortex assisted liquid microextraction have been received considerable attention [2-4]. These last pre-concentration approaches reduce the volume of organic solvent required and simultaneously improve the extraction efficiency with the aim of a dispersive solvent. According to this approach we propose a protocol based on USVA-DLLME for the determination of herbicides and fungicides in waters.

Instrumentation

The chromatographic analysis was performed using a liquid chromatographic system coupled to a triple quadrupole mass spectrometer by an electrospray interface (LC–ESI–MS/MS). The separation was performed using a HyPURITY C18 column (50 x 2.1, 3μm particle size) thermostated at 40 °C. A linear gradient of mobile phase A consisting of a 90:10 water:methanol mixture containing 5 mM of ammonium acetate and mobile phase B a 90:10 water:methanol mixture containing 5 mM of ammonium acetate 10:90 water:methanol containing 5 mM ammonium acetate was employed. The mobile phase flow rate was maintained at 0.2 mL min^{-1} and the injection volume was 10 μL.

Results and discussion

In this study, between two solvents (acetonitrile, methanol) which have the property of miscibility in organic and aqueous phase, methanol was chosen as a disperser solvent. Among solvents which have the abillity to extract organic compounds (octanol, tolouene, dichloromethane, chloroform, n-hexane), chloroform was chosen as an extraction solvent. Chloroform's density being lower than that of water is an important factor because in DLLME, the extraction solvent is collected in the conic bottom of the test tube. Furthermore the volume of the extraction and the dispersive solvent, the salt concentration and the ultrasonic time were studied, so that the optimum extraction conditions could be deduced.

Under the optimum conditions the recovery of 34 herbicides and 31 fungicides studied, ranged from 70 to 120% and relative standard deviations (RSDs) was <20% at the concentration level of 0.1 μg/L which is the limit for residues from a single pesticide in drinking water set by the Directive 98/83/EC [5].

Conclusion

This study illustrated the successful application of the USVA-DLLME coupled to LC-ESI-MS/MS for the simultaneous determination of several herbicides and fungicides at low concentration levels in environmental water samples. Overall, the proposed method is simple, fast and effective and seems an attractive alternative approach for determination of pesticides in waters. Furthermore, it is superior to conventional extraction methods in terms of speed and has more eco-friendly (or green) characteristics.

Acknowledgements

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PP13
ACT1028

DEVELOPMENT AND SINGLE-LABORATORY VALIDATION OF A NEW HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC MULTI-PESTICIDE METHOD OF ANALYSIS OF COMMERCIAL FORMULATIONS CONTAINING ACETAMIPRID, AZOXYSTROBIN, FLUOMETURON, DIFLUBENZURON AND TOPRAMEZONE

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Summary

A multi-pesticide (MP) method was developed and single laboratory validated for the quality control of pesticide formulations containing acetamiprid, azoxystrobin, fluometuron, diflubenzuron and topramezone as active ingredients. A reversed phase high performance liquid chromatographic method with a reversed phase monolithic column was used. The performance characteristics (linearity, specificity, precision and repeatability) of the method satisfied acceptability criteria.

Introduction

A multi-pesticide method is one that determines the active ingredient content of each of a range of commercial pesticide formulations, using the same chromatographic column and elution system [1-5]. In the case of MP methods samples are analysed separately from each other. In one sample, no more than two known active ingredients have to be separated from the impurities of the technical material and components of formulations. In the case of MP methods, instrumental determination is carried out with a notably limited range of liquid chromatographic columns and elution systems, in contrast to the official CIPAC and/or AOAC methods. The present study sought to develop a fast and simple reversed phase multi-pesticide high performance liquid chromatographic method (RP-MP HPLC) method for the determination of five active ingredients (acetamiprid, azoxystrobin, fluometuron, diflubenzuron and topramezone) widely used in Greece. One of the greatest advantages of the proposed method was the use of a rod monolithic column which involves in a considerable reduction in chromatographic analysis time and solvents consumption.

Experimental

The HPLC system used was a Shimadzu LC-10ADVP (Shimadzu, Japan), equipped with a column oven (CTO-10ASVP), a degasser (DGU-14A), an autosampler (SIL-10 ADVP), and a UV-Vis diode-array detector (SPD-M10AVP). The column used for the analysis was a reversed-phase monolithic Chromolith RP-18e (100 x 4.6 mm). The optimal mobile phase for separation of all the above mentioned a.i. was acetonitrile:water (60:40 v/v). The flow rate of the mobile phase was maintained at 0.4 ml min^{-1}. Analysis time varies depending on the active ingredient analyzed with a minimum of 3.87 minutes for topramezone and a maximum of 10 minutes for azoxystrobin. The retention times for each active ingredient were: acetamiprid: 4.08 min, azoxystrobin 6.4 min, fluometuron 4.9 min, diflubenzuron 8.0 min, topramezone 3.87 min.

Results and Discussion

Both CIPAC [6] and EU guidelines [7, 8] recognize that it is necessary to evaluate the following analytical parameters.

Repeatability of injections:

The repeatability of injections was tested for each active ingredient separately, using the medium calibrated working solution following the selected chromatographic conditions. In case the relative standard deviation (RSD) of the peaks area is ≤ 1%, the repeatability test is considered acceptable, as it was in our case.

Specificity: The ability of the analytical method to distinguish the analyte to be determined from degradation products, metabolites or known additives was investigated. In that case specificity was assessed by examination of peak purity using diode array detection system.

Linearity of response: The linearity of response was determined by analysing in duplicate working solutions at different concentrations for each of the tested active ingredients.

Precision of the method: Data obtained from the analysis in duplicate of samples from different batches of each pesticide was used to calculate the experimental RSD_r values. The acceptability of the results should be based on the modified Horwitz equation.

Analysis of commercially available pesticide formulations

In order to assess the applicability of the method in actual commercial pesticide formulations analysis, different batches of commercially available pesticide samples containing the above mentioned active ingredients were analysed. The results obtained from duplicate analysis of the samples from different batches of each active ingredient were compared with the reference values declared by the manufacturers using the paired t-test.

Conclusions

The use of multi-pesticide methods is a practical alternative for the analysis of pesticides. The total operation costs including the eluent consumption, as well as the total analysis time are significantly reduced. Further work will aim at the incorporation of the greatest number possible pesticides in the method.

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PP14
ACT1030

IN-HOUSE DEVELOPMENT OF A POLYCLONAL ANTIBODY FOR THE IMMUNOANALYSIS OF THE POLLUTANT 2,4,6-TRICHLOROPHENOL

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Summary

In this work we present the in-house development of a polyclonal antibody for 2,4,6-trichlorophenol (TCP), a pollutant widely spread in soil and water and possibly carcinogenic to human. 2,4,6-trichlorophenoxyacetic acid (TCPA) was used as starting material for preparing the immunizing hapten. Immunochemical functionality of the antisera was evaluated with titer- and displacement experiments in a (biotin/streptavidin) ELISA system, in which an in-house prepared biotinylated derivative of TCPA was used as the immobilized hapten. The ELISA-titer values obtained were very high (~1:100,000). As shown by the ELISA-displacement experiments, the antibody cross-reacted with the fungicide 2-methyl-4-chloro-phenoxyacetic acid (MCPA), but practically did not cross-react with 2,4,5-trichlorophenol. Due to its discrete immunochemical profile, this antibody can be used as an additional research tool for detecting TCP in environmental and other samples *via* immunoanalytical systems, including various immunosensors.

Introduction

Due to their extensive use -among others as pesticides- chlorophenols are widely spread in the environment and considered as severe pollutants predominantly of natural aqueous sources [1-2]. Significant levels of chlorophenols (2 - 5 μg/L) have been detected in the urine of a great number of people, while urinary excretion from occupationally exposed individuals is even higher [3]. Among other chlorophenols, 2,4,6-trichlorophenol (TCP) has been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer [1,3]. Immunoanalysis of TCP, structurally related micropollutants and/or other pesticides is considered an interesting alternative to instrumental analysis [3-9]. Development of specific antibodies is a challenging step in TCP immunoanalysis, since TCP is a small organic molecule (hapten) that should be conjugated to a carrier protein in order to elicit an immune response [10]. To our knowledge anti-TCP antibodies are not easily available and most TCP-immunoanalytical assays are based on in-house developed antibodies. In the present work we describe the in-house development and ELISA-evaluation of a polyclonal antibody for TCP, which may be applied as an additional research tool to the immunoanalysis of this pollutant.

Materials and Methods

TCPA (Sigma-Aldrich) was conjugated to the carrier protein keyhole limpet hemocyanin (KLH, Thermo Scientific) through a suitable spacer, by using the glutaraldehyde method [10-12]. New Zealand white rabbits were immunized with the afore mentioned KLH-conjugate following a well-established procedure [13]; care of animals was in accordance to the corresponding European legislation. The antisera were evaluated in a (biotin/streptavidin)-ELISA, as follows: <u>ELISA titration experiments</u>: ELISA microwells were coated with streptavidin (Sigma, 1 μg/mL in coating buffer, overnight, 37°C). The following day, the wells were washed with 0.01 M PB, pH 7.4, blocked with a 2% BSA solution in PBS-T (1 h, room temperature), washed with PBS-T and incubated with an in-house prepared biotinylated derivative of TCPA (100 ng/mL in diluting buffer A, 2 h, 37°C); then, the wells were washed as above described, incubated with serial dilutions of the anti-TCP antisera (four consecutive bleedings) in diluting buffer A (2 h, 37°C), washed, incubated with anti-rabbit IgG/HRP (Sigma-Aldrich), diluted 1:3,000 in diluting buffer A (2 h, 37°C), washed, and finally incubated with ABTS (30 min, 37°C). The OD was measured (405 nm) in a microtiter plate reader (Sirio S, SEAK) and the titer curves were plotted. <u>ELISA displacement experiments</u>: ELISA microwells were coated, blocked and incubated with the biotinylated derivative of TCPA as described above. Then, the wells were washed and incubated (2 h, 37°C) with a 1:1, v/v, mixture of the anti-TCP antiserum, suitably diluted in diluting buffer A, and a standard solution (1 - 20 μg/mL) of either TCP or of putative cross-reacting molecules (preincubated overnight, 4°C); all standard solutions were prepared by diluting a 10 mg/mL stock solution in ethanol with diluting buffer B. Afterward, the procedure described in the ELISA-titration experiments was followed and finally the displacement curves were plotted. [ELISA Buffers: *Coating buffer*: 0.01 M phosphate buffer (PB), pH 7.4; *Washing buffer* (PBS-T): 0.01 M PBS, pH 7.4, containing 0.05 % (v/v) Tween-20; *Diluting Buffer A*: PBS-T containing 0.2 % (w/v) BSA; *Diluting Buffer B*: PBS-T containing 0.2 % (w/v) BSA and 10 % (v/v) ethanol].

Results and Discussion

In this work we present the in-house development of a polyclonal antibody for TCP, a pollutant possibly carcinogenic to human and widely spread in the environment for which there are no easily available antibodies. TCPA was used as starting material for preparing the immunizing hapten. Immunochemical functionality of the antisera was evaluated with titer- and displacement experiments in a (biotin/streptavidin)-ELISA, using an in-house prepared biotinylated derivative of TCPA as the immobilized hapten. The ELISA-titer values obtained were very high (~1:100,000). The antibody developed could recognize TCP at all concentrations tested in the ELISA-displacement experiments (1-20 μg/mL). Interestingly, this antibody cross-reacted with the fungicide MCPA, but practically did not cross-react with 2,4,5-trichlorophenol; thorough cross-reactivity studies with other polychlorinated phenols and various pollutants, structurally similar with TCP, are currently underway. Due to its discrete immunochemical profile, which may be attributed to the structure of the corresponding immunizing hapten, this antibody can be used as an additional research tool for detecting TCP in environmental and other samples *via* immunoanalytical systems, including highly sensitive immunosensors [14-16].

Acknowledgements

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PP15
ACT1059

CYANOWATER - CYANOTOXINS IN FRESH WATERS, ADVANCES IN ANALYSIS, OCCURRENCE AND TREATMENT

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Summary

Cyanotoxins are compounds produced by numerous cyanobacterial species, with different chemical structures and physicochemical properties. Due to their widespread distribution and high toxicity, they pose a threat to public health. The objective of the CYANOWATER PROJECT in the frame of ARISTEIA, is to fill current research gaps in the areas of cyanotoxin analysis in environmental samples, the presence and characterization of toxin-producing cyanobacteria and their toxins in fresh water ecosystems and advanced methods of water treatment for cyanotoxins removal.

Introduction

Cyanotoxins comprise a large group of potent toxins (e.g. peptides, alkaloids, amino-acids) produced by cyanobacteria that are common in surface waters and tend to form blooms in lakes and other freshwater bodies. Exposure through drinking or bathing in waters containing cyanotoxins can seriously affect human and animal health: cyanotoxins may damage the liver, kidneys, central nervous system or may cause respiratory and gastrointestinal problems. Cyanotoxins are now recognized as an important emerging threat for water resources by WHO, USEPA and other authorities.

The Present Reseach Project cofounded by Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – ARISTEIA, titled: "CYANOWATER - CYANOTOXINS IN FRESH WATERS, ADVANCES IN ANALYSIS, OCCURRENCE AND TREATMENT", is divided into various working packages including the development of analytical methods, treatment processes and biological tools for the assessment of cyanotoxins' ecological footprint in the environment.

Working packages are as follows:

WP1: Development of new advanced analytical methods for the determination of cyanotoxins in environmental samples. Development of a multi-class cyanotoxin method of analysis, where cyanotoxins belonging to different chemical groups (microcystins, cylindrospermopsin, anatoxin-a, saxitoxins and BMAA) will be detected and quantified in a single analysis with use of LC-MS/MS.

WP2: Identification of the toxin-producing cyanobacteria species in freshwater bodies. Monitor the occurrence and toxic bloom forming cyanobacteria in freshwaters which have high cyanotoxin diversity and/or density by combining the diversity by standard microscopic analysis and phylogenetic analysis after PCR amplification of the 16S rRNA gene with cyanobacterial-specific primers.

WP3 : Use of Advanced Oxidation Processes (AOPs) for the detoxification of water containing cyanotoxins. Degradation/detoxification of cyanotoxins in water with use of AOPs based on the $OH^{\cdot}$ radical, to include toxins that are not yet studied (anatoxin-a, BMAA, cylindrospermopsin, microcystins) or AOPs that have not yet been applied to cyanotoxins. Synthesis and characterization of novel hybrid Titanium Dioxide-Polyoxometalate (TiO_2-POM) nano- catalysts for the photocatalytic degradation of cyanotoxins and intermediate products identification during the process.

WP4 : Exploitation and dissemination of results. Exploitation and dissemination the project's results to the research community, water authorities, policy makers and stakeholders in order to foster public health protection and better management of cyanobacteria and cyanotoxins.

Progress beyond state-of-the-art

The proposed project will fill these gaps by:

-Upgrading of already existing methods for MCs, CYN and anatoxin-a in terms of both number of variants included and matrices analyzed (water, fish tissue, plant tissue).

-Development of methods for the determination of emerging cyanotoxins such as BMAA for which there is no method available for its determination at low levels in water.

-Development of fast, accurate and sensitive new multi-toxin methods for the simultaneous determination of as many as possible toxins of different groups "in one run".

-Development of a method for the determination of unknown MCs (where no standard compounds are available).

Regarding the occurrence of cyanotoxins freshwater bodies, the project will investigate their occurrence in a large number of lakes that not been studied so far and associate the presence of toxins with the prevalence of toxin-producing cyanobacterial species. These studies will increase our understanding of the prevalence of (invasive) cyanobacteria and emerging toxins in the European region that is likely to increase in the next years also due to climate change.

Novel Advanced Oxidation Processes (AOPs) using combinations of polyoxometallate (POM) and TiO_2 photocatalysts under UV light will be studied for the degradation and detoxification of cyanotoxins in water. These studies are expected to lead to a better understanding of the mechanisms of degradation of the diverse cyanotoxin molecules that usually proceed through hydroxyl radicals. Ultimate goal of the proposed research is the development of an efficient water treatment method for cyanotoxin removal.

Research Team

The research team consists of:
Dr. Anastasia Hiskia – Research Director – CCPL-NCSR Demokritos, P.I.
Prof. Dionysios D. Dionysiou - Department of Civil and Environmental Engineering, University of Cincinnati, OH, USA.
Prof. Maria Moustaka-Gouni - Department of Botany, School of Biology, Aristotle University of Thessaloniki, Greece.
Prof. Konstantinos A. Kormas - Department of Ichthyology & Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Greece.
Dr. Theodoros Triantis, CCPL-NCSR Demokritos.
Dr. Triantafyllos Kaloudis, EYDAP SA, as expert in the field, (not representing EYDAP in this project)
Dr. Christophoros Christophoridis, Post-doc Fellow
Dr. Theodora Fotiou, Post-doc Fellow
Ms Sevasti Zervou, PhD Student

Acknowledgments

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PP16
ACT1079

COMPARISON OF LLE AND SPE FOR THE DETERMINATION OF LIDOCAINE IN URINE SAMPLES BY GAS CHROMATOGRAPHIC (GC-MS)

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Summary

Two simple, fast and inexpensive procedures, a liquid–liquid extraction and a solid phase extraction, were applied and compared for the determination of lidocaine in urine samples in order to study their efficiency and applicability.

Introduction

Lidocaine is a widely used amide-type local anaesthetic. It also has antiarrhythmic effects and is used as a therapeutic agent in the treatment of cardiac disorders. The various addictive substances and their metabolites are commonly detected in urine at high concentrations and for this reason are the sample of choice for intensifying analyzes. Also, this substrate can be used as such in many reactions because is freedom to a large extent on the presence of interfering substances such as proteins and lipids.

Materials and Methods

Diethyl ether, 2-propanol, methanol, dichloromethane and ethyl acetate were reagent grade solvents. Lidocaine were supplied by sigma.

Urine samples were collected in polypropylene containers and stored at -20 °C when not in use. For the LLE method, urine (5 mL) was pipetted into a glass centrifuge tube containing internal standard. Five hundred milligrams of a solid buffer (NaHCO3:K2CO3, ratio of 2:1) were added in order to reach a pH of approximately 9–10. An ethyl ether : isopropanol (9:1) solution (3 mL) was added and the mixture was shaken in an oscillating table for 10 min. After centrifugation (5 min at 2000 rpm), the organic phase (top layer) was transferred to a glass tube containing approximately 0.5 g of anhydrous sodium sulfate. The solvent was dried at 40 °C in a heating module under a stream of nitrogen. The residue was reconstituted with methanol (100 mL), vortexed and 1 mL was injected into the GC-MS instrument.

For the SPE method, in 2 mL urine sample was added 3 mL buffer (phosphate buffer, 0.1 M, pH = 9.5). The sample was vortexed for 30 sec followed by centrifugation for 10 min at 4000 rpm. The supernatant was passed through a C18 column and remain there for about 10 min. After trapping the analytes in the adsorbent material, followed by elution with a mixture resulting solutions dichloromethane: isopropanol (9:1 v / v) (2x6 mL). The final eluate was evaporated with nitrogen to dryness and redissolved to a final volume of 0.1 mL with ethyl acetate prior to analysis of the GC-MS.

Results and Discussion

A statistical comparison between SPE method and LLE was performed to verify the agreement of the two results at 95% confidence level (N=5). For simplicity, the variance was considered to be constant among the various samples. The samples were fortified at 10 µg L-1 to ensure the robustness of the measurements. Neither the individual F-test nor the T-test revealed that statistically significant differences, were not found.

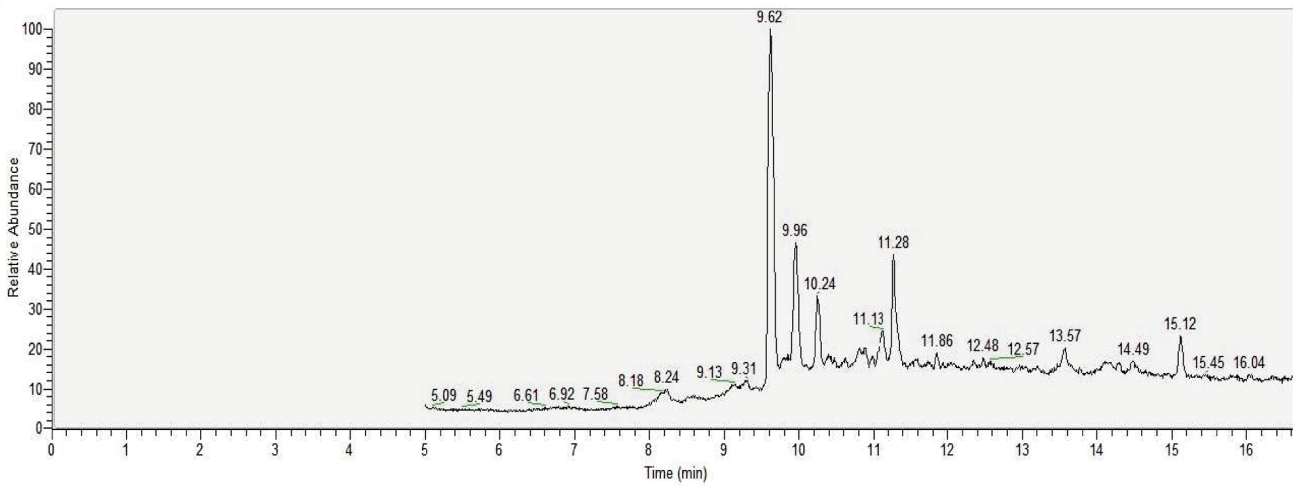

Fig.1:A representative chromatogram of a spiked urine sample after LLE extraction.

Conclusions

The proposed methods were suitable for the analysis of the target analyte in urine samples.

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PP17
ACT1082

DETERMINATION OF NICOTINE IN SALIVA SAMPLES USING SINGLE DROP MICROEXTRACTION COMBINED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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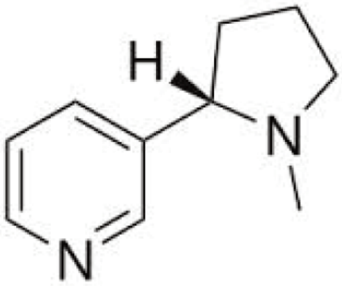
Summary

In this work a simple, sensitive, and inexpensive single-drop microextraction (SDME) method has been developed for the extraction of nicotine in human saliva samples. Then GC-MS was used for quantification of this alkaloid. Experimental parameters influencing the extraction efficiency of alkaloids including extraction organic solvent, pH of sample solution, stirring rate, salting-out, and extraction time were studied with the aid of liquid chromatography-mass spectrometry.



Chemical structure of nicotine

Introduction

Nicotine and anabasine are the main pharmacologically active alkaloids found in tobacco [1]. These alkaloids are absorbed in the human body through skin and lungs [2]. Due to the presence of nicotine in cigarette at relatively high concentrations, its addictiveness properties, and also the primary precursors for the highly carcinogenic tobacco-specific nitrosamines, make these chemicals important from a public health standpoint [1]. Nicotine and its major metabolite cotinine can be found in urine, blood and saliva samples [2, 3]. Single-drop microextraction (SDME) was developed as a sample preparation method due to its simplicity, efficiency, low cost, negligible volume of solvent used and excellent sample cleanup ability [4].

Materials and Methods

Sample preparation

A microsyringe with an angled-cut tip was used for the extraction procedure. The sample vial, containing 5mL (4.5mL water and 0.5mL saliva) of sample solution and analyte was sealed with a screw cap with PTFE silicon septum. Before extraction, a known volume of extraction organic solvent (1μL) was withdrawn into the microsyringe. The microsyringe was fixed above vial with a clamp. The needle of the microsyringe was inserted through the septum of the sample vial and directly immersed into the sample solution. The plunger of the microsyringe was depressed to expose the extraction organic solvent drop to the sample solution. Then the solution was stirred for 20 min using a magnetic stirrer bar. After the extraction, the drop was retracted back into the microsyringe and injected into the GC-MS for further analysis.

Results and Discussion

The effects of several important parameters influencing the extraction efficiency such as extraction organic solvents, pH of sample solution, stirring rate, salting-out, and extraction time were studied. After optimization the proposed method was validated in terms of accuracy, precision, linear range, limit of determination, limit of quantitation, and results demonstrated the suitability of the proposed method for the extraction and determination of the target analyte.

Conclusions

A single-drop microextraction method followed by GC-MS was developed for the determination of nicotine in human saliva samples. The proposed method is simple, inexpensive, sensitive, and accurate. The results from validation indicate the proposed method can be applied for routine the determination of nicotine in saliva samples.

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PP18
ACT1064

PASSIVE SAMPLING: A TOOL FOR ASSESSMENT BROAD SPECTRUM ORGANIC MICROPOLLUTANTS IN STRYMONAS RIVER, N. GREECE

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Summary

Three types of passive samplers (SPMDs, POCIs-Pharm, POCIs-Pest) were employed along Strymonas River, N. Greece, for the assessment of hydrophobic and hydrophilic organic micropollutants along the river. A multi-residue analytical method based on gas chromatography-tandem mass spectrometry was used for the determination of seventy organic compounds (pesticides, alkylphenols, benzotriazoles, pharmaceuticals, PAHs and PCBs) in the framework of Water Directive 2000/60/EC [1] aiming at the good chemical status and protection of surface waters. The occurrence of organic micropollutants in Strymonas River during three sampling campaigns and possible impacts from pollution sources are discussed.

Introduction

Organic micropollutants entering the aquatic environment may pose severe threat on aquatic ecosystem and human health. According to the Water Framework Directive (WFD), monitoring of water quality is necessary to ensure the maintenance of the good status of surface waters. WFD introduces a list of 33 priority substances with defined environmental quality standards (EQS) (EC 2000; EC 2008) [1-2]. In addition to these priority pollutants, there is also concern for various hazardous chemical compounds that may be released into aquatic environment (EC 2006) [3] and consequently the identification of specific pollutants at the river basin.

Most conventional environmental screening methods for pollutants in water involve grab sampling. The analysis of grab samples provides only a snapshot of the levels of pollutants at the time of sampling. However, the concentrations of pollutants could fluctuate very rapidly depending on occasional releases or environmental conditions. In this case, frequent sampling is required that is time consuming, laborious and has high cost. Moreover, conventional sampling techniques are inherently limited in their ability to provide a holistic exposure assessment of pollutants since they lack the capability to integrate sampling through time and they are often insufficient to detect and quantify ultra-trace levels of pollutants in water.

Passive sampling has gained growing attention as a sampling approach that overcomes these limitations. It is based on the free flow of pollutants from the sampled medium to a receiving phase of the sampling device. Passive samplers offer the ability to integratively sample a wide range of environmental pollutants over an exposure period, permit the sequestration of residues from episodic events, not always detected with grab sampling, allow the preconcentration of ultra-trace, yet toxicologically relevant, compounds for chemical analysis and biologically based assays, mimic biological uptake while potentially avoiding problems implicit with the use of aquatic organisms, and finally offer significant handling, use and economy benefits compared with conventional grab sampling techniques [4-5].

Water Sampling and Analysis

Strymonas is a transboundary river in the Balkan Peninsula (runs through Bulgaria, Serbia, Fyrom and Greece). The river is responsible for draining approximately 17,152 km^2 and can be considered as representative of river basins exposed to the impacts from agricultural, industrial and urban activities. Moreover, it is an excellent ecosystem and certain sites are included into the EU 'Natura 2000' network. Three types of passive samplers: POCIS-Pharm, POCIS-Pest and SPMDs were deployed at four sites along Strymonas River for a period of four weeks. Three sampling campaigns were conducted in the year 2013 in order to cover changes in hydrological conditions (high-low flow) as well as possible impacts from seasonally dependent inputs (ie agriculture runoff).

A multi-residue analytical method based on gas chromatography-tandem mass spectrometry was employed for the simultaneous analysis of a broad spectrum of hydrophobic and hydrophilic organic micropollutants in the framework of Water Directive 2000/60/EC [1] aiming at the good chemical status and protection of surface waters.

The compound list included seventy organic micropollutants: 48 pesticides (organochlorinated, organophosphorous, triazines, carbamate and urea compounds), 9 PAHs, 6 PCBs, 4 phenols, 2 pharmaceuticals etc. Thirty three pollutants were identified in Annex X and Annex III of the Directive 2008/105/EC [2].

Results and Discussion

Various organic micropollutants were determined in Strymonas river (organochlorinated, organophosphorous, carbamate and urea pesticides, PAHs, pharmaceuticals). Pesticides are the most ubiquitous pollutants.

Spatial and temporal variations were observed suggesting different inputs from pollution sources along the river as well as influence of hydrological conditions.

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PP19
ACT1106

INCLUSION COMPOUNDS OF AUXINS IN CYCLODEXTRINS

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Abbreviations

indole-3-butyric acid	IBA
4-chlorophenoxyacetic acid	4-CPA
2-methyl-4-chlorophenoxyacetic acid	MCPA
2,4-dichlorophenoxyacetic acid	2,4-D
2,4,5-trichlorophenoxyacetic acid	2,4,5-T
2-napthoxyacetic acid	2-NOA
2-naphthaleneacetic acid	2-NAA
1-naphthaleneacetic acid	1-NAA
α-, *β*-, *γ*- cyclodextrin:	*α*-, *β*-, *γ*-CD

The low aqueous solubility of the auxins causes major problems in their administration. In order to overcome these problems water soluble salts of auxins are used or auxins are dissolved in ethyl alcohol (or another organic solvent). It has been shown that ethyl alcohol may have modifying effects in auxin action even at low concentration [1]. In addition, the alcohol evaporates during use, making the solution more and more concentrated and thus stunted or abnormal growth is observed.

An alternative way to increase the water solubility of the auxins is via their encapsulation inside suitable amphiphilic molecular capsules. Cyclodextrins (CDs) are oligosaccharides consisting of six, seven or eight α-(1→4)-linked D-glucopyranose units called *α*-, *β*- or *γ*-CD respectively, having a toroidal shape with a lipophilic inner cavity and a hydrophilic outer surface. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their hydrophophic cavity and thus exerting a profound effect on the physicochemical properties of guest molecules [2]. Moreover, due to the weak non-covalent interactions between the host and the guest molecules, these supramolecular complexes are not fixed or permanent but rather in a dynamic equilibrium with their free components in solution, thus offering a slow release of the guests and sustaining their action.

CDs are used in agriculture, food, pharmaceuticals, cosmetics, environment protection and it is generally acceptable that the inclusion complex formation of auxins with CDs could improve their bioavailability, enhance their stability and reduce considerably the side effects following administration. The low cost, biocompatible and effective degradation makes *β*-CDs the best candidates for such molecular encapsulants. Commercial available derivatives of *β*-CDs are also very interesting because of their increased aqueous solubility and inclusion capacity.

In the present work, studies on the molecular encapsulation of eight auxins (1-NAA, 2-NAA, 4-CPA, MCPA, 2-NOA, IBA, 2,4-D and 2,4,5-T) have been extensively reviewed. The molecules that have been used as hosts are mainly native *β*-CDs, methylated or hydroxypropyl *β*-CD derivatives and in a few cases *α*- or *γ*-CDs. The combined use of different analytical techniques has provided a complete characterization of the inclusion complexes. The structures of some of these complexes have been accurately determined in crystalline state by X-ray crystallography. NMR, spectrofluorometric and phase solubility studies have given solid evidences about the formation of the complexes in solution and estimations of the binding constants. DSC and FT-IR studies have checked the formation of a true solid inclusion compound and they have determined the stoichiometry of the formed complexes in the solid state.

This inclusion complexation could be regarded as an important step in the design of novel formulations of auxins with enhanced chemical properties and the gathered results can be regarded as an essential step to the development of controlled release agricultural formulations containing auxins. In addition, the inclusion of the auxins inside the CDs' hydrophobic cavity can be regarded as a simple prototype for ligand-enzyme systems, involving mainly van der Waals and hydrophobic interactions and therefore their structural study can provide some insight about the auxins binding into the pockets of the protein receptors.

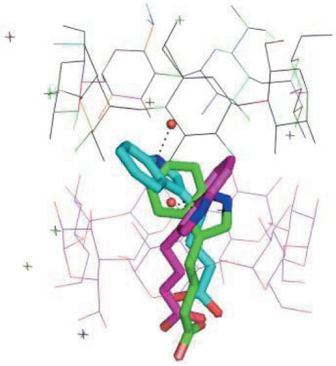

Figure 1: IBA/β-CD inclusion complex

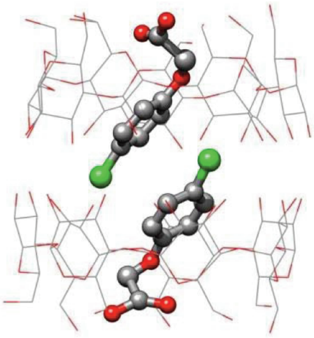

Figure 2: 4-CPA/β-CD inclusion complex

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PP20

ACT1117

QUANTITATIVE DETERMINATION OF PESTICIDE RESIDUES IN SURFACE WATERS USING THE INNOVATIVE HYBRID LINEAR IONTRAP- ORBITRAP MASS SPECTROMETRY

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Summary

The analysis of pesticides residues using a last generation high resolution and high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap-MS) was explored in this study. The proposed approach included a simple off-line solid phase extraction (SPE) step using polymeric cartridges (Oasis HLB) with 250 mL of water sample loaded, followed by ultra-high pressure liquid chromatography (UHPLC)–LTQ-Orbitrap mass spectrometric analysis in positive ion mode. The overall method performance was satisfactory with limits of quantification at the ppt level while recoveries over 70% were achieved for all analytes. This method enables the simultaneous identification of the selected pesticides having various physic-chemical properties and their possible metabolites, providing high accuracy, selectivity and robustness.

Introduction

The widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices. Pesticide residues reach surface water mainly through agricultural runoff. Both parent pesticides and metabolites may exert a toxic action in organisms of freshwater systems, whenever the concentration of a compound is sufficient to trigger such effect. Hence, the analysis of pesticide residues in a variety of environmental matrices is necessary to ensure their safety and quality about health standards [1]. Particularly, the chemical status of water resources is in the spotlight since the European Water Framework Directive (WFD) entered into force one decade ago [2]. State-of-the-art studies focus on the development of reliable analytical methods, which enable a wide-scope straightforward, sensitive and selective determination of priority pollutants in environmental samples. Most of these methods have been conducted by LC-MS/MS by monitoring selected MS/MS transitions (precursor–product) of the compounds of interest.

As an alternative, the use of high-resolution mass spectrometers (LC-HRMS) enables the acquisition of a theoretically unlimited number of species by means of accurate mass measurements in full-scan mode. This allows obtaining the elemental composition of acquired ions, useful for identification of targeted and untargeted compounds, metabolites, or transformation products [3]. The Orbitrap$^{\text{TM}}$mass analyser, developed by Makarov ten years ago and commercially introduced in 2005, implements the principles of Fourier transform (FT) through an electrostatic axially harmonic orbital trapping technique to yield high resolution mass spectra [4].

An SPE sample enrichment procedure was evaluated in order to be applied for the estimation of the pollutant load and its seasonal distribution in natural waters of the Prefecture of Epirus (Aracthos and Louros rivers, Amvrakikos gulf), N.W. Greece. The results obtained confirm that high-resolution mass spectrometry is a helpful and reliable tool for the identification and quantitation of pesticide residues, providing at the same time high accuracy.

Sample preparation and extraction

Water samples (250 mL) were vacuum passed through an Oasis HLB SPE cartridge (200 mg sorbent/6 mL cartridge) previously conditioned. The cartridges were dried under vacuum for 5 min and the analytes eluted with 10 mL of the appropriate solvent mixture. Extracts were evaporated to dryness and then reconstituted with the solvents of the initial mobile phase. The identification of the positive findings was accomplished with the data from accurate masses of the target ions, based on the full-scan exact mass measurement of [M+H]+ ions, along with retention time data and characteristic on-source fragment ions.

Conclusions

In the present work, an analytical methodology using Hybrid LTQ Orbitrap mass spectrometry for the ultra-trace detection and quantification of target pesticides in environmental water samples has been validated and successfully applied to the analysis of river waters. The overall performance of the analytical methodology proved accurate and sensitive enough reaching ppt levels, while it confirmed that high-resolution mass spectrometry is a helpful and reliable tool for the identification and quantitation of pesticide residues, providing at the same time high accuracy.

ACKNOWLEDGEMENTS

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The authors would like to thank the Unit of Environmental, Organic and Biochemical high resolution analysis-ORBITRAP-LC-MS of the University of Ioannina for providing access to the facilities.

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PP21
ACT1123

SYNTESIS OF C18 MAGNETIC NANOPARTICLES FOR THE DETERMINATION OF C60 FULLERENE IN WATER

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Summary

The present study investigates the preparation, characterization and application of C18 coated iron oxide magnetic nanoparticles as an adsorbent for magnetic solid-phase extraction of trace amounts of C_{60} fullerenes from environmental water samples.

Introduction

Interest in studying nanostructured materials has grown significantly in recent years due to unique size and physical properties. Magnetic nanoparticles (MNPs) have attracted much research interest due to many potential technological applications in different areas such as catalysis, adsorption processes, environmental remediation etc. Recently, a new procedure based on the use of magnetic or magnetically modified adsorbents has been developed, adopting magnetic particles as adsorbents, that endow extraction with some unique features and solve some problems associated with conventional extraction methods. A distinct advantage of this technology is that magnetic materials can be readily isolated from sample solutions by the application of an external magnetic field. Most nanomaterial research is centered on developing new uses for nanosized products with unique properties, but on the other hand, there is also significant concern regarding nanomaterials as environmental contaminants. Among them, fullerenes, molecules of pure carbon (e.g., C_{60} and C_{70}) are increasingly investigated for use in biomedical, cosmetic and industrial applications. The marketable value of fullerenes is anticipated to lead to increased human and environmental exposure on a global scale. This process is already under way, most notably in the widespread dermal application of cosmetic products, inhalation of dust particles or soot from combustion processes, and discharge of fullerene-containing products into waterways [1].

Materials and Methods

Chemicals

Fullerene C_{60} (99.5%) was purchased from SIGMA - Aldrich (Germany). Ethanol, toluene were trace analysis grade obtained from Pestiscan (Labscan Ltd., Dublin, Ireland). Analytical-grade $FeCl_2.4H_2O$, $FeCl_3$, ammonia and trimethyl octadecyl silane were supplied by SIGMA-Aldrich (Germany).

Prepation of Magnetic Nanoparticles

0.7 g of $FeCl_3$ and 0.42 g of $FeCl_2·4H_2O$ were dissolved in 100 mL deionized water, degassed continuously with N_2 .After 10 min of intense bubbling, 5 mL of 25% (w/w) NH_3 were added and the mixture was held at 80°C in a water bath for 30 min, with vigorous magnetic stirring (5000 rpm).After cooling down to room temperature, the black iron oxide nanoparticles were rinsed several times with deionized water and ethanol. The next step was the silica coating of magnetic iron oxide nanoparticles. First, 0.5 g Fe_3O_4NPs were dispersed in a mixture containing 12.5 mL ethanol and 4 mL deionized water with the aid of ultrasonication, for 5 min. Then, 250 µLTEOS and 500 µL ammonia 25% (w/w) were added dropwise undervigorous stirring (5000 rpm) in a period of 10 min. The reaction was allowed to proceed for 12 h. Finally, the nanoparticles were rinsed several times with ethanol and dried under vacuum for 24 h thus obtaining a gray–black powder of Fe3O4@SiO2 nanocomposite.The iron oxide magnetic nanomaterial was further treated to load the C18. 0.2 g silica-coated NPs were dispersed in 70 mL toluene with the aid of ultrasonication. The slurry was heated to boiling and 200 μL of trimethoxy(octadecyl)silane were added under vigorous stirring (5000 rpm). The mixture was then refluxed for 12 h, at 80°C. After cooling down to room temperature, the black product (Fe_3O_4@SiO_2@C18) was rinsed with toluene and ethanol several times and dried under vacuum at room temperature, before use.

Results and Discussion

The resulting functionalized magnetic nanoparticles were characterized by XRD and SEM while the surface area and the pore size distribution of NPs were calculated based on N_2 adsorption–desorption porosimetry according to the BET method. The carbon loading was estimated by elemental analysis.

Extraction

10 mg of the functionalized magnetic nanoparticles were quickly added to a vial containing 10 mL of aqueous nC_{60} stock solution .The mixture was sonicated for 5 minutes. Subsequently, a magnet was held next to the bottom of the vial to attract and isolate the nanoparticles and the sample solution was discarded simply by decanting it. Thereafter, the magnet was removed, and an aliquot of toluene was introduced to the vial to desorb the nC_{60}s from the nanoparticles by sonication for 4 minutes. Finally, the magnet was again placed next to the vial, and the supernatant was collected into an Eppendorf tube for analysis.

Conclusions

In this work, we synthesized magnetic C18-functionalized iron oxide NPs which were demonstrated to be easily separable by an external magnetic field. The NPs were successfully applied for the determination of trace amounts of C_{60} fullerenes from environmental water samples.

Acknowledgments

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013)

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PP22
ACT1008

HETEROGENOUS PHOTOCATALYTIC DEGRADATION AND MINERALIZATION OF THE HERBICIDE THIACLOPRID

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Summary

Degradation and mineralization of thiacloprid, a neonicotinoid insecticide reported to occur in drinking water, by heterogeneous photocatalytic oxidation in the presence of UV-A and visible light has been studied. The effect of various operating conditions such as different commercial TiO_2 products, the addition of H_2O_2, the effect of initial pH on the degradation and the organic content reduction (DOC) of the insecticide was examined. The addition of H_2O_2 resulted in all cases in higher initial degradation rates, while the use of TiO_2 P25 in the presence of UV-A, led to higher initial degradation and mineralization rates in comparison to other commercial TiO_2 photocatalysts.

Introduction

Agrochemical compounds including pesticides are widely used as a result of intensive agricultural practices [1]. As these contaminants are in many cases toxic and non-biodegradable, they have the ability to accumulate in the environment and to magnify through the global trophic network with unpredictable consequences for the mid-term future [2]. Thiacloprid ({(2Z)-3-[(6-Chloropyridin-3-yl) methyl]-1,3-thiazolidin-2-ylidene} cyanamide) is an insecticide of the neonicotinoid class, used on agricultural crops to control a variety of sucking and chewing insects, primarily aphids and whiteflies. Thiacloprid is highly soluble in water and has been detected in drinking water [3].

Alternative treatment methods currently employed for the degradation of agrochemicals in water and wastewater include Advanced Oxidation Processes (AOPs). Among them, heterogeneous photocatalytic oxidation (TiO_2/UV-A) in the presence of artificial or solar light has been effective for the degradation of various contaminants found in industrial or domestic wastewaters. A variety of toxic agrochemical substances, such as insecticides and pesticides has been studied in regard to their photocatalytic degradation, in the presence of artificial or solar illumination, with very encouraging results [4]. Our current study investigates the heterogenous photocatalytic decomposition and mineralization of thiacloprid, in order to assess the effect of various operating conditions on pesticide degradation and mineralization.

Materials and Methods

Photocatalytic experiments were performed in a closed Pyrex cell of 600 ml capacity, under constant magnetic stirring. The reaction vessel was fitted with a central 9 W lamp. The spectral response of the UV-A irradiation source, ranged between 350-400 nm (max: 366 nm), while that of the visible irradiation source ranged between 400-520 nm. Prior to heterogeneous photocatalysis, the catalyst suspension was left for 30 minutes in the dark, to achieve maximum adsorption of the insecticide onto the semiconductor surface. During photocatalysis samples were withdrawn and filtered through a 0.45 μm filter. The catalysts employed in the study were TiO_2 P-25 (Degussa, anatase/rutile=3.6/1, BET: 50 m^2g^{-1}, nonporous), TiO_2 UV 100 (Hombikat, 100% anatase, BET: 300 m^2g^{-1}), TiO_2 Kronos 7000, TiO_2 Kronos 7500 (Kronos Worldwide, Inc., 100% anatase, BET: 250 m^2g^{-1}) and ZnO (Merck, BET 10 $m^2\ g^{-1}$). Changes in the concentration of thiacloprid were monitored via its characteristic absorption band at 280 nm using a UV-Visible spectrophotometer (UV-1700, Shimadzu). Determination of the dissolved organic carbon (DOC) was conducted according to standard methods by a TOC analyzer (Shimandzu VCSH 5000).

Results and Discussion

Photocatalytic degradation of thiacloprid in the presence of TiO_2 P25 and UV-A irradiation was enhanced by the addition of H_2O_2, resulting in all cases in higher initial degradation rates of the insecticide. Among the various photocatalysts employed in the study, TiO_2 P25, ZnO and UV-100 in the presence of UV-A, resulted in higher initial degradation rates of the insecticide. In contrast, Kronos 7000 in the presence of either UV-A or visible light, led to low initial degradation rates. Moreover, mineralization of carbon was faster in the case of TiO_2 P25 in comparison to other photocatalysts. The determination of inorganic chlorine, nitrogen and sulfur produced during the oxidation of thiacloprid, of ecotoxicity and of major intermediate by-products is currently in progress, aiming to the determination of possible photocatalytic degradation pathways.

Acknowledgments

The present study is implemented within the framework of the research project entitled "A novel method for detoxification and reuse of wastewater containing pesticides by solar photocatalysis and constructed wetlands" (project No: 957) of the Action ARISTEIA of the Operational Program "Education and Lifelong Learning" (Action's Beneficiary: General Secretariat for Research and Technology), and is co-financed by the European Social Fund (ESF) and the Greek State.

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PP23
ACT1013

COMPUTATIONAL INVESTIGATION OF THE DEGRADATION MECHANISM OF METOLACHLOR

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Summary

The degradation mechanism of metolachlor in aqueous surroundings is examined computationally using DFT and ab Initio electronic methods.

Introduction

Metolachlor, [2-chloro-N - (2-ethyl-6 - methylphenyl)-N - (2-methoxy-1 -methylethyl) acetamide] and similar chloroacetamides are widely used to control annual grasses and broad leaf weeds. The widespread use has resulted in their frequent detection in water sources near agricultural areas, raising serious ecological problems and public health issues.[1] These concerns have led to extended experimental investigations of their soil persistence, the general degradation mechanism and the possible dissociation products of metolachlor and related herbicides.[2-8]

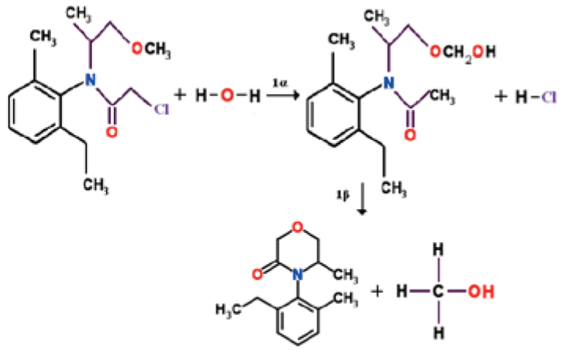
Calculations

The calculations have been carried out using the Gaussian 03 suite of programs.[9] The optimization of the structures examined has been performed at the B3LYP/6-31G(d,p) level of theory. Reaction enthalpies have been evaluated using the advanced coupled-cluster method.

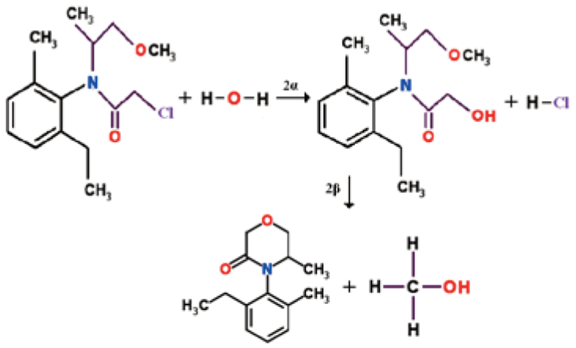
Results and Discussion

Confirming the experimental evidence, the computational investigation has shown that the degradation mechanism of metolachlor begins mainly with the elimination of the chlorine atom and the production of a variety of nitrogen containing aromatic degradates. Two main types of the Cl elimination have been examined :

A) Example of metolachlor degradation via HCl removal (formation of 4-(2-ethyl-6-methyl phenyl)-5-methyl-3-morpholinone)



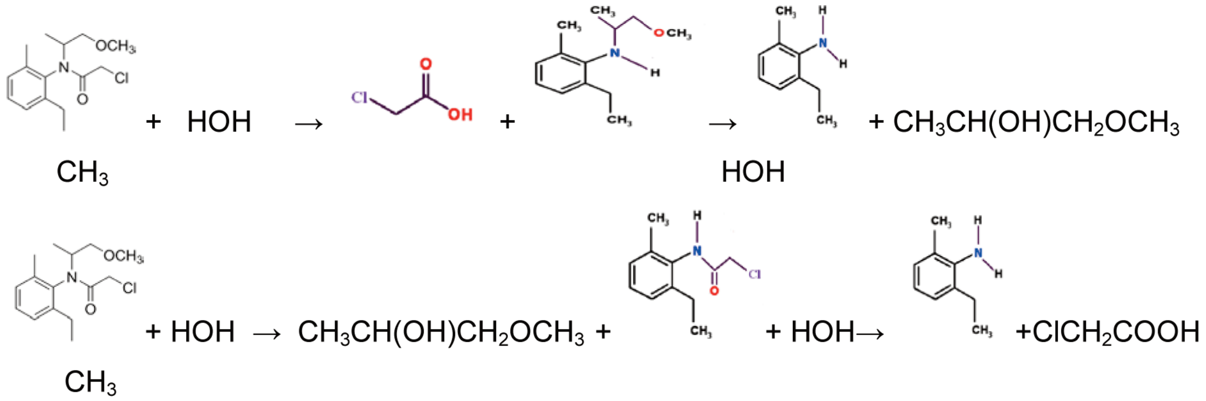
1° Through acetamide1



2° Through acetamide 2

Calculated overall reaction enthalphy $\Delta Hr = 5.94$ *kcal /mol*

B) Example of metolachlor degradation via chloroacetic acid formation



Calculated overall reaction enthalpy ΔHr = 6.45 kcal/mol

(a) Removal of HCl (b) Removal of chloroacetic acid

Calculation of the reaction enthalpy, ΔHr, for two typical such examples of dissociation mechanism using DFT and ab Initio quantum chemical methods, has given valuable information on the preferred pathways of the transformation process. Specifically, the calculated values indicate that both dechlorination mechanisms are endothermic and consequently, they are assisted by UV irradiation.

The results also point out that removal of Cl atom through HCl elimination presents a slightly lower reaction enthalpy over chloroacetic acid formation. Therefore, the HCl removal must be the favoured starting step in the degradation process.

Conclusions

The mechanism of metolachlor degradation in aqueous environment has been studied theoretically, using DFT and ab Initio computational methods. The calculations confirm the experimental hypotheses that the degradation is an endothermic process and preferably starts with the chlorine atom elimination. The computed reaction enthalpies show that the Cl elimination is energetically slightly favoured through HCl release rather than $ClCH_2COOH$ formation.

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PP24
ACT1016

A COMPUTATIONAL STUDY OF THE WATER CATALYSED DEGRADATION OF BROMOMETHANOL

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Summary

We present a computational investigation of the catalytic activity of the water molecules in the oxidation of bromomethanol which is a known contaminant of surface waters, coming from the degradation of brominated organic compounds in the natural environment. The computational investigation shows clearly the substantial reduction of the activation energy of the oxidation to formaldehyde that occurs when explicit coordination of water molecules is taken into account.

Introduction

Halogenated alcohols and other halogenated species are frequent contaminants of surface waters, formed in the degradation process of a rich variety of halogenated pesticides.[1,2] The transformation to carbonylic products has been a subject of intense interest in the recent decades, found to be greatly assisted in a water-solvated environment that acts very effectively in the acceleration of the degradation process in comparison with the water-free system.[3-9]

The detailed examination of the oxidation mechanism of haloalcohols in particular, has emphasized the key role that the water molecules play in their oxidation by forming hydrogen-bonded water complexes. In the present work, a systematic computational investigation is carried out to characterize possible hydrogen-bonded water adducts of bromomethanol and the role of these adducts in the mechanism of the oxidation of bromomethanol into formaldehyde and HBr.

Calculations

The calculations have been carried out using the Gaussian 03 suite of programs.[10] The optimization of the structures examined and the evaluation of the activation energy barriers have been performed at the MP2/6-311++G(3df,2p) level of theory.

Results and Discussion

First, the computational characterization of the reaction in water-free surroundings

$CH_2BrOH \rightarrow TS0 \rightarrow HCHO + HBr$

has been performed. The activation energy barrier, $E_{act}{}^{0}$, corresponding to the transition state TS0, as shown in Fig. 1 below, is computed to be 38.6 kcal /mol above the reactant level. This is a high activation energy barrier which underlines the stability of bromomethanol in a water-free environment.

Next, the activation energy barrier has been calculated assuming that bromomethanol forms water bound complexes in aqueous surroundings. The simplest system to examine is the complexation with one water molecule. More specifically, we examine the energetics of the reaction

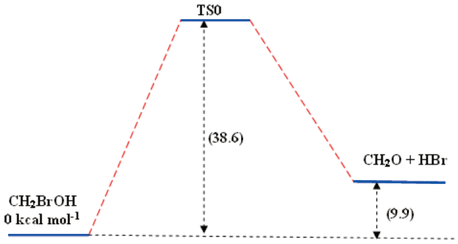

Fig.1. Reaction energy profile in MP2/6-311++G(3df,2p) level of theory, indicating the degradation mechanism of bromomethanol. Relative energies (kcal mol^{-1}) + ZPE, in parentheses.

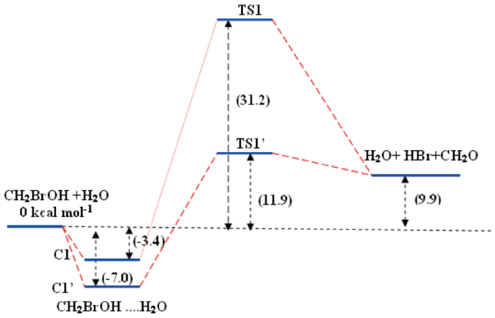

Fig.2. Reaction energy profile in MP2/6-311+G (2df, 2p) level of theory, indicating the water catalysed reaction of degradation mechanism of bromomethanol. Relative energies (kcal mol^{-1}) + ZPE, in parentheses.

$CH_2BrOH + H_2O \rightarrow C1\ (CH_2BrOH—H_2O) \rightarrow TS1 \rightarrow HCHO + HBr + H_2O$ (1)

$CH_2BrOH + H_2O \rightarrow C1'(CH_2BrOH—H_2O) \rightarrow TS1' \rightarrow HCHO + HBr + H_2O$ (1′)

Two types of water complexes $CH_2BrOH—H_2O$ have been identified, C1 and C1′ (Fig. 2). In C1, hydrogen bonding is formed between the O atom of the water molecule and a methylic H. The complex is found to be located -3.4 kcal/mol below the reactants and the corresponding activation barrier related to the transition state TS1 is 31.2 kcal/mol above reactants, i.e., it has been reduced by 7.4 kcal/mol compared to the water-free state. However, a most spectacular reduction takes place when the formation of complex C1′ is considered where the hydrogen bonding takes place between the O atom of the water molecule and the hydroxylic H atom. The binding energy of C1′ is 7.0 kcal/mol, i.e., twice that of C1 and the activation barrier drops to just 11.9 kcal/mol above reactants.

Conclusions

The present computational investigation elucidates the detailed molecular mechanism of the oxidation of bromomethanol to formaldehyde in water-solvated surroundings. The calculations demonstrate most clearly the striking selectivity of the hydrogen bonding complexes that may be possibly formed in principle and the strong reduction of the activation energy barrier, that takes place in aqueous environment compared to the water-free system.

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PP25
ACT1017

INFLUENCE OF HERBICIDE TRIASULFURON ON SOIL MICROBIAL COMMUNITY IN UNAMENDED AND AMENDED SOILS

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Summary

The effect of organic amendments and pesticides on soil microbial community has gained a great interest currently due to the relationship of this community with soil quality. The impact of triasulfuron and sewage sludge on soil microbial community, determining different parameters related with their activity and abundance, as dehydrogenase activity, microbial biomass and respiration of soils, was evaluated. The changes in the microbial community structure were studied by the analysis of phospholipids fatty acids (PLFAs). Results indicated that amendment and high dose of herbicide had effects on the soil microbial parameters studied.

Introduction

The use of pesticides to increase production and quality of crops is a common practice nowadays. These pesticides applied to crops can affect the soil microbial community, and this topic has become of interest in recent years given that this soil community is involved in numerous reactions of soil conservation and maintenance. On the other hand, organic amendments are usually applied to improve fertility or increase the organic matter (OM) content of soils, although OM could influence fate of pesticides in soils.

The aim of this work is to study the effect of the simultaneous addition of an herbicide, triasulfuron, at three doses and an organic amendment, sewage sludge (SS), on soil microbial community. Degradation kinetics, dehydrogenase activity (DHA), soil microbial biomass and respiration, as indicators of abundance and activity of soil microbial community were determined, and the profile of phospholipid fatty acids (PLFAs) was evaluated as an indicator of microbial community structure.

Material and Methods

Triasulfuron is an herbicide of Pestanal grade (>98% purity) and was supplied by Sigma-Aldrich Química SA (Madrid, Spain). Its water solubility and log Kow (pH 7, 25°C) are: 815 mg/L and -0.59.

Soil samples were collected from the surface horizon (0-30 cm) of an agricultural soil in Toro, Zamora (Spain). Soil was amended in situ with sewage sludge at rate of 50 t ha^{-1}. Some soil characteristics determined by standard methods of analysis are shown in Table 1.

Soil containers were prepared in triplicate for unamended and amended soils (500 g) and for each of the three herbicide doses applied. For all treatments, a soil container was used as control (no addition of herbicide). Soils were contaminated with the herbicide by applying a volume of solution in water to obtain a final triasulfuron concentration of 2, 10 and 50 mg kg $^{-1}$. Soil samples were incubated at 20 °C in the dark and moisture was adjusted to 40% of the maximum soil water holding capacity. Soil sampling was carried out at different times: 1) to determine degradation kinetics and half-life values. The herbicide was extracted by an optimized method with extraction recoveries >70% and determined by HPLC / DAD / MS, and 2) to determine the soil dehydrogenase activity (DHA) according to the method of Tabatabai (1994) [1], microbial biomass according to the chloroform fumigation extraction method, microbial respiration determined with a respirometer Oxitop, profile of phospholipids fatty acids following the method-

Table 1: Characteristics of unamended and amended soils

Suelo	pH	Sand %	Silt %	Clay %	CO %	N %	C/N	DOC %
S	6.29	83.4	5.96	10.7	0.49	0.04	11.8	0.005
S+SS	6.16	-	-	-	2.20	0.35	6.30	0.068

ology described by Zelles (1999) [2] for extraction and fractionation, prior to their determination by GC-FID. Identification and Quantification of fatty acids was performed by Sherlock ® 6.0 software (MIDI Inc., Newark, DE, USA).

Results and Discussion

Experiment is currently being carried out and preliminary results are presented.

Degradation kinetics of triasulfuron was fitted to a single first-order kinetics model. DT_{50} values were higher for amended soil than for unamended soil for the 10 and 50 mg kg^{-1} doses. DT_{50} values increased in amended soil from 33.0 days for the 2 mg kg^{-1} dose to 51.7 days for the 50 mg kg^{-1} dose.

Preliminary soil DHA results are shown in Figure 1. DHA mean values were higher in amended soil at the beginning of the experiment. Initially, DHA values decreased in soil samples treated with triasulfuron at 10 and 50 mg kg^{-1}, indicating that the herbicide had a toxic effect for microorganism.

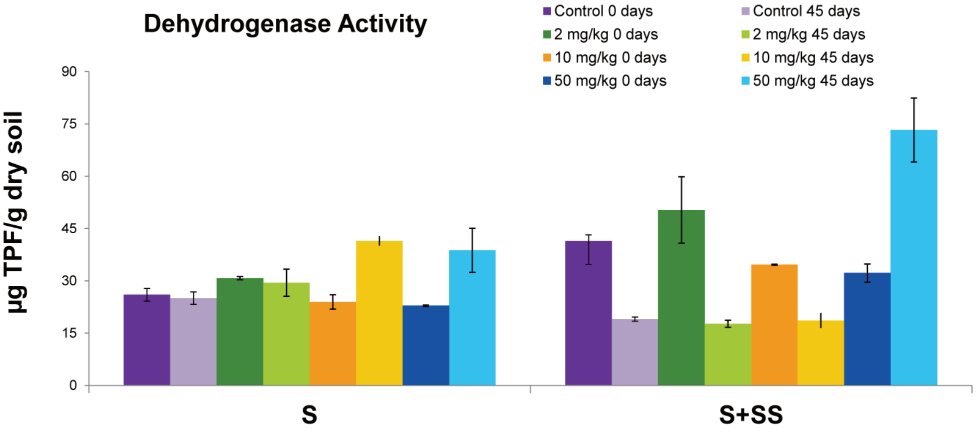

Figure 1. Soil DHA in unamended (S) and amended (S+SS) soils, without herbicide (control) and treated with triasulfuron at doses of 2, 10 and 50 mg kg^{-1} soil, at time 0 and 45 days of incubation.

Microbial biomass increased in amended soils. At the beginning of the incubation, this parameter decreased when the triasulfuron dose added increased and after 45 days the microbial biomass increased as the herbicide was degraded. The changes in microbial biomass with time indicated that the effects of herbicide were reversible. At the beginning of the incubation, respiration increased in the soils treated with 2 mg kg^{-1} of triasulfuron, while decreased in the soils treated with 50 mg kg^{-1}. After 28 days of incubation, respiration decreased in the soils treated with 2 mg kg^{-1} of triasulfuron, while increased in the soils treated with 50 mg kg^{-1}. High dose of herbicide may have a toxic effect on soil microbial community. Soil respiration was higher in amended soil. Identification and quantification of fatty acids is currently being carried out.

Conclusion

The results indicated that the soil amendment has effect on herbicide degradation and soil microbial community parameters. High dose of herbicide had detrimental effects on the soil microbial community, which is important in the case of long-term use of this compound.

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Acknowledgments

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PP26
ACT1027

EFFICIENT PHOTOCATALYTIC DEGRADATION OF ORGANIC POLLUTANT IN WATER USING TITANIA PREPARED BY ELECTROSPINNING TECHNIQUE

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Summary

This study presents a combination of sol-gel method and electrospinning technique for the fabrication of TiO$_2$ fibers of mixed anatase and rutile phase for water purification. The fibers characterized using the BET model, UV-DRS spectroscopy, XRD and SEM techniques. The photocatalytic activity of the titania fibers was investigated by the decomposition of methylene blue in comparison to the reference TiO$_2$ powder Degussa P25.

Introduction

Synthetic dyes have been extensively used in many industries such as textile, printing and dying, cosmetic, petroleum and leather [1]. However, dye contamination has attracted much attention because of their increasing damage to natural ecosystems when dyes are discarded into wastewater. Methylene blue is the most commonly used substance for dying cotton, wood and silk [2]. Though methylene blue is not strongly hazardous, it can cause some harmful effects. Acute exposure to methylene blue can cause increased heart rate, vomiting, shock, Heinz body formation, cyanosis, jaundice, quadriplegia and tissue necrosis in humans [3]. Therefore, the removal of this dye from process effluent becomes environmentally important. Electrospinning techniques have several attractive advantages such as simplicity, comparatively low cost applicability to various materials, and the ability to generate relatively large-scale continuous fibers. Many inorganic micro/nanofibers have been fabricated using electrospinning techniques and sol–gel methods [4].

Materials and Methods

The reagents used were polystyrene (PS), titanium (IV) propoxide (TiPr), dimethylformamide (DMF), acetic acid glacial (AA), urea (U) and methylene blue (MB). A sol of PS, TiPr, AA with (D22U) and without (D22) U in DMF, was electrospun. The used voltage was 17KV, the distance of the tip target to the collector was 8 cm and the flow rate was 1.2 mL/h. The as-spun fibers were calcined at 450 ^{o}C for 3 h. The calcined fibers characterized using the BET model, UV-DRS spectroscopy, X RD and SEM techniques. The photocatalytic activity of the titania fibers was evaluated by measuring the decomposition of MB with an initial concentration of 10mg/L. Before the UV irradiation the dispersion were stirred in the dark for 1h to reach the adsorption equilibrium. The UV source intensity was 2.88 mW/cm^2 and the wave length was 365 nm.

Results and Discussion

The BET analysis showed that the Urea addition makes a small reduction of the specific surface area of 3 m^2/g. The calculated band gaps from UV-DRS spectroscopy of the samples are lower than Degussa P25 that means that are active in the visible region. XRD patterns show that the rutile phase in our samples is in a higher proportion than Degussa P25. SEM images show small bars ranging from 0.6 to 2μm. The MB decomposition showed a bigger half life in our samples in contrast to the reference P25 and this can be explained by the smaller percentage of the anatase phase which is responsible for the photocatalytic procedure.

Material code	TiO_2 Phases (%)		Crystallite Size (nm)		Specific Surface Area (m^2/g)	Band Gap (eV)	MB Degradation (%)	Half Life (min)
	Anatase	Rutile	Anatase	Rutile				
P25	74.5	25.5	28.3	24.7	53	3.09	99.2	17
D22	68	32	15.8	26.5	52	2.97	98.9	33
D22U	67	33	16.9	31.8	49	2.81	98.7	30

***Table 1.** Characterization and photocatalytic ability of samples.*

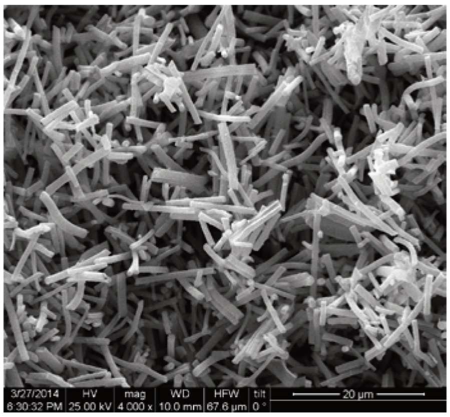

Fig 1. The SEM image of D22U.

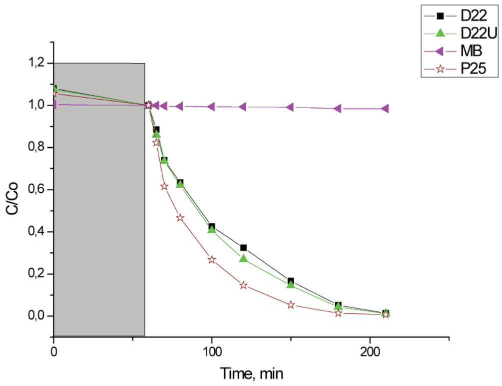

Fig 2. Methylene Blue Degradation.

Conclusions

In this study, TiO_2 and doped N/TiO_2 ultrafine fibers has successfully been prepared by a sol gel/electrospinning technique. Methylene blue was successfully decomposed in UV light by the TiO_2. The photocatalytic experiments showed that N-doping slight improved the photocatalysis process by the reduction of decomposition time of MB. UV-DRS analysis show that N-doping brought about a narrowing in the band-gap of TiO_2 and a hence shifting of the absorption onset to the visible region.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALIS. Investing in knowledge society through the European Social Fund.

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PP27
ACT1090

DEGRADATION OF CONGO RED IN SURFACE WATER SAMPLES AFTER THE SYNTHESIS OF NANOCOMPOSITES OF TiO_2 AND REDUCED GRAPHENE OXIDE

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Summary

In the present study nanocomposites of titanium dioxide (P25) and reduced graphene oxide (RGO), which were prepared by hydrothermal method, were studied as photocatalysts for degradation of congo red.

Introduction

Wastewater effluents from textile and other dyestuff industry contain a large number of toxic dyes and industrial dyestuffs which constitute the largest groups of organic compounds, they are represent an increasing environmental danger. If these effluents are improperly treated, they will pose badly threats to all species on the earth because the hydrolysis of the pollutants in the wastewater can produce a great deal of toxic products. So the disposal of dyestuff wastewaters is necessary to settle the problem of environmental pollution. By now, a lot of researches have been studies the degradation technology of dyestuffs for a long time [1].

In this paper, we reported a catalyst based on TiO_2, which was prepared by hydrothermal method using reduce graphene oxide to improve the catalysis activity of TiO_2. These catalysts irradiation with using simulated solar light as radiation source and compare the catalytic activity of dioxide titanium in different water matrices

Results and Discussion

Preparation and characterization:

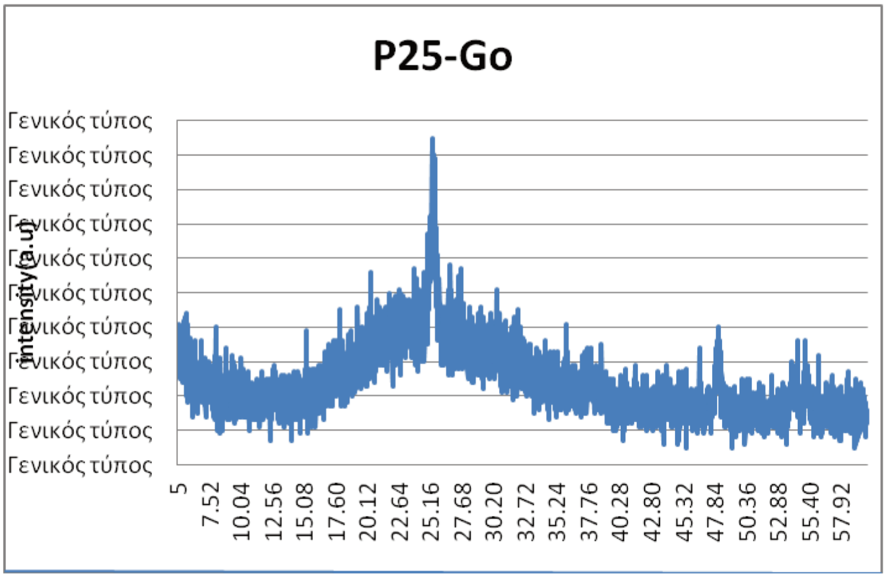
<u>Synthesis of Graphene Oxide (GO)</u>. GO was prepared from graphite powder according to the modified method reported by Hummers and Offeman.35 In brief, 1.0 g of graphite

powder and 0.5 g of $NaNO_3$ were added into 23 mL of cooled (273 K) concentrated H_2SO_4. Then, 3 g of $KMnO_4$ was added gradually with continuous stirring and cooling, and the temperature of the mixture was maintained below 293 K. After the ice bath was removed, the mixture was stirred at 308 K for 30 min. 46 mL of distilled water was added slowly to cause an increase in temperature to 371 K, and the mixture was maintained at that temperature for 15 min. The reaction was terminated by addition of 140 mL of distilled water followed by 10 mL of 30% H_2O_2 aqueous solution. The solid product was separated by centrifugation and washed repeatedly with 5% HCl solution until sulphate anion could not be detected with $BaCl_2$. The resultant solid was dried in vacuum at 323 K to obtain GO.

Preparation of P25_RGO Composites. P25, which contained 20% of rutile and 80% of anatase, was purchased from Degussa.

<u>Hydrothermal Method</u>: GO was first dissolved in H_2O by ultrasonic treatment for 1 h to yield a yellow-brown solution, and P25 was added into the GO colloidal solution. After stirred for another 1 h, the obtained homogeneous suspension was transferred to a Teflon-lined autoclave and was subjected to hydrothermal treatment at 453 K for 6 h. GO could be reduced to RGO during the hydrothermal process. The resultant composite was collected by centrifugation, washed repeatedly with water, and dried in vacuum at 333 K. The sample prepared by this procedure is denoted as P25_RGO-hydrothermal [2].

The synthesized hybrid materials are characterized by several techniques, such as scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform-infrared (FT-IR) and Brunauer–Emmett–Teller (BET). For example, XRD of P25_RGO (*Fig.1*).



Acknowledgments

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PP28
ACT1091

ADSORPTION OF METOPROLOL TARTRATE ONTO IOTA-CARRAGEENAN MODIFIED BY GLUTARALDHEYDE MATRIX AND PREPARED BY SPRAY-DRYING

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Summary

Iota-carrageenans were prepared and modified by cross-linker (glutaraldheyde) using spray-drying in order to adsorptively remove a pharmaceutical compound (metropolol tartrate) from aqueous matrices.

Introduction

Pharmaceuticals are of scientific and public concern as newly recognized classes of environmental pollutants and are receiving considerable attention with respect to their environmental fate and toxicological properties over the last 15 years. Metropolol tartrate (MTT), is a selective β_1 receptor blocker used in treatment of several diseases of the cardiovascular system, especially hypertension. The active substance metoprolol is employed either as metoprolol succinate or as metoprolol tartrate. The tartrate is an immediate-release and the succinate is an extended-release formulation. The residue of MTT in manure andsoils may affect soil microbial and enzyme activities. Adsorption is a potential technique to remove MTT and overcome the pollution of pharmaceuticals in the environment. As adsorbent material, iota-carrageenan as potential super-adsorbent material was tested.

Synthesis of i-carrageenan (ι-CAR)

ι-CAR was first dissolved in distilled water under mechanical stirring and at 60 °C. When dissolution achieved, glutaraldehyde solution (50% in water) was added in order to prepare a solution containing GA 5%wt in ι-CAR. The solution was left to stir for 10 min and then it was spray-dryed. Microparticles were obtained.

HPLC Analysis

Analysis of metoprolol was performed with HPLC-DAD by using a CNW Athena C18, 120 Å, 4.6mm *250mm, 5μm. Water (A) and methanol (B) were the elution solvents. The samples were eluted according to the following gradient: 90% B as initial condition; 50% B for 10 min; 20% B for 2 min, followed by 3 min90% in B equilibration time and the total run time was 16min. The column temperature was 40 oC and the flow rate 1ml/min. Chromatographic data were acquired at the wavelength of 223 nm. Identification of metoprolol was achieved by comparing the retention time and UV spectra of metoprolol standard.

Results and Discussion

Microparticles were at the range of 20-40 μm in size, as SEM showed. XRD patterns showed that Metoprolol is adsorbed on matrix in its amorphous form, due to the observation that no XRD reflex of the drug was found.

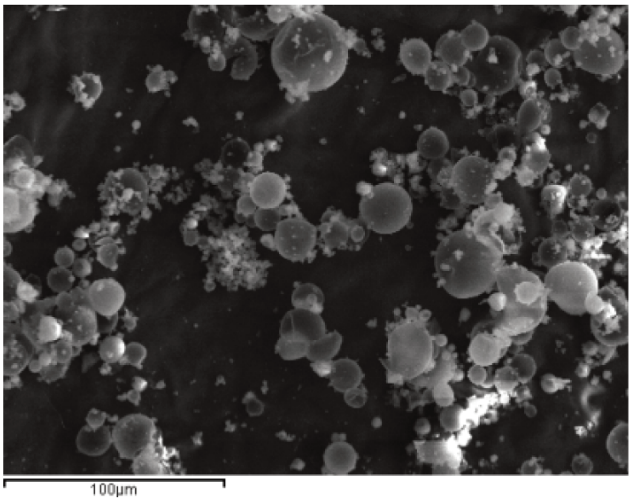

Figure 1. SEM photos of MMT adsorbed.

The experiments for the pH effect showed that the optimum value was at alkaline conditions (pH=6), while the maximum theoretical adsorption capacity was found to be 85 mg/g at 25 °C. The latter was found after fitting to the combinational isotherm model of Langmuir-Freundlich. The adsorption was reached at equilibrium very quickly (~50 min) after testing the optimum adsorption contact time.

Conclusions

Metoprolol tartrate was adsorbed in matrix in its amorphous form. The synthesis of ι-CAR was effectively acted for the isolation (removal) of MMT compound from aqueous matrices, presenting 85 mg/g maximum theoretical adsorption capacity.

Acknowledgments

The support for this study was received from the Greek Ministry of Education (GGET) and through the research program “Excellence II (ESPA2007-2013/EPAN II/Action “ARISTEIA II)” under the title “Advanced microextraction approaches based on novel nano- polymers to measure pharmaceuticals, personal care products and their transformation products in the aquatic environment” (project acronym: PoL-PPCPs-TPs), which is gratefully appreciated.

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PP29
ACT1094

SYNTHESIS AND CHARACTERIZATION OF NANOCOMPOSITES OF TiO_2 AND REDUCED GRAPHENE OXIDE FOR DEGRADATION OF EMERGING POLLUTANTS IN DIFFERENT WATER MATRICES AND COMPARISON WITH TiO_2

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Summary

In the present study nanocomposites of titanium dioxide (P25) and reduced graphene oxide (RGO), which were prepared by hydrothermal method, were studied as photocatalysts for degradation of emerging pollutants. The synthesized hybrid material are characterized by several techniques. This is a comparative study of photolytic degradation about the titanium dioxide and titanium dioxide – reduced graphene oxide in distilled water, lake ioannina water, river and effluent wastewater treatment plant.

Introduction

Advanced oxidation processes (AOPs), which involve the insitu production of highly reactive oxygen species (e.g., hydroxylradical (•OH)) in contaminated environmental media, constitute a promising strategy for the remediation of municipal and industrial wastewater that contains recalcitrant organic substances. In contrast to the substrate-dependent activities of chemical oxidants (e.g., chlorine and ozone) and microbial communities used in conventional water treatment infrastructures, the indiscriminate nature of •OH-induced oxidation enables the application of AOPs to the degradation and mineralization of a wide range of organic compounds. As a result, •OH effectively mediates the chemical transformation of bio-refractory organics into intermediates that are highly vulnerable to microbial degradation, which indicates the potential suitability of AOPs as a pre-treatment for biological processes. AOPs are also considered to be a post-treatment option for the oxidative destruction of emerging organic contaminants (e.g., endocrine disruptors, pharmaceuticals, and personal care products) that survive secondary biological treatment [1].

Results and Discussion

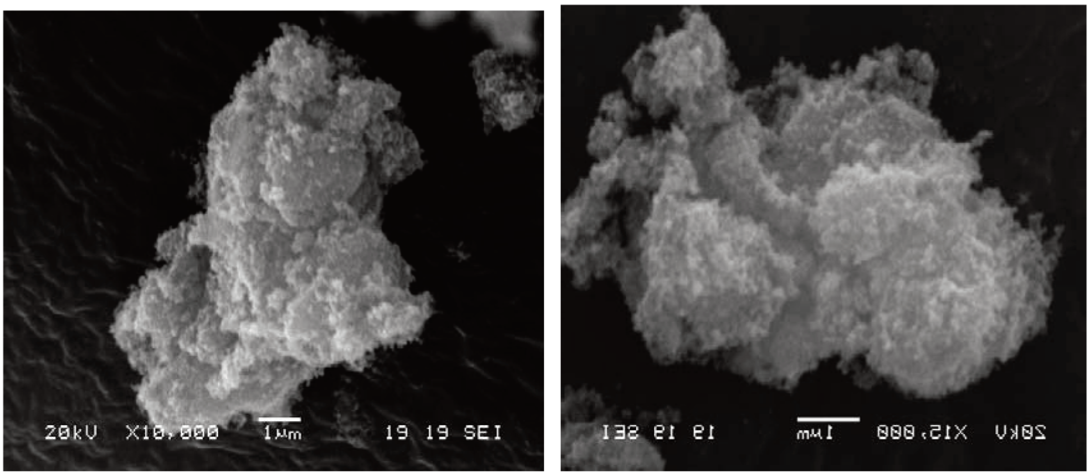
Preparation and characterization:

Synthesis of Graphene Oxide (GO). GO was prepared from graphite powder according to the modified method reported by Hummers and Offeman.35 In brief, 1.0 g of graphite powder and 0.5 g of $NaNO_3$ were added into 23 mL of cooled (273 K) concentrated H_2SO_4. Then, 3 g of $KMnO_4$ was added gradually with continuous stirring and cooling, and the temperature of the mixture was maintained below 293 K. After the ice bath was removed, the mixture was stirred at 308 K for 30 min. 46 mL of distilled water was added slowly to cause an increase in temperature to 371 K, and the mixture was maintained at that temperature for 15 min. The reaction was terminated by addition of 140 mL of distilled water followed by 10 mL of 30% H_2O_2 aqueous solution. The solid product was separated by centrifugation and washed repeatedly with 5% HCl solution until sulphate anion could not be detected with $BaCl_2$. The resultant solid was dried in vacuum at 323 K to obtain GO.

Preparation of P25_RGO Composites. P25, which contained 20% of rutile and 80% of anatase, was purchased from Degussa.

Hydrothermal Method: GO was first dissolved in H_2O by ultrasonic treatment for 1 h to yield a yellow-brown solution, and P25 was added into the GO colloidal solution. After stirred for another 1 h, the obtained homogeneous suspension was transferred to a Teflon-lined autoclave and was subjected to hydrothermal treatment at 453 K for 6 h. GO could be reduced to RGO during the hydrothermal process. The resultant composite was collected by centrifugation, washed repeatedly with water, and dried in vacuum at 333 K. The sample prepared by this procedure is denoted as P25_RGO-hydrothermal [2].

The synthesized hybrid materials are characterized by several techniques, such as scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform-infrared (FT-IR) and Brunauer–Emmett–Teller (BET). For example, SEM of P25_RGO(*Fig.1*).



Acknowledgments

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PP30

ACT1102

FACTORS AFFECTING THE PHOTODEGRADATION OF AQUATIC RESIDUES OF TWO WIDELY USED VETERINARY DRUGS: SULFADIAZINE AND TRIMETHOPRIM

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Summary

To advance the knowledge of the environmental fate of two antibiotics sulfadiazine (SDZ) and trimethoprim (TMP) the photolysis was studied in river water and distilled water. It was also evaluated the effect of initial concentration and the presence of sodium humic acid salt (HNa) in solution in the photolysis rate of antibiotics. Long half –live time for total disappearance of TMP in double distilled water demonstrated that is highly stable in photolysis. While there is a direct photolysis component to the river water photolysis of SDZ. Chromatograms of LC-MS-MS analysis of photolysis solutions revealed the formation of a ketone derivative of TMP as the photolysis proceeds which acts as a photosensitizer.

Introduction

Antibiotic is one important category of these trace organic contaminants. Occurrence of antibiotics in the environment has been reported worldwide [1]. Photodegradation is likely to be the main process controlling the environmental fate of organic pollutants [2]. Trimethoprim is widely used in combination with sulfadiazine. The present study deals with the degradation of these antibiotics and the investigation of their behavior after the release in the environment.

Materials and methods

The photochemical fate of SDZ and TMP was investigated in different matrices, with the addition of HNa, in different initial concentrations under natural sunlight.

Results and Discussion

3.1 Comparing photolysis in double distilled and river water

Photolysis of SDZ followed a pseudo first order kinetics in both matrices while the loss of TMP in sunlit river waters appears to be dependent on indirect photolysis.

1.2 Effect of initial concentration and [HNa]

The photolysis rate of SDZ at initial concentrations of 1.0, 5.0 and 10μM were similar while the photolysis rate of trimethoprim at initial concentrations 1.0, 5.0, 10.0, 20.0μM revealed a strong self -sensitization effect. LC-MS analysis photolysis solution confirmed the formation of a ketone derivative TMP.

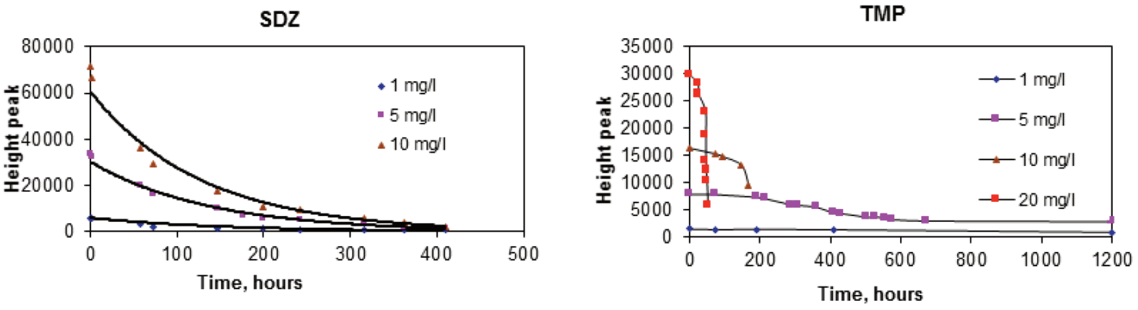

Figure1: Photolytic degradation at different initial concentrations.

HNa promote photodegradation of TMP acting as a photosensitizer. In our study SDZ photolysis was inhibited with increasing [HNa] from 5 to 20 μM.

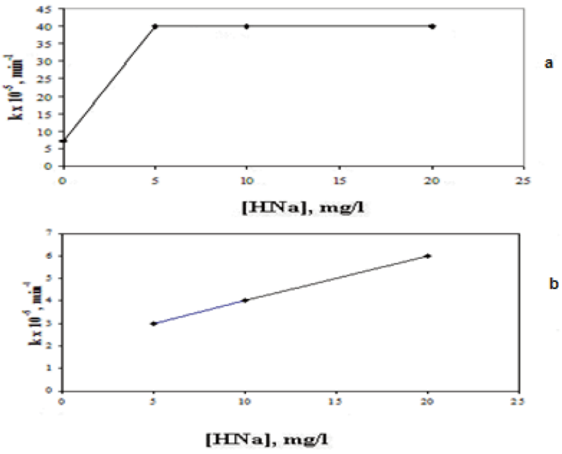

Figure 2: Effect of [HNa] on plotolysis rate of SDZ (a) and TMP (b).

Conclusions

The study highligts that photedegradation is the main process ofremoval of the two antibiotics via different mechanisms.

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PP31
ACT1103

EFFECTIVENESS OF AOPs PROCESSES FOR THE REMOVAL OF EMERGING CONTAMINANTS FROM WASTEWATER: THE MEPANIPYRIM CASE

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Summary

Conventional municipal wastewater treatment plants are not able to entirely degrade some organic pollutants that end up in the environment. For this reason enhanced technologies such as Advanced Oxidation Processes (AOPs) have been employed to reduce the presence of these compounds in the aquatic environment. In this work were studied and compared two AOPs as tertiary treatments for the remediation of micropollutants in aqueous environment, solar photocatalysis using TiO_2-Aeroxide P25, and solar photo-Fenton using peroxymonosulfate as oxidant. The examined pollutant was mepanipyrim a fungicide belonging to the family of the anilino-pyrimidine, active against gray mold (*Botrytis cinerea).* Solar TiO_2 and photo-Fenton process using low iron and peroxymonosulfate doses were proved to be efficient methods for the elimination of this compound with relatively high degradation rates.

Introduction

Increasing number of organic micropollutants (pharmaceuticals, personal care products, steroid hormones, industrial chemicals, pesticides, etc), also called as emerging contaminants, are detected in surface waters. Micropollutants are commonly present in water at trace concentrations, ranging from a few ng/L to several µg/L [1,2]. The "low concentration" and diversity of micropollutants not only complicate the associated detection and analysis procedures, but also challenges for water and wastewater treatment processes [3]. Conventional municipal wastewater treatment plants (WWTP) are not able to entirely degrade some organic pollutants that end up in the environment. Therefore it is necessary to apply additional treatment technologies to remove remaining micropollutants from WWTP effluents. Advanced oxidation processes (AOPs) offer good opportunities; during these processes are generated highly reactive radicals (especially hydroxyl radicals (HO•), or other species of similar reactivity such as sulphate radical anion (SO4•$^-$), in sufficient quantity to influence water purification [4]. The purpose of this work was the evaluation of effectiveness of solar photo-Fenton using peroxymonosulphate (PMS) as oxidant and heterogeneous photocatalysis using TiO_2.

Experimental

The examined pollutant was mepanipyrim (N-(4-methyl-6-prop-1-inylpyrimidin-2-yl)anilin), a fungicide belonging to the family of the anilino-pyrimidine, active against gray mold (*Botrytis cinerea*) on grapevine, strawberry and tomato. Analytical standards of the fungicide were purchased from Dr. Ehrenstorfer. All solvents used for chromatographic analyses, acetonitrile, methanol and ultrapure (MilliQ) water were HPLC grade. The heterogeneous photocatalytic degradation tests were carried out in real wastewater using a slurry suspension of TiO_2 (Aeroxide P25 Evonik) having a specific surface area of 54 $m^2\ g^{-1}$. Photo-Fenton experiments were performed using iron sulphate ($FeSO_4$ x $7H_2O$), Oxone® monopersulphate compound (PMS) ($HKSO_5$ x 0.5 $HKSO_4$ x 0.5 K_2SO_4) and sulphuric acid for pH adjustment (around 3) only when necessary, all provided by Sigma Aldrich. Real samples of wastewater effluents were collected downstream of a biological WWTP effluent with a Total Organic Carbon of 24 mg L^{-1}. A solar panel pilot plant, described in a previous works [5, 6] was used for the photocatalytic experiments. The progress of the photoreactions was evaluated by a fast and reliable analytical method based on a HPLC system.

Results and Discussion

From the calibration curve, it was possible to calculate an LOD of 0.05 µg L^{-1} and an LOQ of 0.16 µg L^{-1}) for mepanipyrim standard solutions prepared in MillQ water.

As the scope of this study was to propose a solar AOP treatment permitting reuse of WWTP effluents, the first step was to compare different approaches, photo-Fenton and TiO_2 powder dispersion. It was also decided to work with real effluents from WWTP. Indeed, the obtained results could describe a real-life situation, as all the tests were done at a typical concentration (10 µg L^{-1}) of the fungicide.

Solar titanium dioxide (0.7g L^{-1}) and photo-Fenton process using low iron and peroxymonosulfate doses ([Fe(II)] = 100 µM ; [PMS] = 200 µM) were proved to be efficient methods for the elimination of mepanipyrim with relatively high degradation rates. Independently of the oxidation method assessed, the photocatalytic degradation of the fungicide with both the solar advanced processes followed apparent first-order kinetics. The evaluated order of the micropollutant elimination efficiency under the experimental conditions was solar heterogeneous photocatalysis with TiO_2 followed by solar PMS/Fe(II). A limiting factor for both processes performed was the presence of the organic carbon in the wastewater, and more specifically of carbonate species (CO_3^{2-} and HCO_3^-), which compete with organic contaminants for hydroxyl or sulfate radical reactions, and significantly decrease the degradation efficiency. In the real wastewater, which was used in our experiments, the concentration of HCO_3^- was high and equal to 180.8 mg L^{-1}. In the case of the photo-Fenton process with peroxymonosulfate, the problem encountered with the scavenging of radicals can probably be avoided using higher amount of iron and PMS. Nevertheless, one must keep in mind that 5 mg L^{-1} represent the maximum concentration that it is possible to release in the environment according the EU regulations.

Acknowledgments

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PP32

ACT1024

DISSIPATION OF PESTICIDES IN AN AMENDED SOIL AT TWO RATES AND ITS IMPACT ON THE SOIL MICROBIAL COMMUNITY

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Summary

The objective of this work was to study the effect of spent mushroom substrate (SMS) on the dissipation of two pesticides in vineyard soils from La Rioja (Spain), and the effect of the addition of the pesticides and SMS on the soil microbial community structure.

Introduction

The dissipation of pesticides in soils can be modified by changes in soil management such as the addition of organic amendments. We have studied the behaviour of two pesticides with different characteristics (azoxystrobin, a synthetic strobilurin fungicide, and pirimicarb, a selective carbamate insecticide) in soils which were unamended or amended with SMS at two rates. Residues of pesticides were extracted with an organic solvent and with a cyclodextrin solution over the course of the experiment to determine dissipation kinetics and to predict pesticide bioavailability in the soils [1].

Soil dehydrogenase activity (DHA) and soil respiration were measured to give an indication of broad-scale impacts of the pesticides and SMS amendment on the microbial community, because according to previous studies [2], the incorporation of organic amendments can induce a change in the spatial and temporal distribution of soil microorganisms. Furthermore molecular methods are being used to determine the responses of soil eukaryotic microbial communities to different concentrations of the pesticides and organic amendment, using Illimuna MiSeq technology, a next generation sequencing (NGS) technique [3].

Material and Methods

The soil used in all experiments was taken from the surface horizon (0–30 cm) of a vineyard farm in La Rioja (NE-Spain). The amended soil was prepared by uniformly mixing soil with SMS at a rate of 2% and 5% on a dry weight basis. Soil and organic residues were mixed without sieving. The initial moisture content of the unamended and amended soil was previously adjusted to 40% of their maximum water holding capacity. Samples of unamended and amended soils were taken and sieved (<2 mm) prior to the addition of pirimicarb and azoxystrobin. The pesticides were dissolved in acetone and added to the soil gradually during a 10 min mixing period to allow the acetone to volatilize. An equal volume of acetone was added to the control samples (without pesticide). The pesticide concentrations used were the agronomic dose and 12.5 times the agronomic dose (2 mg kg^{-1} and 25 mg kg^{-1}). After mixing thoroughly using sterilized spatulas, soil was divided into equal aliquots of 50 g, placed in sterilized bottles and incubated in darkness at 20ºC. The samples were arranged in a randomized design (split plot design), in which there were three replicate blocks. Soil moisture content was maintained throughout by the addition of sterile distilled water as necessary.

To determine how pesticide persistence in soil is affected by SMS, methanol extraction was carried out. Samples of incubated soil (5 g) were shaken with 10 mL of methanol for 24 h. HPLC/MS analysis was used to monitor the pesticides and/or metabolites over the course of the experiment. Chemical extraction techniques like non-exhaustive extraction with hydroxypropyl-b-cyclodextrin (HPCD) were also conducted to measure the bioavailable fraction of azoxystrobin and pirimicarb in soil. Soil samples (1 g) were shaken with 10 mL of a HPCD solution (50 mM) and the aqueous phase was analyzed with HPLC-DAD.

Soil dehydrogenase activity was determined using the Tabatabai method [4] at the beginning and at the end of the dissipation period, and microbial soil respiration analysis was performed with Oxi-Top equipment, according to ISO

16072. Moreover analysis of eukaryote communities by sequencing the 18S rRNA gene from DNA extracted from soil samples is underway using Illumina MiSeq technology.

Results and Discussion

Azoxystrobin and pirimicarb dissipation from all soil samples followed first order kinetics. The degradation of both pesticides was slower when pesticides were added at 25 mg kg^{-1} relative to 2 mg kg^{-1}. DT_{50} values in the unamended soil were 23 and 35 days for pirimicarb and 69 and 87 days for azoxystrobin, when applied at the low and high doses, respectively. In all cases degradation rate of azoxystrobin was slower than that of pirimicarb.

In amended soils, DHA was higher than in unamended soils; however, this effect was transient and was observed only at the beginning of the experiment. At the end of the experiment a significant decrease in DHA was observed in the soils treated with pirimicarb. The stimulation observed by the addition of SMS could be a result of extra C provided by this amendment for the soil microbial community. However, the stimulation of DHA by SMS could also be a result of direct change in microbial activity due to altered physicochemical properties such as sorption of inorganic and organic compounds including enzymes, changes in soil water retention and pore structure. The decreased DHA values in soils treated with pirimicarb might have resulted from the death or metabolic inhibition of a fraction of the microbiota sensitive to the pesticide.

Conclusions

SMS application to soil did not have an effect on the degradation rate of azoxystrobin or pirimicarb. SMS and fungicides had positive and negative impacts respectively on the soil microbial activity in the amended soils treated with fungicides. Sequencing of unamended and amended soil samples treated with the pesticides at the different doses is currently in progress and the impacts of pesticides and organic residues on the soil microbial community will be understood.

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PP33
ACT1074

DEVELOPMENT OF NOVEL CATALYTIC BIOSCAVENGERS FOR HERBICIDE DETOXIFICATION

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Summary

Glutathione transferases (GSTs) from tau class are unique in plants.They play important roles in stress tolerance and the detoxification of herbicides in crops and weeds. Protein engineering studies were used for the creation of GST variants with higher selectivity and activity towards the chloroacetanilide herbicide alachlor. A library of tau class GSTs was constructed by DNA shuffling using the DNA encoding for GSTs from *Glycine max*. The library of variant GST sequences was expressed in *E. coli* and the enzymatic activity of selected clones was screened and determined. Important structural determinants for activity were recognized by means of primary structure analysis and activities of purified enzymes chosen from the screening. Chimeric enzymes with enhanced detoxifying activities were isolated and characterised.

Introduction

GSTs are phase II detoxification enzymes involved in cellular detoxification by catalyzing the nucleophilic attack of the tripeptide glutathione (GSH) on the electrophilic center of a number of herbicides including the chloroacetanilide herbicide alachlor [1-3]. This catalytic activity can be the ground of the development of catalytic bioscavengers and 'green' bioremediation tools for the decontamination and herbicide detoxification.

GSTs comprise a large family of ubiquitous detoxifying enzymes. GSTs are known as promiscuous enzymes capable of catalyzing the conjugation of GSH with a broad range of electrophilic substrates including xenobiotics and endogenous electrophilic compounds such as pesticides, chemical carcinogens, environmental pollutants, leading to their detoxification. There has been a particular interest in plant tau class GSTs with regard to herbicide selectivity and environmental safety [1-3].

A protein design experiment requires a suitable protein scaffold. Robustness and tolerance towards mutations are desirable as well as easy of expression, purification and long-term stability are advantageous properties. The GSTs are good model systems, for protein design experiments, due to their modular feature with two binding sites, one that is promiscuous and the other relatively stringent. In addition, they are stable and well-studied with respect to structure-function relationships. Sequence and structural data are available for many GSTs and they have been successfully used in engineering experiments. GSTs are very adaptable platform for engineering new or improved catalytic activities, particularly with respect to the detoxification of synthetic compounds [4-7].

Results and Discussion

Protein engineering was used for the creation of GST chimeric variants with higher selectivity and activity towards the chloroacetanilide herbicides alachlor. cDNA libraries were created from *Phaseolus vulgaris* and *Glycine max* stressed plants using degenerated primers and reverse transcription-PCR. Large diversity in GST genes was accomplished employing directed evolution through DNA shuffling. The shuffled library of chimaeric GST genes was cloned and expressed in *E. coli*. Screening of the library led to the isolation of novel GST enzymes that displays enhanced detoxifying activity towards alachlor. The enzymes were purified by affinity chromatography and characterized by kinetic analysis. The study demonstrates the power of DNA shuffling in identifying segments of primary structure that are important for catalytic activity with a targeted substrate. From an evolutionary perspective, the results show that recombination of segments from homologous proteins gives rise to a large proportion of functionally competent enzymes with a wide range of activities. Knowledge of activity-determining segments in the structure is valuable in the protein engineering of GSTs for altered specificity.

Conclusions

In the present work, we describe a combinatorial protein engineering approach for the creation of GST variants with higher selectivity and activity towards the herbicide alachlor. The approach could provide a novel strategy for modulation of plant GSTs towards new binding specificities and enzyme activities.

Acknowledgments

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PP34
ACT1076

GLOBAL SENSITIVITY ANALYSIS OF PESTICIDE LOSSES IN STRUCTURED TILE-DRAINED SOIL

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Summary

In order to understand which parameters influence pesticide exportation in a drainage context, a global sensitivity analysis was conducted with the model MACRO on one of the six European drainage scenarios from [1] called "La Jaillière" and based on a western french site. Sixteen hypothetical substances were selected to study how influent parameters fluctuate with the pesticides physico-chemical characteristics . Morris' and Sobol's methods were used in two successive steps, the first one as a screening method and the second (based variance analysis), on the selected parameters, since the initial parameters number was too high. Results show a strong influence of the proportion of sorption sites in macropores (called Fracmac), whatever the characteristics of the substance. Another important conclusion is that non linearity and interaction between parameters increase with adsorption and half life values.

Introduction

Diversity of processes involved in pesticide fate leads to create complex modelling tools. Interactions between processes and parameters can make results interpretation difficult or superficial. Sensitivity analysis (SA) appears as a usefool tool to identify key parameters. However, the most used SA practice seen in the literature is that of 'one-factor-at-a-time' (OAT) which consists in analysing one parameter variability influence on various models outputs while the other inputs are fixed. As criticized by [2] OAT strategy is definitively not a sufficient option. Thus, this work objective is to use global sensitivity analysis methods to determine key sensitive parameters in regards of water and pesticide fluxes in drainage and runoff flows with a calibration perspective.

Material and methods

The field study called la Jaillière is located in the North West of France. The soils are classified as stagnic luvisol (FAO) and a drainage network (10 m space and 0.9 m depth) was implemented to prevent soil water logging in winter. It is representative of a large part of drained land in France and is one of the six European drainage scenarios [1]).. The model MACRO [3] was chosen as it's widely used in Europe to study pesticide exportations in drainage flow, and especially for homologation. Two complementary sensitivity methods have been selected. The first one called : "Morris method" was used to analyze influence of 48 main parameters on water and solute fluxes. This screening method provides qualitative information and is here performed to select the most significant parameters to be analyzed with a more quantitative method. Secondly, Sobol method was chosen as it's a very robust one which provides first and total sensitivity indices [4].Simulations were performed with the software CEMAFOR [5] which couples MACRO, used as slave model, with the optimisation tool PEST [6]. This study is focused on the evolution of parameters influence depending on adsorption and degradation initial value. For that, a set of 16 hypothetical substances were evaluated. A C.V of 20 % was used for adsorption and degradation parameters. Others inputs values (distribution and ranges) were based on literature values as far as possible.

Results

Morris results for pesticides varied from one substance to another, depending on their physico-chemical characteristics. For example, increasing adsorption and persistence lead to an increase in non linearity and interaction between parameters (above 1:1 line on Fig. 1). Moreover, influent parameters vary between each scenario, in particular when adsorption changes. The only exception is Fracmac which strongly influences all substances exportation. There is no evident correlation between physico-chemical characteristics and related macroporosity parameters. Yet,. Finally, influent parameters for water dynamic and balances in drainage do not fit with influent parameters for pesticides.

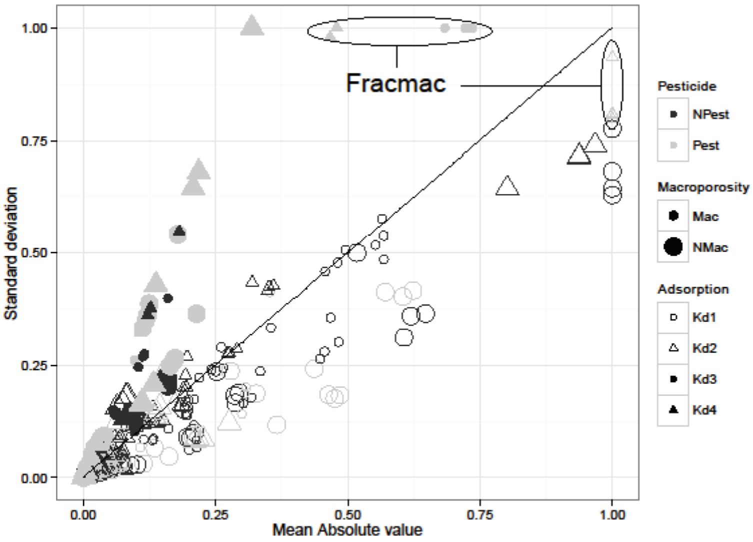

Figure 1 : Absolute mean (representing parameter effect) and standard deviation (representing interactions and/or non-linearities) obtained with Morris method on exportation of pesticide in drainage, for the 16 test substances, as function of Kd. Here Kd1 : 0.5, Kd2 : 5, Kd3 : 15, Kd4 : 50 L.kg^{-1}. The variable "Pesticide" indicates if parameters are related to physico-chemical characteristics (Pest) or not (Npest). Same thing for macroporosity related parameters.

Conclusion

Global sensitivity analysis was performed on 16 hypothetic pesticides, representative of a large set of physico-chemical characteristics. Results show a strong influence of these parameters on key processes that govern pesticide exportations. Thus, calibration of pesticide leaching seems to be highly substance specific. Influent parameters related to water output (balance and dynamic) don't necessarily fit well with solute results. Thus, this study tends to prove that water and pesticides transfers should be calibrated in a unique step, as was suggested by [7].

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ACT1086

FATE OF CYPRODINIL AND FLUDIOXONIL RESIDUES IN STORED APPLES AT OZONE-ENRICHED ATMOSPHERE

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In this paper the fate of two fungicides, cyprodinil and fludioxonil, residues on cold stored apples at continuously ozone-enriched atmosphere or at air storage conditions was studied. Ozone is one of the most potent sanitizers against a wide spectrum of microorganisms; it has been found to control various diseases during fruit or vegetable conservation/storage, to decrease mycotoxin contamination and pesticide residues and often can have physiological action to fruit and vegetable.

Granny Smith apple trees were sprayed with Switch WG (cyprodinil 37.5%, fludioxonil 25%) at 100g/100L at October and apple samples collected 24 hours after application were transported to cold rooms at 0-1°C and 95% RH for storage of 6 months. The rooms were continuously flushed with 300 ppb or not. During cold storage the fate of targeted pesticide residues was monitored monthly by analysing apple samples (three 15-fruit replicates for each treatment). Residue analysis and determination were performed by HPLC in a Nova Pack C18 150x3.9mm column with detection at 245 nm. A mixture of hexane and ethyl acetate was used for extraction followed by clean-up in SPE cartridges. The extract was evaporated until dry in a rotary evaporator under vaccum and the residues reconstituted in 1mL of methanol-water solution for liquid chromatographic analysis.

One day after application initial pesticide residues measured in apples sampled from experimental field were 1.09 ± 0.15 and 0.92 ± 0.14 mg/kg for cyprodinil and fludioxonil, respectively. These values are very close to MRL value imposed by EE for cyprodinil in apple (1.0 mg/kg), but significantly lower than the MRL value for fludioxonil (5 mg/kg). During the 5 months storage the measured concentration of residues in apples did not change for both pesticides and storage conditions. In recent study[1], after a single fumigation of table grapes with very high doses of ozone (10000 μL/L ozone for 1 h), iprodione and boscalid residues were not significantly reduced, while fenhexamid, cyprodinil, pyrimethanil, and pyraclostrobin residues were reduced by 68 to 100%. In our experimental condition the behaviour of studied pesticides during storage conditions is attributed mainly to their stability at low temperature conditions. The behavior of others pesticides in similar apple storage conditions is under study.

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PP36
ACT1089

REMOVAL OF TRIMETHOPRIM AS ORGANIC MICROPOLLUTANT IN AQUEOUS MATRICES USING MODIFIED CHITOSAN SUPER-ADSORBENT BEADS

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Summary

Cross-linked chitosan beads were prepared and evaluated in order to adsorptively isolate a pharmaceutical compound (trimethoprim) from aqueous matrices.

Introduction

Pharmaceuticals are of scientific and public concern as newly recognized classes of environmental pollutants and are receiving considerable attention with respect to their environmental fate and toxicological properties over the last 15 years [1,2]. Trimethoprim (TMP), a dihydropteroatesynthetase inhibitor, is commonly used in combination with sulfonamides for broad-spectrum antimicrobial therapy. It blocks the folic acid metabolism, and thus produces a synergistic antibacterial activity. The residue of TMP in manure andsoils may affect soil microbial and enzyme activities. Adsorption is a potential technique to remove TMP and overcome the pollution ofantibiotics in the environment. As adsorbent material, a polymeric super-adsorbent has been synthesized namely chitosan. Chitosan (poly-β-(1→4)-2-amino-2-deoxy-D-glucose) is an amino-polysaccharide, a cationic polymer produced by the N-deacetylation of chitin. Due to its molecular structure, chitosan exhibits many characteristics that have been the cause of much recent attention, since the range of its applications has enormously expanded in various fields including biotechnology, water-treatment, medicine and veterinary medicine, membranes, cosmetics and food industry. Chitosan presents high swelling degree in aqueous matrices, which usually leads to plugging of columns. In order to overcome this problem chitosan was cross-linked both covalently, by glutaraldehyde (GLA) and ionically, by a polyanion such as tripolyphosphate sodium (TPP).

Synthesis of cross-linked chitosan beads (CCB)

CCB were prepared by initially dissolving chitosan ($1.41\text{x}10^{-6}$ moles) in 50 mL of aqueous solution of acetic acid (2% v/v). The solution was added dropwise from a pipette into an aqueous solution of glutaraldheyde ($5\text{x}10^{-2}$ mol/L), which also contained tripolyphosphate ($1.36\text{x}10^{-3}$ moles) at pH=6, adjusted with an aqueous HCl solution. The formed gelled beads were stirred overnight at room temperature in the aforementioned solution. Then, after filtration and purification by extraction with water in a Soxhlet apparatus for 24 h, a conventional drying at 60 °C was realized. The resulting beads, (CCB) were used for the adsorption experiments.

HPLC Analysis

Identification and quantification of trimethoprim was accomplished with HPLC-DAD and CNW Athena C18, 120 Å, 4.6mm *250mm, 5 μm column. Binary gradient mobile phases were used, with water as solvent A and methanol as solvent B. The elution started with 70% methanol and 30% water. All the other parameters remained stable, flowrate was 1 mL/min, the column temperature was 40 °C and the injection volume was 20 μL. The detection wavelength was set at 287 nm. Further analysis was conducted for linearity, LOD and LOQ, repeatability and reproducibility. The UV spectra and the retention time of trimethoprim standard were used for the identification.

Results and Discussion

SEM showed that Trimethoprim adsorbed on chitosan modified beads wasabout 5μm in size and seemed to be in amorphous phase. This observation noted also from XRD patterns, which showed that Trimethoprim adsorbed is mainly in its amorphous form. However, there are four peaks of the drug present at the adsorbed matrix at 2theta 34.02, 37.84, 39.57 and 44.06 deg indicating that the drug is not completely amorphous. FT-IR was used to examine if bonds between drug and matrix were formed. The spectra of TMP exhibited bands at 3470 and 3319 cm^{-1} corresponds to v(N.H)asy and v(N.H)sy, respectively. These bands were sifted at 3445 and 3262 cm-1 respectively showing that hydrogen bonds with hydroxyl groups of matrix were formed.

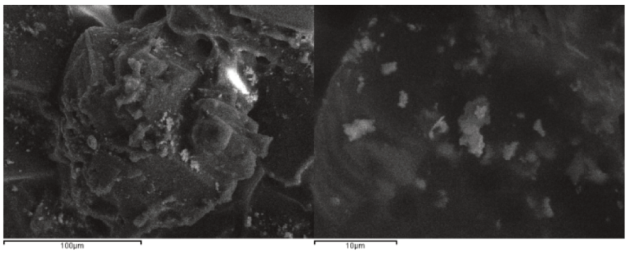

Figure 1. SEM photos of TMP adsorbed.

The experiments for the pH effect showed that the optimum value was at alkaline conditions (pH=10), while the maximum theoretical adsorption capacity was found to be 112 mg/g at 25 °C. The latter was found after fitting to the combinational isotherm model of Langmuir-Freundlich. The adsorption was reached at equilibrium very quickly (~80 min) after testing the optimum adsorption contact time.

Conclusions

The synthesis of CCB was effectively acted for the isolation (removal) of TMP compound from aqueous matrices, presenting 112 mg/g maximum theoretical adsorption capacity.

Acknowledgments

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PP37
ACT1093

SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS (MIPS) FOR THE SELECTIVE REMOVAL OF IBUPROFEN FROM BIOMEDICAL WASTEWATERS

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Summary

Molecularly Imprinted Polymers (MIPs) were prepared for the selective isolation of ibuprofen (pharmaceutical compound) from aqueous matrices of biomedical wastewaters.

Introduction

One of the most hot-topics of recent research is the reuse of some compounds existed as pollutants in environment. These compounds (molecules, ions, complexes, etc) are of high-added value and it will be ideal to selectively bind them with any environmental application and reuse them in their initial or modified form. The latter can be achieved using molecular imprinting. The whole process is based on adsorption technology, which is already one of the most successful techniques for pollutants removal.Pharmaceuticals are of scientific and public concern as newly recognized classes of environmental pollutants and are receiving considerable attention with respect to their environmental fate and toxicological properties over the last 15 years [1,2]. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) used for relieving pain, helping with fever and reducing inflammation. Ibuprofen has an antiplatelet effect, though it is relatively mild and somewhat short-lived compared with aspirin or prescription antiplatelet drugs. In general, ibuprofen also has a vasodilation effect.

Synthesis of (IBU-MIP)

For the preparation of IBU-MIP, 2mmol of MAA, 10 mmol EGDMA, 1.6 mmol IBU and 0.05 g AIBN were dissolved in 50 mL of the organic solvent DMF. The functional monomer and cross-linker were determined by the ratio of 2:10. The mixture of reagents was stirred for 2 h, followed by nitrogen sparging (~5 min) to remove oxygen. After then, the flask was immediately sealed and stirring was continued at 75 °C for 12 h. The flask was then smashed and the monolithic polymers obtained, were ground in a laboratory mortar and pestle. Next, the obtained particles were washed with acetone and hot water, followed by extraction in a Soxhlet apparatus using methanol, in order to remove residuals of the monomers and template molecules. The extraction lasted for 16 h, a sum of 30 – 35 solvent cycles (each cycle lasts approximately 30 min). At the end of the 16-h period, the higher percentage of template molecules (97%) was removed from the polymer matrix, giving that for non-covalent imprinting 85 – 95% was reported to be the ideal range of the template extraction from the polymer. Non-imprinted polymers (NIPs) were prepared with the same procedure just in the absence of the template molecule.

Results and Discussion

SEM showed that the surface of MIPs was not smooth but it had many channels and cavities.

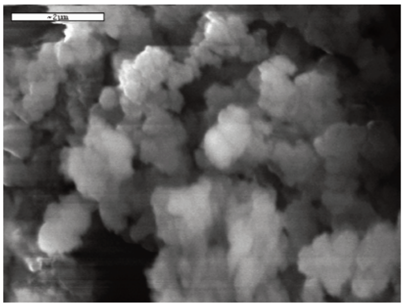

Figure 1. SEM photos of IBU-MIP adsorbed.

The experiments for the pH effect showed that the optimum value was at alkaline conditions (pH=10), while the maximum theoretical adsorption capacity was found to be 54 mg/g at 25 °C. The latter was found after fitting to the combinational isotherm model of Langmuir-Freundlich. The adsorption was reached at equilibrium slowly (~6 h) after testing the optimum adsorption contact time. The selectivity tests with other similar pharmaceutical compounds showed extremely large selectivity to IBU molecules, revealing the excellent use of this material as selective adsorbent.

Conclusions

The synthesis of IBU-MIPs was effectively acted for the isolation (removal) of ibuprofen compound from aqueous matrices, presenting 54 mg/g maximum theoretical adsorption capacity and very high selectivity degrees.

Acknowledgments

The support for this study was received from the State Scholarships Foundation (IKY) of Greek Ministry of Education and Religious Affairs (in the framework of the Hellenic Republic - Siemens Settlement Agreement) through the research program "IKY Fellowships of Excellence for Postgraduate Studies in Greece - Siemens Program" under the title "Advanced Molecularly Imprinted Polymers (MIPs) as materials for the selective binding and recovery of various high-added value environmental targets with application to industrial-scale adsorption columns", which is gratefully appreciated.

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PP38
ACT1109

SOIL DISSIPATION AND TRANSPORT OF THE HERBICIDE QUIZALOFOP-P-ETHYL IN SUNFLOWER CULTIVATED FIELD PLOTS

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Summary

Soil dissipation and transport in runoff water and sediment of quizalofop-p-ethyl (QpE), were investigated in a sunflower cultivation. The cultivation was carried out in silty clay soil plots with two surface slopes, 1% and 5%. The soil dissipation rate of QpE can be described by both first-order (SFO) and Gustafson and Holden (FOMC) kinetics. The half-life ranged from 0.55 to 0.68 and 0.43 to 1.04 days when SFO and FOMC kinetics were applied, respectively. QpE can be transferred by the runoff water, if a runoff event occurs in the first days after application (DAA) and, in small amounts, from sediment even up to 45 DAA.

Introduction

Sunflower is the mostly cultivated energy crop in Greece. Its cultivation is expected to increase as the demands for biofuels are growing. QpE, a systemic, post-emergence herbicide for the control of annual and perennial grass weeds, is one of the few herbicides registered for sunflower in Greece. QpE belongs to the arylphenoxy-propionic acid group and has low water solubility (0.61 mg/L, 20°C), low vapor pressure (1.1×10^{-4} mPa, 25 °C), high K_{OC} (log K_{OC} = 3.26) and high K_{OW} (log K_{OW} = 4.61) [1]. Limited data have been reported about the dissipation of the QpE in soil [2, 3] and no data exists in literature regarding the transport in runoff water and sediment.

Materials and Methods

The experimental field area (700 m^2) (silty clay soil) was divided in two groups of six plots each with a plot dimension of 4x10 m^2. One group of plots was used for the cultivation of rapeseed and the other group was the control plots (bare soil). Two different slopes (1% and 5%) were formed in each group. In the lower side of plots a surface runoff collection reservoir was established. Soil samples were randomly taken at two depths: 0–10 and 10–20 cm. Runoff water and sediment samples were collected, after every sufficient rainfall or irrigation event. QpE was applied as a water emulsion of a commercial EC formulation at a rate of 150 g/ha. Water samples (0.5 L) were extracted using SPE method with Oasis HLB SPE cartridges. For soil and sediment extraction a modified QuEChERS method was used [4]. All samples were analyzed by gas chromatography–mass spectrometry instrumentation (GC-MS).

Results and Discussion

QpE was detectable in upper soil layer (0–10 cm) for 6 days and its dissipation rate can be sufficiently described by both SFO and FOMC kinetics (Fig. 1). Using SFO kinetic model the half-life was estimated from 0.55 to 0.68 days when using FOMC kinetic model the half-life was from 0.43 to 1.04 days. No residues were detected at the depth of 10–20 cm.

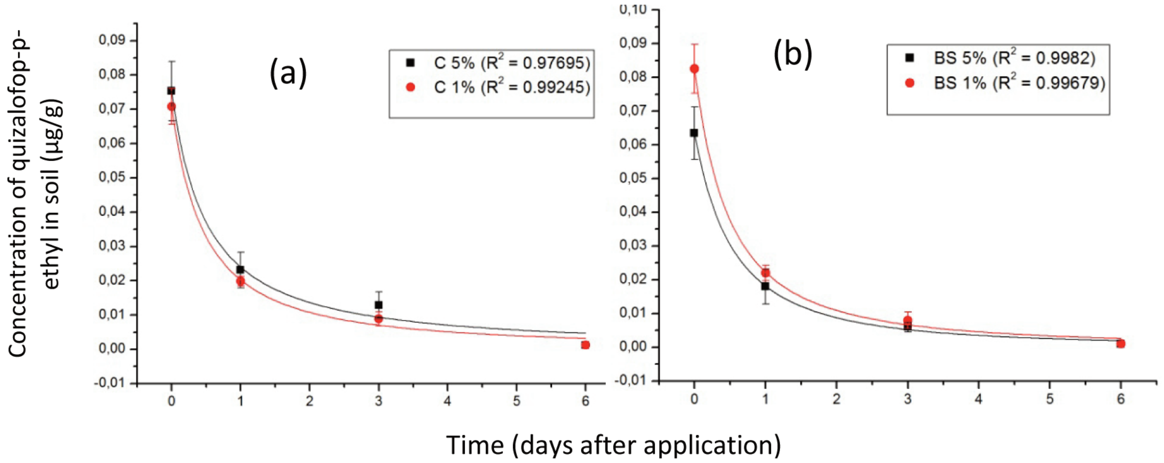

Figure 1. The dissipation of quizalofop-p-ethyl in soil layer of 0–10 cm in tilled (a) and untilled (b) plots with surface slops 5% and 1%. The lines fitted describing herbicide dissipation according to the FOMC kinetics.

QpE is present in runoff water in one sampling, 3 DAA, in concentrations between 1.70 and 2.04 μg/L. The cumulative losses from tilled and untilled plots with soil slope 5% were estimated at 0.0212% and 0.0140% respectively, of the initially applied active ingredient (IAAI); the losses from plots with 1% slope were estimated at 0.0022% and 0.0025% respectively. In the sediment, QpE was detected in 3 samplings, 3, 25 and 46 DAA. The residual concentrations 3 DAA ranged from 0.085 to 0.126 μg/g, 25 DAA from 0.013 to 0.022 μg/g and for 46 DAA the herbicide was detected in concentrations <LOQ. The cumulative losses in sediment from tilled and untilled plots with 5% soil slope were estimated at 0.0070% and 0.0048% of the IAAI; while with 1% slope the respective values were 0.00035% and 0.00031%.

Conclusions

Quizalofop-p-ethyl in soil has low half-life. The herbicide can be transferred by the runoff water if a runoff event occurs in the first days after application. Contrary, it can be found in small amounts in sediment up to 45 days after application.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

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PP39
ACT1110

ENVIRONMENTAL FATE OF METAZACHLOR HERBICIDE IN RAPESEED CULTIVATED FIELD PLOTS

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Summary

The environmental fate of the herbicide metazachlor (MTZ), was investigated under field conditions in a rapeseed cultivation over a period of 225 days. The cultivation was carried out in silty clay soil plots with two surface slopes, 1% and 5%. The soil dissipation rate of the herbicide was better described by first-order kinetics, the half-life was between 11.4 and 12.7 days and it was detectable up to 170 days after application (DAA). The herbicide was detected below the 10 cm soil layer 5 DAA up to 48 DAA. MTZ can be transferred in relatively high amounts by runoff water and sediment with maximum concentrations of 79.67 μg/L and 0.57 μg/g, respectively.

Introduction

Rapeseed is a growing energy crop cultivation in Greece during the last decade as the demand for biofuels is increasing. MTZ and quinmerac are the only herbicides registered in Greece for rapeseed cultivation. MTZ is a pre-emergence and early post-emerged herbicide for the control of annual grass and broad-leaved weeds. MTZ belongs to the chloroacetanilide group with a water solubility and vapor pressure of 450 mg/L and 0.093 mPa, respectively, K_{OC} value of 134 and log K_{OW} = 2.49. Until now very few studies have been conducted for the dissipation of MTZ in soil [1–3]. The transport of MTZ by runoff has been studied only under laboratory conditions [4]. No data exists in the literature regarding the transport in sediment.

Materials and Methods

The experimental field area (700 m^2) (silty clay soil) was divided in two groups of six plots each with a plot dimension of 4x10 m^2. One group of plots was used for the cultivation of rapeseed when the other one was the group of control plots (bare soil plots). Two different slopes (1% and 5%) were formed in each group. In the lower side of every plot a surface runoff collection reservoir was established. Soil samples were randomly taken at two depths: 0–10 and 10–20 cm. Runoff water and sediment samples were collected, after every sufficient rainfall or irrigation event. MTZ was applied as a water emulsion of a commercial SC formulation at a rate of 940 g/ha. Water samples (0.5 L) were extracted using Oasis HLB SPE cartridges. For soil and sediment extraction a modified QuEChERS method was used. All samples were analyzed by gas chromatography–mass spectrometry (GC-MS).

Results and Discussion

The dissipation of MTZ in soil was monitored for a time period of 225 days (Fig. 1). In upper soil layer the rate of dissipation was better described by the SFO model. The half-life in the cultivated plots was at 12 and 11.4 days for soil slopes 5% and 1%. In uncultivated plots the respective values were 11.4 and 12.7 days. The herbicides concentrations fell below LOQ 170 DAA. The herbicide was found present below the 10 cm soil layer at low concentrations 5 DAA and was detectable up to 48 DAA.

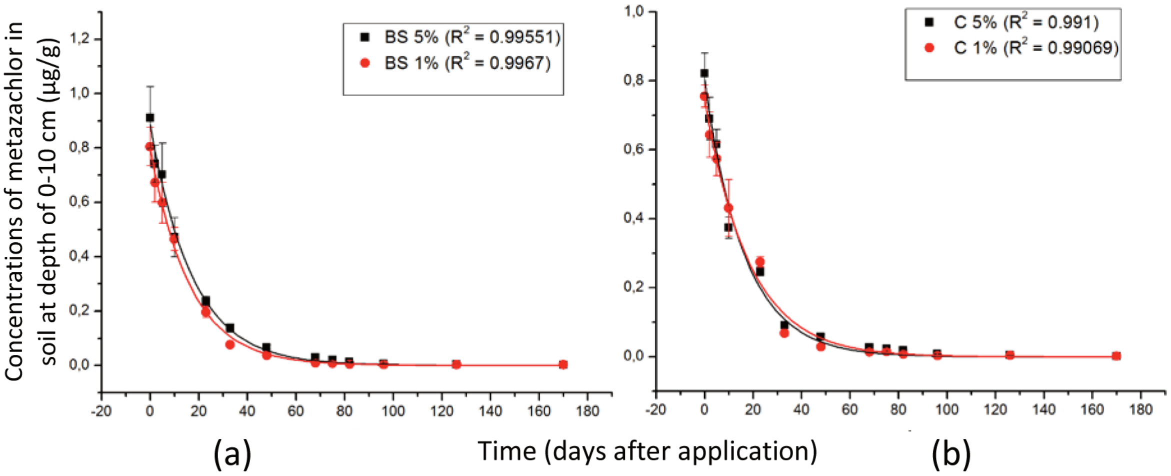

Figure 1. The dissipation of metazachlor in soil layer of 0–10 cm in untilled (a) and tilled (b) plots with surface slops 5% and 1%. The lines fitted describing herbicide dissipation according to the SFO kinetics.

In the first runoff event, 12 DAA, high amounts of MTZ were transferred by runoff water (70.14 to 79.67 μg/L). The concentrations of MTZ in runoff decreased in later runoff events and reached LOQ level at 210 DAA. The cumulative losses of MTZ from tilled and untilled plots with soil slop 5% were estimated at 0.31% of the initially applied active ingredient (IAAI) and the losses from plots with 1% slope were estimated at 0.13% and 0.12% respectively. In sediment the initial values of herbicide's residual concentrations (12 DAA) were ranged from 0.48 (cultivated 5% soil slope) to 0.57 μg/g (uncultivated 5% soil slope). The herbicide's concentrations were reached LOQ levels 207 DAA. The cumulative losses in sediment from tilled and untilled plots when soil slope was 5% were estimated at 0.13% and 0.12% of the IAAI; while when the slope was 1% the respective values were 0.024% and 0.022%.

Conclusions

Metazachlor in soil has low half-life between 11.4 and 12.7 days. The herbicide can be transferred by the runoff water and sediment in relatively high amounts, especially if a runoff event occurs in the first days after application.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

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PP40
ACT1026

MULTIRESIDUE METHOD FOR THE SIMULTANEOUS DETERMINATION OF 55 PESTICIDES IN WATERMELONS

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Summary

A liquid chromatography - tandem mass spectrometry technique combined with electrospray ionization (ESI), (LC–MS/MS) was applied for the detection of 55 pesticides in lettuce. The extracts were obtained using the acetonitrile-based QuEChERS sample preparation technique (EN 15662). For the LC analysis, an Agilent 1200 HPLC system with a binary pump was used. The mobile phase was methanol and Milli-Q water with 0.1% formic acid in gradient mode, with the flow rate of 0.6 ml/min. For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was applied. Agilent Mass Hunter Data Acquisition; Qualitative Analysis and Quantitative Analysis software was used for method development and data acquisition. The optimized analytical conditions were evaluated in terms of recoveries, repeatability, limits of quantification (LOQ), linearity and matrix effects for the investigated pesticides in watermelon. The matrix influence on linearity and recoveries and its effects on ionization were evaluated for this matrix. The calibration range was from 0.01 to 0.25 mg/kg. Recoveries were investigated at the 0.01, 0.05, and 0.1 mg/kg levels. Average recoveries for all analites were 61.0 to 114.2% (RSDs 7.41 -12.93%).

Introduction

Watermelon (*Citrus vulgaris* sin. *Citrus lanatus Thumb.*) is juicy, sweet and rich in β-carotene, B_1, C and E vitamins, and cooking oil, rich in vitamin D, can be extracted from seeds [1]. On our market there are 7 compounds registered for the use in watermelon protection out of which 1 is an insecticide, 4 herbicides and 4 fungicides. Since watermelons are predominantly used as fresh food and to a lesser extent in food processing there is a justified concern that, due to treatments, it can contain pesticide residues above the maximum residue levels – MRLs.

To evaluate the negative effects of pesticides in watermelons and to ensure the consumers safety, the validation of multiresidue LC/MS-MS method must be developed. The LC/MS-MS method has high selectivity and sensitivity, simplicity of sample cleanup, and easy and reliable indentification and quantification, even at trace levels [2]. The pesticide extraction was carried out by the most promising sample preparation techniques: Quick Easy Cheap Effective Rugged and Safe (QuEChERS) [3, 4].

The aim of this study was to develop a multiresidue method for the determination of 55 pesticide residues in watermelons.

Experimental

Chemicals: All solvents used were chromatography grade and were obtained from Merck (Darmstadt, Germany). The certified pesticide analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), most of them of purity ≥98%. The internal standard carbofuran-d3 (99.7%) was purchased from Pestanal, Fluka (Germany). The composite mixtures of all pesticides at appropriate concentrations were used to spike samples in recovery experiments.

Instrumentation. Agilent 1100 Series HPLC system with Zorbax XDB C18 analytical column of 50×4.6mm and 1.8 mm particle size (Agilent Technologies) column. For LC analysis, an Agilent 1200 HPLC system with a binary pump was used. For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was used. Agilent MassHunter Data Acquisition, Qualitative Analysis and Quantitative Analysis software were applied for method development and data acquisition.

Validation: The method was validated according to SANCO/12571/2013.The LOD was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated mathematically. The linearity was checked using matrix matched standards (MMS) at concentrations of 5, 10, 25, 50 and 100 ng/mL. The recovery was checked by enriching 10 g of a blank sample with the mixture of pesticide standard of 10 mg/ml in the amount of 100 and 50 µl (final mass concentration 0.10 and 0.05 mg/kg) and with the mixture of pesticide standard of 1 mg/mL in the amount of 100 µl (final mass concentration 0.01 mg/kg.) with the addition of the internal standard carbofuran-d3.

Results and Discussion

The LC-MS/MS was used for the simultaneous residue determionation of 55 pesticides in watermelon. The extraction was done using QuEChERS. The calibration was carried out in watermelon matrix in order to overcome the matrix effect. The R^2 were >0.99 for all the studied pesticides ranging from 5 to 100 ng/mL. The obtained mean values of the responds were in the range from 77. 5 to 102.4% with RSD <20%. The LOQs were 0.01 mg/kg.

Conclusions

An efficient, sensitive and reliable LC-MS/MS method has been developed which can be applied in the analysis of real samples to the 55 pesticide residues in watermelon.

Acknowledgments

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PP41
ACT1033

HIGH ACCURACY ON SINGLE RESIDUE ANALYSIS OF SELECTED PESTICIDES IN CUCUMBER BY GC-MS/MS

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The achievement of high precision, trueness and low uncertainties for analytical methods is of utmost importance when they are destined for the certification analysis of matrix reference materials. The introduction of hyphenated systems, coupling gas chromatography (GC) to triple quadrupole mass spectrometry analyzer (MS/MS) has been compared to single quadrupole mass spectrometry analyser (MS). This comparison opened the possibility for further improvement of selectivity and hence the sensitivity when quantifying the target compounds. These extended capabilities are being gradually introduced in the field of pesticide analysis in control and routine laboratories, which usually develop and validate analytical methods for the simultaneous determination of hundreds of pesticides.

In this work, this comparison has been investigated with the particular objective to certify a cucumber matrix reference material for pesticide residue analysis. A method based on GC-MS/MS, using stable isotopic labeled standards as surrogate, was developed and fully validated for a selected short list of representative pesticides (azoxystrobin, chlorpyrifos, cypermethrin, diazinon, α+β-endosulfan, fenitrothion, iprodione and malathion) in a cucumber matrix. The performance results obtained during validation were considered satisfactory for the purpose of certification for all target compounds with the exception of chlorpyrifos. Estimated expanded uncertainties ranged between 1.9 % for azoxystrobin, and 7.5 % for α+β-endosulfan inside a mass fraction working range between 0.004-0.100 mg/kg. Furthermore, for comparison purposes, samples were injected in parallel in a GC-MS single quadrupole system employing two different injection techniques, namely split-splitless (SSL) and programmed temperature vaporizing injection (PTV). The performance of both in-house developed methods, evaluated and critically assessed for their suitability during the production of a certified reference material, is presented here.

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ACT1070

DEVELOPMENT AND VALIDATION OF A METHOD FOR THE SIMULTANEOUS DETERMINATION OF PAHs AND NDL-PCBs IN FISH USING GAS CHROMATOGRAPHY COUPLED TO MASS SPECTOMETRY

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Summary

A new method for the simultaneous determination of PAHs and non dioxin like PCBs in fish was developed and validated using a GC-EI-MS system. The efficacy of different solvents for a quantitative extraction was studied, as well as the efficacy of different sorbents (either using dispersive solid phase extraction (d-SPE) or a usual solid phase extraction (SPE) for sample clean up. The method was statistically validated at two concentration levels by assessing the following parameters: accuracy, precision, limit of quantification, uncertainty. Calibration curves were established for all analytes and regression analysis was performed at 95% confidence level. Matrix effect was also studied and the method was used for monitoring purposes analysing fresh or frozen fishe from Greek retail market as well as for smoked fish products.

Introduction

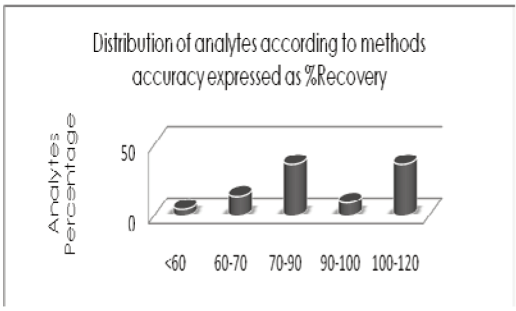
PAHs (products of incomplete combustion and pyrolytic processes) and PCBs (synthetic organic compounds) are widespread organic pollutants, which can be detected almost everywhere (water, soil, air) and can be highly toxic and mutagenic. They can accumulate mainly in invertebrates and fishes and enter the food chain. The maximum levels for their presence in fish are set by directives 1881/2006/EC and 1259/2011/EU [1,2]. Benzo[a]pyrene is used as an indicator for the presence of PAH's in non smoked fish products, whereas for smoked products the sum of four PAHs is used. Many methods have developed for the determination for both categories in fish which mainly include a Soxhlet extraction, supercritical fluid extraction and microwave assisted extraction. For sample clean up, gel permeation chromatography or solid phase extraction is usually used. Determination takes place mainly with GC/MS system. Nonetheless LC-UV or LC-FLD can be used for PAHs determination [3].

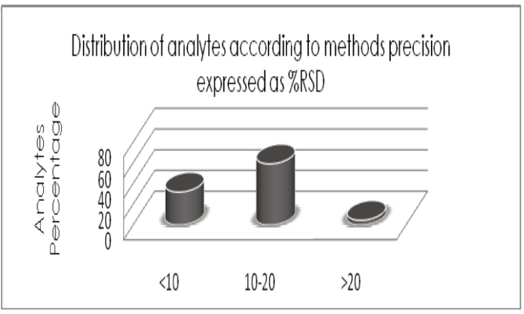
Method Development

2 g of homogenized fish tissue were weighed in a 50 mL Teflon tube. 10 mL of ethylene chloride are added and the tube is shaken for ten minutes. A mixture of magnesium sulphate and sodium chloride is added and the tube is shaken for five more minutes and centrifuged. 6 mL of the supernatant are evaporated under a gentle stream of nitrogen, reconstituted in 0,2 mL of n-Hexane and loaded to a previously conditioned and equilibrated Isolute® SI cartridge. The analytes were eluted by 3 mL of DCM:n-Hexane 3:7. The eluate was evaporated and reconstituted in 0,5 mL of ethyl acetate. The final extract was injected into a GC-EI-MS system in selected ion monitoring (SIM) acquisition mode.

Results and Discussion

Accuracy and precision were calculated from recovery experiments at two different concentration levels with 5 replicates per level (0.0033 µg/g and 0.0066 µg/g for PAHs and 0.0016 µg/g and 0.0032 µg/g for PCBs). A summary of results is shown on the graphs below.





The limit of quantification was set as the lowest concentration level with acceptable accuracy and precision, thus 0.0033 μg/g and 0.0016 μg/g for PAHs and PCBs respectively. Calibration curves were established for all analytes and regression analysis was performed at 95% confidence level. For each case the correlation coefficient was > 0.99. Matrix effect experiments showed that matrix influences detector's response, so for quantification purposes matrix matched standards have to be used.

Application of developed method to real samples

Ten samples were analysed, 3 of which were smoked fish products. No NDL-PCBs were detected at concentrations greater than 0.0016 μg/g. 70% of the samples were found positive for PAHs and most frequently detected one was benzo[b]fluoranthene. The highest concentration detected was for naphthalene (28.5 μg/Kg) in smoked salmon fish.

Conclusions

A new method for the simultaneous determination of PAHs and non dioxin like PCBs in fish was developed and statistically validated using a GC-EI-MS system. The method includes extraction by methylene chloride and sample clean up by SPE using Isolute® SI cartridges. The method was used for monitoring purposes analysing fresh and frozen fishe as well as smoked fish products.

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ACT1092

APPLICATION OF GC-MS AND LTQ-ORBITRAP-MS FOR THE DETERMINATION AND RISK ASSESSMENT OF EDCS IN FRUITS AND VEGETABLES

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Summary

In the present study endocrine disrupting pesticides belonging to different chemical classes were analyzed in fruits and vegetables from the region of Epirus (NW Greece) by two extraction methods and two chromatographic techniques for the determination of pesticide residues. A UHPLC-LTQ Orbitrap Mass Spectrometer technique and gas chromatography-mass spectrometry (GC-MS) were applied in the detection of thirteen endocrine disrupting compounds. Chronic dietary exposure to EDCs residues was assessed for the Epirus population.

Introduction

An endocrine disrupting compound (EDC) is defined as "exogenous substance or mixture that alters the function of the endocrine system and generate noxious effects on the health of a safe body, its descendants, or its sub-population" [1, 2] at the European Union level, EDCs are included in the list of so-called emerging contaminants [3]. In terms of adverse health effects, there is concern that substances with endocrine-disrupting properties may be causally involved in a number of diseases or conditions, such as hormone-dependent cancer, reproductive disorders, a decline in fertility, or obesity [4]. EDCs are ubiquitous in the environment because of their very frequent use in residential, industrial, and agricultural applications in particular, the origin and fate of these contaminants can lead to their transmission in the food chain [5]. It is widely accepted that food and diet are among the most important exposure routes for EDCs. There are many types of EDCs in food, ranging from natural compounds (e.g., hormones, phytoestrogens, and mycotoxins) to synthetic compounds (e.g., pesticides, pharmaceuticals, and industrial or process chemicals) [6]. An urgent demand appears for the development of analytical methods to monitor EDCs in food so that regulatory limits may be enforced.

The aim of the present study is to evaluate the endocrine disrupting pesticides of fruits and vegetables by two extraction methods and two chromatographic techniques. A UHPLC-LTQ Orbitrap MS technique and GC-MS technique were applied in the detection of thirteen targeted endocrine disrupting compounds and non-targeted compounds. For UHPLC-LTQ Orbitrap MS extraction method was evaluated using QuEChERS sample preparation technique. For GC/MS technique Ultrasound assisted emulsification microextraction (USAEME) was developed and optimized for the determination of targeted analytes activity in fruits and vegetables.

Sampling and Analysis

Fruits and vegetables were collected from the local supermarkets in the region of Epirus (NW Greece). The samples were blended using a multi machine to obtain a homogeneous mixture and stored them at 4 °C for at least 30 minutes [7]. In the USAEME method, the mixture was centrifuged and then the supernatant was diluted by ten times. Chloroform used as extraction solvent and was added to water sample in a conical centrifuge tube. The centrifuge tube was then immersed in the US water bath. After ultrasonification a coarse dispersion system named as emulsion could be formed. Then the coarse dispersion sample was centrifuged. Accordingly, the dispersed extraction solvent was sedimented in the bottom of the tube. Then the extraction solvent was injected to GC for further analysis. For UHPLC-LTQ Orbitrap MS extraction method was evaluated using the QuEChERS sample preparation technique, followed the standard approach, for non-targeted screening of 510 pesticides (included thirteen targeted EDCs) in food samples.

Results and Discussion

Optimisation of USAEME method was performed by evaluating the different parameters (pH, ionic strength, solvent, temperature, extraction time) that influence the recovery of the analytes in the extract. After optimization step, the analytical characteristics of the method were evaluated according to the guidelines included in the SANCO/12571/2013 document. High level of linearity for all targeted analytes was recorded with coefficient of determination values (R^2) above 0.990, while repeatability (intra-day) and reproducibility (inter-day) varied from 7% to 12% and 9% to 17%, respectively. Limits of detection (LODs) and limits of quantification (LOQs) were found to range in the low ppb level. The obtained extraction recoveries ranged from 88 to 119%. The proposed method provides high selectivity, enrichment and reproducibility. USAEME was applied for the determination of the targeted EDCs in fruit and vegetables samples taken from traditional and local markets from the region of Epirus (NW Greece). For non-targeted screening of 510 pesticides was achieved within twelve minutes and the high mass resolution and accuracy of the Exactive mass spectrometer enabled identification of all compounds.

Acknowledgments

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program “THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013” of the National Strategic Reference Framework (NSRF 2007-2013)

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ACT1098

NON-TARGETED SCREENING AND ACCURATE MASS CONFIRMATION OF PESTICIDES AND MYCOTOXINS ON THE UHPLC-LTQ-ORBITRAP MASS SPECTOMETRY

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Summary

Two rapid, sensitive, accurate and reliable multiresidue methods for screening and confirmation of 510 common agricultural pesticides in fruits and vegetables and 32 mycotoxins and their metabolites in European beers, with full spectral Orbitrap MS detection using electro spray source ionization (ESI) were applied. Prior to instrumental analysis of multi-class pesticides, an extraction procedure based on QuEChERS method (quick, easy, cheap, effective, rugged and safe) was used. As concerning to the multi-mycotoxin analysis in beer samples, a liquid-liquid extraction method was applied.

Introduction

Pesticides are applied to crops throughout the world but they can be toxic and can thus be harmful to human health. More than 800 pesticides belonging to over 100 different chemical classes are used. The residue of pesticides left after treatment may penetrate plant tissues and appear in the pulp and juice of fruits and vegetables, although their concentrations are, in general, lower than those observed in whole fruit [1]. Pesticides can also remain as residue in foodstuffs after their application and can spread in the environment. The presence of pesticide residues in food is one important concern for consumers, due to their possible long-term adverse health effects. Government agencies and international organizations limit the amount of pesticides in food establishing maximum residue limits (MRLs), with the aim of protecting consumer's health. Several European Union (EU) directives have set different MRLs for pesticide residues in vegetables and fruits at the low microgram per kilo level [2].

The production and consumption of beers is a widespread practice. Maize is the most common ingredient for the brewing process. This commodity is prone to pre- and post-harvest toxigenic fungal colonization and mycotoxin contamination. Mycotoxins are of public concern due to their association with a wide array of adverse health effects. The risk of mycotoxin contamination increases during the malting step which involves raising moisture content of the grains and the humidity of the environment. Some studies have reported almost 100% carry-over of mycotoxins from malted grains into beer and/or fermentation residues [3]. In contrast, other workers have reported significant reduction of mycotoxins following alcoholic fermentation [4]. Nonetheless, there are numerous reports of mycotoxin incidences in beers worldwide [5] [6].

The aim of this study, therefore, was to quantitatively determine pesticides and mycotoxins present in foods and drinks and to estimate associated dietary exposures. The data presented in this study may be useful in facilitating improved pesticide and mycotoxin risk assessment in Greece.

Sampling and Analysis

Fruits and vegetables were collected from the local supermarkets in the region of Epirus (NW Greece). The samples were blended using a multi machine to obtain a homogeneous mixture and stored them at -20ºC ºC for at least one hour. Samples were prepared for analysis using QuEChERS method: 10 g sample was extracted with 10 mL acetonitrile. The mixture was shaken vigorously in a vortex for 1 min. Afterwards, the extract was partitioned by adding $MgSO_4$, NaCl, $C_6H_5O_7$ $2H_2O$.3Na, $C_6H_8Na_2O_8$ another step of manual agitation and vortex followed. Then the sample centrifuged. An additional cleanup step was performed, 6 mL extract MgSO4, PSA and C18 were used. After that, it was shaken vigorously in a vortex for 1 min and centrifuged. In supernatant MeCN and an amount of 8mM Ammonium formate buffer was added. An aliquot of 10 μl was injected into the UPLC-LTQ- Orbitrap MS.

Several bottled commercial beer samples were purchased from local stores from the region of Epirus, and kept at 4 °C at freezer. Each beer sample was gently shaken and approximately 4 ml was degassed by ultra-sonication. After the addition of 16 mL acetonitrile, the content was shaken for 1min. The dark colored matrix precipitated under these conditions and was then separated by centrifugation. In the next step, 5 mL of the supernatant was evaporating to dryness and reconstituted in 1 mL MeOH: H_2O (50:50, v/v). Finally, microfiltration was performed prior to injection.

Conclusions

The proposed rapid and robust methods for multi-residue screening analysis in combination with full spectral Orbitrap MS detection using electro spray source ionization (ESI) are ideally suited for the routine monitoring of non-targeted pesticides and mycotoxins by regulatory laboratories.

Acknowledgements

This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

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ACT1011

NEW ORGANOMETALLIC COMPOUNDS OF TIN: ASSESSMENT OF THE *IN VITRO* GENOTOXICITY

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Summary

In the present study, four new organometallic compounds of tin were tested for their possible geno- and cyto-toxic effect towards human lymphocyte cultures using the cytokinesis block micronucleus assay (CBMN assay). The results obtained indicate that at low concentrations there is no increase in micronuclei (MN) frequencies by these organotin compounds, when compared with control cultures. Cytotoxic effects of organotin compounds were evaluated by the determination of cytokinesis block proliferation index (CBPI). Our observations on the CBPI values of the tested compounds indicate that they are cytotoxic in several tested concentrations to human lymphocytes treated *in vitro*. The observed differences in cytotoxicity of each tested compound might reflect differences in their chemical structure.

Introduction

The indiscriminate use of pesticides in the past decades and their bioaccumulation in the ecosystem and the food chain led to adverse effects on humans and the environment.

In an effort to produce environmentally friendly pesticides a large amount of organometallic compounds was designed. Among them, organotin compounds constitute a special class due to the wide broad of applications they present, such as coating agents, stabilizers, catalysts in the plastics' industry, wood preservatives and agricultural biocides [1-5].

Towards this goal, four new organometallic Sn(II) and Sn(IV) compounds namely $L_{OEt}SnCl$ (**1**), $L^*_{OEt}SnCl$ (**2**), $L_{OEt}SnPh_3$ (**3**), $L^*_{OEt}SnPh_3$ (**4**), incorporating the oxygen tripodalligands $[(\eta^5\text{-}C_5R_5)Co\{P(OEt)_2O\}_3]^-$, {R = H, ($L_{OEt}^-$); Me ($L^*_{OEt}{}^-$) were synthesized recently.The choice of the ligand is based on the chemical inertness of these ligands along with their thermal and hydrolytic stability [6-8].

In a recent study eight tin (II) and tin (IV) compounds, used as precursors for the synthesis of organotin compounds did not induce statistically significant micronuclei in the absence of metabolic activation, indicating that they are not genotoxic [9].

The above referred four new organotin compounds were tested for their possible genotoxic and cytotoxic activity towards human lymphocytes with the CBMN assay.

Materials and Methods

The CBMN assay was performed according to the standard procedure, with minor modifications [10-11]. All tested compounds were studied in human peripheral blood lymphocytes cultures at final concentrations of 1, 5, 10, 20, 50 and 75μM. In total, 4000 BN cells, with preserved cytoplasm, were scored per experimental point. To determine possible cytotoxic effects, the cytokinesis block proliferation index (CBPI) was calculated by counting at least 2000 cells for each experimental point.

Results and Discussion

Induced MN frequencies (genotoxic effect) in comparison with the control were seen only at the higher tested concentration of **1** (75 μM) as well as at the concentration of 50 μM of **2**, while **2** at the highest concentration (75 μM) was lethal for the lymphocytes. In the meantime **3** and **4** revealed not to be genotoxic at the concentrations of 1, 5 and 10 μM. However **3** appeared to be lethal towards the lymphocytes at 50 and 75 μM and **4** revealed genotoxic effects in comparison with the control in the MN frequencies at 20, 50 and 75 μM.

Overall, at low concentrations no genotoxic effect was observed for the **1** and **2** compounds, while compounds **3** and **4** were genotoxic at 20 μM and higher. Compound **1** appeared to be cytotoxic at the concentrations of 20, 50 and 75 μM, while **2** is cytotoxic at all tested concentrations. Compound **3** appeared cytotoxic at the concentrations of 5, 10 and 20 μM but extremely toxic at 50 and 75 μM, while **4** appeared cytotoxic at all tested concentrations.

Compounds	Genotoxicity						Cytotoxicity					
	Concentration (μM)						Concentration (μM)					
	1	5	10	20	50	75	1	5	10	20	50	75
$L_{OEt}SnCl$ (**1**)	-	-	-	-	-	+	-	-	-	+	+	+
$L^*_{OEt}SnCl$ (**2**)	-	-	-	-	+	NC	+	+	+	+	+	NC
$L_{OEt}SnPh_3$ (**3**)	-	-	-	-	NC	NC	-	+	+	+	NC	NC
$L^*_{OEt}SnPh_3$ (**4**)	-	-	-	+	+	+	+	+	+	+	+	+
-: negative; +:positive; NC: No Cells												

Conclusion

At low concentrations the above mentioned organotin compounds did not induce any genotoxic effect towards human lymphocyte cultures. On the contrary, our results revealed increased cytotoxicity in several concentrations for all of the tested organotin compounds. Their cytotoxicity pattern indicates that they should be used cautiously. Their possible future agricultural utilization is under investigation.

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ACT1012

CHANGES IN ANTIOXIDANT SYSTEMS IN SOYBEAN AND ASSOCIATED WEEDS TREATED WITH LINURON AND DIMETHENAMID

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Summary

Changes in antioxidant systems in soybean and associated weeds were studied in relation to treatment with herbicides linuron and dimethenamid. Differences in the total superoxide dismutase (SOD) and catalase (Cat) activities were observed in plants after application of herbicide formulation. Quantities of superoxide ($O_2^{\cdot -}$) and hydroxyl ($^{\cdot}OH$) radicals and malondialdehyde (MDA), reduced glutathione (GSH) and total polyphenols (TP) content were also determined. In addition to this, potential antioxidant activity of the plant EtOH extracts were assessed based on the scavenging activity of the stable DPPH free radicals.

Introduction

Linuron (3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea) (trade names include Afalon, Afalon Inuron, DuPont 326, Linex *etc.*) is a substituted urea herbicide used to control annual and perennial broadleaf and grassy weeds on crop and noncrop sites. It is used as a pre- and a postemergent herbicide and works by inhibiting photosynthesis in target weed plants [1]. In our study, linuron was combined with another herbicide, dimethenamid ((*RS*)-2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl) acetamide, trade name Frontier) which is used for control of annual grasses and certain broadleaf weeds, primarily in corn and soybeans. It belongs to the chemical class chloroacetamide.

Since influence of linuron and dimethenamid on antioxidant systems in weeds is so far unsufficiently investigated the aim of this study was to determine the effect of herbicide formulation on different plant`s enzymatic and and non-enzymatic antioxidants.

Experimental procedures

The Serbian high productive soybean cultivar Sava was used for the experiment together with four important weed species commonly growing in soybean fields: *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Convolvulus arvensis* L. and *Sinapis arvensis* L. Experimental field was divided in two blocks – one treated with herbicide formulation (Afalon, active compound linuron 2 L ha^{-1} + Frontier, active compound dimethenamid 1.25 L ha^{-1}). The nontreated one was used as a control. Leaves from 21-day-old plants were collected for the biochemical assays.

$O_2^{\cdot -}$ level was determined by the inhibition of adrenaline autooxidation, $^{\cdot}OH$ was measured by the inhibition of deoxyribose degradation and the SOD activity was determined by the method of Misra and Fridovich [2]. The lipid peroxidation (LP) was measured as malondialdehyde (MDA) production with thiobarbituric acid (TBA) [3]. The GSH quantity was determined with Elman reagent and Cat activity according to Malenčić et al. [4]. TP were determined after reaction with Folin-Ciocalteu reagent [5] and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was assessed to evaluate the antioxidant potential of test samples [6].

Results and Discussion

Results show that the plant species examined were partially susceptible to the action of toxic oxygen species. $^{\cdot}OH$, in the leaves of *A. artemisiifolia*, *C. album* and *C. arvensis* were produced in larger quantities after herbicide treatment, as well as $O_2^{\cdot -}$ in *C. album* (Table 1). LP occurred in all weeds but not in *Ambrosia*, as well as in soybean. SOD and Cat activities increased in most of the specimen, especially in *Chenopodium*. GSH and TP did not change significantly among treatments and plants. All weed extracts exhibited much higher DPPH-radical scavenging activities (in % of control) compared to soybean.

Table 1. Effect of linuron and dimethenamid on the biochemical parameters in soybean plants and associated weeds (C – control; T – treated)

	soybean		*Ambrosia*		*Chenopodium*		*Convolvulus*		*Sinapis*	
	C	T	C	T	C	T	C	T	C	T
$O_2^{\cdot -}$ (μM g^{-1} fr.m.)	43.3	32.5	17.5	17.0	12.5	32.0	22.9	13.0	31.0	24.4
$^{\cdot}$OH (nM g^{-1} fr.m.)	540	633	378	588	520	880	127	483	1422	611
MDA (nM g^{-1} fr.m.)	159	168	112	108	115	330	59.7	136	88.5	93
SOD (U g^{-1} fr.m.)	875	588	223	380	801	1200	388	577	875	975
Cat (U g^{-1} fr.m.)	68.8	96.3	90.7	100	375	492	105	41.3	339	197
GSH (μM g^{-1} fr.m.)	10.8	12.3	14.2	10.8	8.5	10.8	7.3	7.7	15.0	8.9
TP (mg g^{-1} dr.m.)	1.06	1.5	0.75	0.90	1.77	1.70	4.12	4.10	1.39	1.47
DPPH (%)	6.78	24.3	30.0	45.0	35.6	37.1	43.7	45.4	30.1	34.2

Conclusions

On the basis of our results several conclusions could be drawn: 1) investigated plants expressed different antioxidant systems in response to herbicide treatment; 2) enzymatic and non-enzymatic protective mechanisms were complementary; 3) some weeds showed distinctive and combined activity of several biochemical parameters, such as *A. artemisiifolia*. Results obtained for antioxidant properties of this species could partially explain its biological viability and ecological success. Further monitoring of antioxidant status of this plant, along with other weed species growing in the region is necessary.

Acknowledgments

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PP47
ACT1014

EFFECTS OF NATURALLY OCCURING ALUMINOSILICATES ON SERUM BIOCHEMICAL INDICES OF CHICKENS INTOXICATED BY PARAQUAT

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Summary

In this study experiments with the herbicide paraquat were carried out on broilers. The concentration of selected biochemical indices in the blood serum of broilers chronically exposed to pararaquat was examined. The influence of feed additive (AntiToxic Nutrient-ATN) based on natural occurring aluminosilicates (zeolites and clays), on paraquat-induced changes was also examined. Serum concentration of creatinine was increased after oral intake of paraquat. Aluminosilicates alone did not provoke any adverse effect and did not disturb normal biochemical homeostasis in blood serum. The combined data showed that chicken fed aluminosilicates received protection against the effects of the paraquat for measured parameter.

Introduction

Paraquat (PQ) (methyl viologen, 1,1′-dimethyl-4,4′-bipyrydinium dichloride) is a non-selective contact herbicide widely used for broadleaf weed control [1]. PQ is highly toxic to humans and animals and notorious because of the large number of human deaths worldwide that have followed its ingestion [2]. Since there are no known pharmacological antagonists for PQ, the management of PQ poisoning has been limited to decrease its absorption, to enhance its elimination or to prevent or reverse LP at the cellular level with antioxidant compounds. It was concluded from adsorption studies that the mineral adsorbents could be an effective adsorbent for removing PQ from water solutions [3]. Clays and zeolites are hydrated and composed mostly of aluminium and silica: belonging to the group of aluminosilicates [4-5].

In this study, we investigated effect of feed additive based on natural occurring hydrated aluminosilicates (Antitoxic nutrient-ATN) in preventing or minimizing the oxidative stress in liver, kidney and pancreas induced by chronic administration of paraquat in broiler chickens.

Material and methods

Eighty four 1-day-old, broiler chicks were divided at random into four groups: 1. Control: basal diet; 2. ATN: basal diet plus 5.0 g ATN kg^{-1} diet; 3. PQ: basal diet plus 70 mg paraquat kg^{-1} diet; 4. PQ + ATN: basal diet plus 70 mg paraquat plus 5 g ATN kg^{-1}. ATN (Antitoxic nutrient) is a fine powder containing mostly zeolitic ore and bentonite, together with small amounts of activated charcoal (ratio 60:20:1/zeolite:bentonite:charcoal). When the chicks reached 3 weeks of age, the feeding trial was terminated and broilers were bled via cardiac puncture for serum biochemical analyses. Serum concentrations of total protein, creatinine, glucose, and α-amylase (AMY) activity were determined on a clinical chemistry analyzer. Results were expressed as mean of determinations ± standard error (SE). Statistical significance was tested by analysis of variance followed by comparison of means by Duncan's multiple range test ($P < 0.05$).

Results and Discussion

Data presented in Table 1 show the effects of the dietary treatments on selected serum biochemical values and AMY activity of broilers. There were no differences in the serum concentrations of total protein, glucose, calcium and AMY activity between the 4 groups, whereas there were differences between PQ and other groups in the term of serum creatinine. The significant increase in creatinine level noted in this investigation is classical sign of adverse affects of

PQ administration on kidney [7]. Kidney dysfunction and nephrotoxicity induced by PQ in the present study are probably mediated through oxidative stress. Unlike other authors [7-8], we did not observe any change in the concentration of total serum protein and glucose.

Table 1. Effects of paraquat and aluminosilicates on serum biochemical values and α-amylase activiy of broiler chicks.

	Experimental group			
	Control	ATN	PQ	PQ + ATN
Protein [g/l]	$24.57^{a} \pm 0.75$	$24.80^{a} \pm 0.74$	$25.71^{a} \pm 0.42$	$23.86^{a} \pm 1.06$
Glucose [μmol/l]	$13.88^{a} \pm 0.21$	$13.90^{a} \pm 0.17$	$12.44^{a} \pm 0.39$	$13.29^{a} \pm 0.64$
Creatinine [mmol/l]	$20.67^{a} \pm 0.49$	$21.80^{a} \pm 0.86$	$23.80^{b} \pm 0.37$	$20.43^{a} \pm 1.31$
Ca^{2+} [mmol/l]	$2.24^{a} \pm 0.08$	$2.36^{a} \pm 0.20$	$2.44^{a} \pm 0.05$	$2.42^{a} \pm 0.7$
α-amylase (AMY) [IU/ml serum]	$888.2^{a} \pm 40.1$	$1044.8^{a} \pm 40.1$	$919.6^{a} \pm 62.5$	$927.6^{a} \pm 119.9$

The data are mean values ± standard error
^{a,b} Values without the same superscripts differ significantly ($P < 0.05$)

It was observed that oral intake of ATN did not cause impairment of the selected biochemical indices. These results are in agreement with our previous works [9]. There was no significant difference in serum indices in animals treated with PQ along with ATN. This result indicates that ATN provides full protection to the broiler chickens exposed to harmful PQ treatment.

Conclusions

Co-administration of naturally occurring aluminosilicates (ATN) offers protection against PQ-induced adverse effects in the blood serum of broiler chickens. It is possible that ATN has the ability to absorb PQ in the lumen of the digestive tract.

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PP48
ACT1021

ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN AUTOPSY LUNGS FROM EPIRUS, GREECE

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Introduction- Aim

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are synthetic organic compounds characterized as Persistent Organic Pollutants (POPs). These chemicals are monitored worldwide, because they bioaccumulate in human bodies resulting in consequent long term toxic effects. The aim of this study was the determination, with a validated analytical methodology, of the concentrations of selected OCPs and PCBs in autopsy lungs from Epirus, a relatively restricted region in north-western Greece.

Materials and Methods

Lungs were collected at autopsy from thirty routinely autopsied deceased bodies of both sexes and different ages, during the period 2010-2012. Sample preparation was carried out with extraction by matrix solid phase dispersion (MSPD); separation and determination of target compounds was performed by gas chromatography - electron capture detector (GC-ECD). The procedure allowed the simultaneous determination of 20 OCPs (aldrin, endrin, dieldrin, α- β- γ- and δ-BHC, α- and γ-chlordane, *p,p'*-DDE, *p,p'*-DDT, *p,p'*-DDD, endosulfan I and II, endosulfan sulphate, endrin aldehyde, heptachlor, heptachlor epoxide, endrin ketone and methoxychlor) and six PCBs (PCB 28, 52, 101, 138, 153 and 180) [1, 2].

Results

Thirty lungs collected from respective number of autopsy cases were analyzed; 19 males and 11 females residents of the Region of Epirus. The age range of individuals were from 14-91 years (mean ± SD = 61.8 ± 22.5 y). Twenty two cases (73 %) were positive simultaneously for at least one POP while eight cases were negative (27%). Based on the age of the deceased positive cases were distributed as follows: 2 out of 6 positive cases within age range 14-45 years (33.3%); 5 out of 7 cases within age range 46-65 years (71%); and, 15 out of 17 cases with age >66 years (88%). PCBs were the most abundant class of contaminants detected in 15 out of the 30 cases (50%). DDTs and HCHs were second and third in abundance, with 9 (30%) and 8 (27%) positive cases, respectively.

Conclusions: The frequency of detection of OCs shows a tendency to increase with age of individuals. The patterns of OCs found in human autopsy lungs were similar to those reported previously for other human specimens. Our results suggest that a similar trend in contamination sources and distribution has occurred, and given their high diversity and persistence in the environment, they enhance the possibility of exposure of individuals through environmental routes, mainly the diet. The results indicate that human autopsy material could be used for biomonitoring studies of xenobiotics.

Keywords: organochlorine pestisides, polychlorinated biphenyls, human lung, autopsy

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PP49
ACT1029

HIGHER-TIER RISK ASSESSMENT OF INSECTICIDE IMIDACLOPRID IN PORTUGUESE RICE PADDIES: PART I – CALIBRATION OF RICEWQ MODEL

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Summary

Rice production presents a unique environmental scenario with respect to pesticide off-site movement. Given the lack of studies on Mediterranean countries into (higher-tier) fate modelling of insecticides in rice paddies and effect assessment on field communities required to ensure protection of aquatic life from pesticide stress, this study aims to evaluate the fate of imidacloprid after application to an experimental rice plot in Portugal and evaluate the accurateness of higher-tier models, as recommended by MED-Rice.

Introduction

To protect sensitive freshwater ecosystems against pesticide side-effects, a so-called tiered approach has been adopted in the European Union (EU) for the admission of pesticides on the market. The first tier is based on the comparison of the toxicity (NEC: No Effect Concentration) and the predicted exposure (PEC: Predicted Environmental Concentration; (EC) No 1107/2009). The PEC is calculated using environmental fate modelling as developed by FOCUS group. Higher tier studies have been performed mainly in Atlantic Central Europe/North America, results have been extrapolated to other climatic regions (e.g. Mediterranean) [1] but the climatic/ecological conditions are quite different, so it may be expected that fate and effects of pesticides are also different [2].

Rice production presents a unique environmental scenario with respect to pesticide off-site movement [3] and mathematical methods as developed by FOCUS are not applicable to rice cultivation due to the constant flooding conditions. In order to address this problem, a group of experts the MED-Rice Group produced guidelines for risk assessment, at exposure higher level indorsing the use of Rice Water Quality (RICEWQ) model. One of the major recommendations is that National scenarios may be developed by the Southern EU countries to evaluate the pesticides applied in rice (SANCO/1090/2000). Moreover, a recent study points the lack into (higher-tier) fate modelling of pesticides in rice paddies and effect assessment on field communities [4].

Since no insecticide was authorized in Portugal for use in rice fields to control aphids, the neonicotinoid imidacloprid was proposed for this end. In spite of its widespread use in agriculture and the fact of being ranked in the top-10 of substances that exceed water quality standards for surface water [5], the lack of consistent data on the environmental fate in aquatic ecosystems has frequently been discussed [4].

In these regards the present study is divided in two parts: **I)** assessment of imidacloprid fate to evaluate/validate pesticide RICEWQ fate model; **II)** monitoring of aquatic ecosystem following insecticide application in paddy water and surrounding waterways. The present experiment focuses on **Part I** and its main aim is contribute to evaluate how well the proposed model RICEWQ predict environmental concentrations under Portuguese Mediterranean conditions.

Materials and Methods

The study was performed in a rice area on Portugal, "Leziria Grande de Vila Franca de Xira", in the vicinity of the River Tagus Estuary Natural Reserve. This rice field area receives water from the water catchment *of Conchoso (Tagus River)*, which is distributed through an irrigation canal (by water adduction) over the different rice plots. The experiments were carried out in three experimental rice plots (C, C1, and C2) with a surface area of 2.76, 2.73 and 2.39 ha respectively. They were set up according to standard agricultural practices, but no insecticides were used prior to the study. The soil is a silty-clay with pH 5.9 and an organic matter content of 3.8%. In order to achieve the proposed objective the following methodology was implemented: **1)** Parameterization of the RICEWQ using field data resulting from specific field measurements, including soil properties, irrigation/drainage management data, daily meteorological data, description of the initial/boundary conditions; **2)** Water pesticides contents monitoring; **3)** Calibration of water and pesticide balance parameters; **4)** RICEWQ calibration against field monitored pesticide concentration data.

Results achieved so far

Initial RICEWQ parameterization using field data resulting from specific field measurements. Water sampling (in the irrigation canal, rice plots and watershed) for chemical analysis a day before imidacloprid application on rice plots, as well as 0.125, 1, 3, 6, 13 and 27 days after treatment. Calibration of water balance: In order to calibrate the water only the irrigation amounts were varied to match the observed ponded water depth. Initially irrigations were applied using the "fixed volume" facility, which allows input of specified amounts, in order to make the water balance as accurate as possible. Irrigations were also applied using the "automatic" facility, which fills the bay to a set level when the water level in the bay drops to a critical level. The results of the model calibration were well matched to the observed water depths.

Status of the work

After the water balance was adequately calibrated the pesticide balance is being calibrated against field monitored pesticide concentration data. The basic data used for the water balance calibration were again used and the soil and chemical parameters were added.

All achieved results until September will be in the poster presentation.

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PP50

ACT1035

INFLUENCE ON FIELD SOIL INVERTEBRATES OF PESTICIDES APPLICATION AND SOIL MANAGEMENT PROCEDURES OF THREE CROPS UNDER MEDITERRANEAN CONDITIONS

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Summary

Sustainable agricultural production relies on soil communities as the main actors in key soil processes necessary to maintain sustainable soil functioning. Nevertheless, agricultural practices such as pesticides and fertilizer applications and soil tillage, may affect soil biodiversity causing an impact on soil ecosystem. The present study aimed to evaluate the influence of several agricultural practices of three crops (potato, onion and maize) under Mediterranean climate conditions on soil macro- and mesofauna during their entire crop cycles. Effects on soil invertebrates were assessed in the field and control site by testing indigenous edaphic communities in a selected study-site located in a major agriculture region of Central Portugal. The community of soil macro- and mesofauna of the three crops field varied *versus* control site along the crops cycles. The feeding activity of soil fauna between control site and crop areas varied only for potato and onion crops vs. control site but not among crops. Concentration of pesticides residues in soil did not cause apparent negative effects on the soil communities for the three crops. The results indicate that soil communities recovered from the agricultural disturbances associated with crops management.

Introduction

Sustainable agricultural production relies on soil communities as the main actors in key soil processes necessary to maintain sustainable soil functioning. Soil biodiversity influences soil physical and chemical characteristics and thus the sustainability of crop and agro-ecosystems functioning. Agricultural practices (e.g.: soil tillage, pesticides and fertilizer applications, irrigation) may affect negatively or positively soil biodiversity and abundances by modifying the relationships between organisms in the soil ecosystem. The present study aimed to study the influence of agricultural practices of three crops (potato, onion and maize) under Mediterranean climate conditions on soil macro- and mesofauna during their entire crop cycles.

Materials and Methods

Effects on soil invertebrates were assessed at a higher tier of environmental risk assessment comprising field testing of indigenous edaphic communities in a selected study-site located in a major agriculture region of Central Portugal, Ribatejo e Oeste, neighbouring protected wetlands. A reference site near the agricultural field site was selected as a Control site to compare the terrestrial communities' composition and variation along the crops cycle. The field soil and Control site soil are a sandy loam soil. Crops irrigation was performed by center-pivot and by sprinklers. Soil macro- and mesofauna were collected at both sites using two methodologies through pitfall trapping and soil sampling. Soil fauna feeding activity was also measured [1].

Results and Discussion

The community of soil macro- and mesofauna of the three crops field varied *versus* control site along the crops cycles. Main differences were due to arachnids, coleopterans, ants and adult Diptera presence and abundance. The feeding activity of soil fauna between control site and crop areas varied only for potato and onion crops vs. control site but not among crops. Concentration of pesticides residues in soil did not cause apparent negative effects on the soil communities [2-3]. Similarities of soil communities were observed at the last sampling date after harvesting for the three crops indicating that soil communities recovered from the agricultural disturbances associated with crops management.

Conclusions

An integrated approach such as the one adopted in present study, taking into consideration soil community's abundances, feeding activity and time variations along entire crop cycles under Mediterranean conditions, as well as soil exposure to pesticides residues in soil, may contribute to decision making towards a sustainable use of crop areas, including pesticide use and management practices.

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PP51
ACT1045

METABOLOMIC STUDY OF THE IMPACT OF IRON, COPPER AND IRON-COPPER NANOPARTICLES ON GRAM POSITIVE AND NEGATIVE BACTERIA

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Summary

The rapid evolution of nanotechnology inevitably has resulted in the release of nanoparticles (NPs) into the aquatic environment, which has caused the exposure of living organisms to them. Due to their large surface area, enhanced chemical reactivity and potential for easier penetration of cells, nanoparticles may be more toxic than larger analogues of the same chemical composition. The aim of this study is to evaluate the antibacterial effects of zero-valent iron and copper nanoparticles as well as of their composite material. Moreover, an attempt is made to explore metabolic changes, which occur in bacteria like *E. coli* and *S. aureous* after their exposure to nanoparticles by employing chromatographic and nuclear magnetic resonance techniques.

Introduction

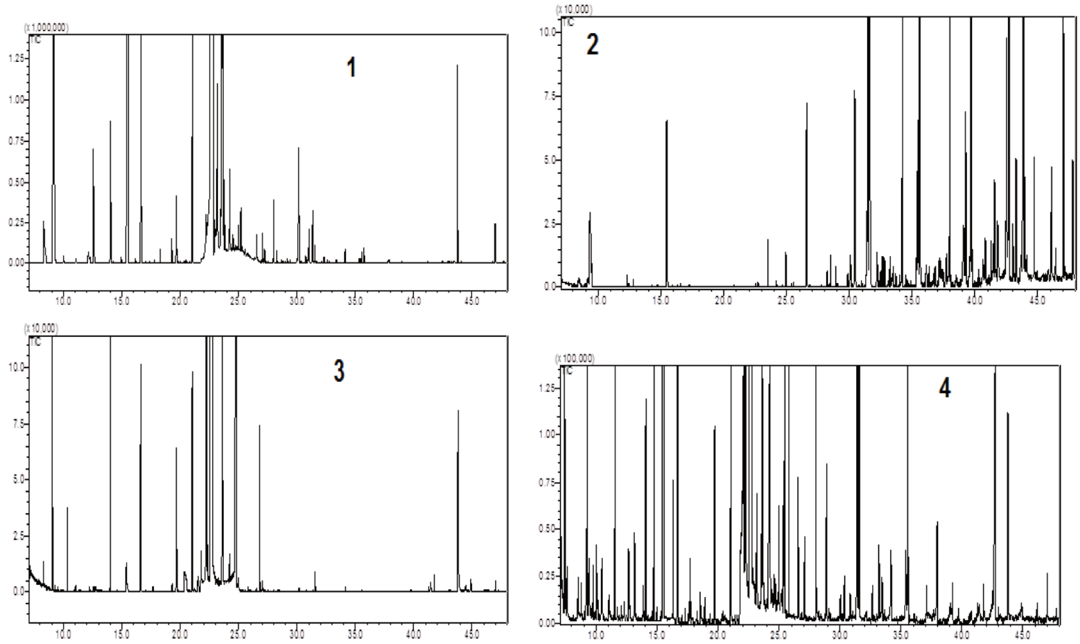
With the development of nanotechnology, a multitude of nanomaterials have been used in a wide range of applications. The increased use of NPs consequently has been leading to greater and uncontrolled disposal to the environment. Copper and iron are known for their biocidal properties and therefore they have been widely used in antimicrobial textiles, hospital equipment and antifouling paints for years [1,2]. NPs have a large surface area, which leads to increased reactivity and thus to enhanced bactericidal properties. However, their use as antibacterial agents calls for extra attention because they can be hazardous or toxic to other organisms that may be co-exposed to them [2]. Plenty of mechanisms have been proposed to clarify the toxic effects of the nanoparticles such us the generation of reactive oxygen species, oxidation, lipid peroxidation etc. [1,3]. Therefore, there is a need for further understanding of the mechanisms through which antibacterial properties are achieved. For this assay two different microorganisms have been chosen to study the effect of the nanoparticles on them: *Escherichia coli* a Gram-negative non-pathogenic species and *Staphylococcus aureus* a Gram-positive pathogenic species. Both of them are commonly found either on human skin or in the lower intestines and they are a normal part of the human micro-flora.

Experimental

Cultures of the bacteria were grown in Lauria Broth overnight, at 37°C. After the culture reached the stationary phase, the bacteria were collected with centrifugation. The bacterial pellet was re-diluted in an aqueous solution of the nanomaterial and shaken thoroughly for 1 hour, at 37°C. Bacteria were collected again with centrifugation. The metabolism was quenched and the metabolites were extracted with cold ethanol after three freeze-thaw cycles, in liquid nitrogen [4]. The metabolites were obtained after centrifugation and separation and they were analysed using Nuclear Magnetic Resonance, HPLC-DAD and GC-MS. The antibacterial properties of the three nanoparticles were tested by exposure followed by consequent dilutions and plating on dishes. The number of the colonies was counted and compared to the number of the colonies of the non-exposed bacteria.

Results and Discussion

Zero-valent iron, copper and iron-copper nanoparticles have shown great bactericidal properties. In an amount of 100μg/mL, a death rate of 99% was observed. The metabolic profiles of the bacteria after the treatment with the nanoparticles exhibit some pronounced differences.



GC-MS chromatograms after silylation of the metabolites of (1) non-exposed *E. coli*, after exposure at 100µg/mL of (2) Fe-Cu NPs, (3) Fe NPs and (4) Cu NPs, respectively.

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PP52
ACT1047

PESTICIDE LEVELS AND ENVIRONMENTAL RISK ASSESSMENT IN THE SURFACE WATERS OF LAKE VISTONIS BASIN

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Summary

A study was undertaken for the evaluation of the pollution caused by the agricultural and other activities in the basin of the Vistonis Lake during the years 2010-2012. A total of 327 compounds (pesticides, degradation products) were analyzed. Herbicides were the most frequently pesticides detected (55%) followed by the insecticides and the fungicides. Concentrations exceeding the 1 μg/L were recorded in some of the samples. Environmental Risk assessment revealed that in several cases the danger to aquatic life caused by the pesticide residues was significant.

Introduction

Management of water resources is of great importance in the EU countries. Water Framework Directive 2000/60/EC [1] established a community policy for the protection of surface and ground waters, and Directives 2006/118/EC [2] (setting groundwater quality standards) and 2008/105/EC [3] (establishing specific Environmental Quality Standards) were also issued.

Lake Vistonis is a swallow coastal lagoon of Thrace in Eastern Greece. It is an important biotope for many bird species and fish and it is protected by the Ramsar convention and included in the EU Natura 2000 network.

Monitoring network and sample analysis

Vistonis lake extends in an area of approximately 45 km^2 and a catchment area of 1350 km^2. The main rivers of the area are Kosynthos, Kompsatos and Aspropotamos, which discharge in Vistonis lake and the river Lissos which discharges in the Aegean Sea. Several other small creeks exist. Water samples were collected from the lake Vistonis (4 samplings), the aforementioned rivers, various small streams and drainage canals (25 samplings). Water samples were extracted by SPE and the extracts analyzed by GC-MS/MS and LC-MS/MS

Results and Discussion

72 different pesticides and conversion products were detected: 29 herbicides, 26 insecticides, 13 fungicides and 4 other pesticides/conversion products. Herbicides were the most frequently detected pesticides (55%), followed by the insecticides (27%) and the fungicides (14%). 12 pesticides were detected in Lake Vistonis. Fluometuron was the only pesticide which was detected consistently (max 0.088 μg/L). Alphamethrin (alpha cypermethrin) was detected at levels exceeding 0.1 μg/L in Spring 2012 Lamda—cyhalothrin was detected in low concentrations in all samples in the Spring of 2011. 69 different pesticides were detected in the rivers and drainage canals. Fluometuron was detected far more frequently than any other pesticide (median 0.063 μg/L; max 317.6 μg/L). Chlorpyrifos and prometryne were the only other pesticides with detection frequencies above 10%. Quintozene, lindane, chlorpyrifos, prometryne, malathion and terbutryn were detected above the 0.1 μg/L level.

AA-EQS were exceeded six times for lindane and two times for chlorpyrifos, whereas MAC-EQS were exceeded two times for chlorpyrifos. For Vistonis lake, alphamethrin and Lamda-cyhalothrin exhibit RQ values higher than 1, both when median and maximum concentrations are used. RQs higher than 1 were also calculated for many pesticides found in the rivers and drainage canals of the basin.

Conclusions

Herbicides were the most frequently detected pesticides in the surface aquatic systems in the basin of the Vistonis lake. However, environmental risk was mainly posed by the group of insecticides.

Acknowledgments

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[3] Directive 2008/105/EC

PP53
ACT1073

DISSIPATION KINETIC OF CLODINAFOP-PROPARGYL IN RESISTANT AND SUSCEPTIBLE BIOTYPES OF *PHALARIS BRACHYSTACHYS*

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Summary

Among the most common weeds in the cereal crops of the Mediterranean fields is short-spike canarygrass (*Phalaris brachystachys*). In Greece, clodinafop-propargyl has been used routinely to control short-spike canary grass for more than 10 years. This study focuses on two field populations of *Phalaris brachystachys* collected from the central and northern Greece. An accurate and well validated analytical method for the determination of clodinafop-propargyl and clodinafop free acid in wheat (Triticum aestivum L.) and *Phalaris brachystachys* was developed. Two different extraction and clean-up approaches were tested. The QuEChERS method seemed advantageous compared to SPE technique for the determination of both analytes. The extracts were analysed by GC-NPD/ECD and HPLC/DAD systems. Different dissipation kinetic of clodinafop-propargyl and formation of its major metabolite clodinafop free acid was observed in wheat, susceptible (Sitohori) and resistant (Karla) biotype of short-spike canarygrass.

Introduction

Phalaris brachystachys is a common weed of barley and wheat crops of Greece. Yield reduction and quality deterioration due to this annual grass (up to 95%) has been observed worldwide [1].

Arylophenoxypropionates (APP) are acetyl co-enzyme A carboxylase inhibitors that are applied post-emergence to control grass weeds in wheat fields. Clodinafop-propargyl (CP) is a main member of APP herbicides [2] that is commonly used to control *P. brachystachys*. Susceptible and resistant biotypes of *P. brachystachys* to clodinafop-propargyl from Sitochori (North Greece) and Karla (Central Greece) have been identified in a previous study [3], respectively.

The aim of this study was to develop and validate of an analytical method for the determination of CP and clodinafop acid (CA) in wheat and the two biotypes of *P.brachystachys*. Moreover, dissipation of clodinafop-propargyl and formation of clodinafop acid in the above mentioned plants was studied by a pot experiment.

Materials and methods

A SPE and a QuEChERS modified methods for the determination of both analytes were developed and compared. Spraying of wheat and weeds and determination of the dissipation kinetics of CP was conducted under pot experiment. The formulation Sword 240® EC was dissolved in water and sprayed with experimental sprayer at the recommended dose of 170 cm^3/ha. The experiment was conducted when the wheat plants were at the three leaf stage and weeds at two leaf stage. Samples were collected randomly before spraying, 15 min, 3h, 8h, 16h, 2 days and 8 days, after spaying.

Results and Discussion

CP was detected on NPD and ECD while clodinafop acid on DAD at 240 nm. Recovery values by the SPE (C-18 and florisil) method were 46% and 40% for the CP and CA, respectively. Both analytes showed better recovery values by the QuEChERS method (90-98%) with RSD lower than 9%. QuEChERS method performance was similar in wheat and

P. brachystachys biotypes. The dissipation kinetic of CP and formation of CA in all substrates is presented in the following table.

Table: Concentrations (μg/g) of CP and CA in wheat and two biotypes of *P.brachystachys* .

Intervals after application	Triticum aestivum		*P.brachystachys* (Sitohori)		*P.brachystachys*	
	CP	CA	CP	CA	CP	CA
Control	nd	nd	nd	nd	nd	nd
15 min	6.25 (±0.03)	0.05 (±0.00)	6.35 (±0.02)	0.08 (±0.00)	6.30 (±0.02)	0.06 (±0.00)
3 hours	4.05 (±0.04)	0.28 (±0.01)	5.28 (±0.03)	1.05 (±0.00)	4.28 (±0.03)	0.52 (±0.03)
8 hours	3.22 (±0.02)	0.89 (±0.01)	4.54 (±0.00)	1.68 (±0.01)	3.59 (±0.01)	0.73 (±0.01)
20 hours	1.28 (±0.00)	1.56 (±0.02)	3.85 (±0.00)	2.02 (±0.01)	2.69 (±0.03)	1.38 (±0.04)
2 days	1.01 (±0.02)	1.89 (±0.01)	2.95 (±0.01)	2.35 (±0.00)	1.56 (±0.00)	1.70 (±0.03)
4 days	0.45 (±0.04)	1.95 (±0.02)	2.02 (±0.02)	2.98 (±0.02)	0.95 (±0.03)	1.98 (±0.04)
8 days	0.06 (±0.00)	0.85 (±0.03)	1.58 (±0.04)	3.22 (±0.02)	0.32 (±0.02)	1.45 (±0.05)

Conclusions

The QuEChERS method was appropriately modified for the determination of the clodinafop-propargyl and its herbicidal metabolite, clodinafop in wheat and two short-spike canary grass biotypes. Similar dissipation rates of clodinafop-propargyl were observed in wheat and resistant short-spike canary grass biotype. Clodinafop-propargyl showed lower dissipation rate in susceptible short-spike canary grass biotype and the respective formation of clodinafop acid was increased up to the final sampling, 8 days after the application of the parent compound.

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ACT1006

ADSORPTIVE CAPACITIES OF CHEMICALLY PREPARED CARBON FROM WOOD WASTE

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Activated Carbon (AC) was prepared by chemically carbonizing waste wood using phosphoric acid for chemical activation. The activity of (AC) was evaluated by adsorption measurement of potassium permanganate, Iodine, phenol and Methylene blue from their aqueous solution.

The adsorptive capacities of activated carbon for major organic constituents (Target compounds) of wide variety of wastewater were measured in pure and actual wastewater samples.

The study successfully yielded activated carbon from low value or refuse materials with high adsorption capacities.

Keywords: Activated Carbon (AC), Wood Wastes

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TOOLS FOR BIOPESTICIDES DEVELOPMENT WITHIN THE EUROPEAN LEGISLATIVE FRAMEWORK

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Summary

In order to highlight the importance biopesticide development, the purpose of this presentation is to carry out an analysis of the current tools for the development of plant protection products from natural products, within the European legislative framework.

Introduction

The development of less harmful active substances is stimulated by the novel European Pesticide Regulation (EC) No. 1107/2009. In this sense, biopesticides are gaining interest to be used in pest control within the Integrated Pest Management practices [1] thanks to the novel mode of action of the active substances obtained from natural sources. Therefore, the development of biopesticides in the last years is influenced by the current strict legislative requirements and in the availability of chemical tools to achieve their study and monitoring during their usage.

Discussion

The last major revision of the law regulating plant protection products in the European Union (EU) was mainly enacted through the introduction of the current Regulation (EC) No. 1107/2009, which entered into force on 14 December 2009, and started to be applied from 14 June 2011. This Regulation has been developed with the aim to ensure a high level of protection of both human and animal health and the environment and to improve the functioning of the internal market through the harmonisation of the rules on the placing on the market of plant protection products, while improving agricultural production. Consequently, several active substances will not be reapproved because of the new registration requirements, purely as a result of their classification (cut-off criteria). This fact has induced that many biopesticides have gained favour in recent years because usually they are safer than the synthetic pesticides. However, it is important to be aware that biopesticides are still pesticides and fall under the scope of the same regulations as their synthetic counterparts.

In a general way, it could be said that there are two approaches of how to face the development of new biopesticides to satisfy the current legislative requirements: a) by obtaining crude extracts (e.g. from lignocellulosic materials); b) by chemical synthesis of the active substance aided by technologies in computational chemistry. Furthermore, the availability of analytical methods which allow monitoring in field of these active substances play a key role in the approval for marketing of these plant protection products.

These chemical tools are used by researchers and companies within the agrochemical sector to biopesticide progress. The first approach commented above is extensively used in the search of new active substances from natural sources with multiple purposes [2], while the second with tools such as the approximations of Structure-Activity Relationship (SAR) and Quantitative Structure-Activity Relationships (QSAR) allow the study of the mode of action (e.g. QSAR correlate quantitative data of concern for specific compounds, such as toxicity, with several structural parameters of these compounds, such as ionisation potentials, octanol-water partition coefficients, aqueous solubility, dipole moment, etc).

However, all these efforts to develop biopesticides would not have sense without suitable analytical methods to cover the current legislative requirements.

Conclusions

Two chemical approaches will be essential to discover and develop new biopesticides with novel modes of action: extraction and chemical synthesis aided by computational chemistry.

The availability of whole analytic methods, from pre-treatments to final determination by modern instrumental techniques, has a decisive influence in the approval for marketing of biopesticides.

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ACT1032

SALT MARSH PLANTS AS POTENTIAL AGENTS FOR THE CONTAINMENT OF PAHS CONTAMINATION IN SEDIMENTS OF PORTUGUESE LAGOONS AND ESTUARIES

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Summary

Strategies for remediation of PAHs contaminated sediments are subject of intense research since they constitute a secondary source of PAHs for the water column and for incorporation in living organisms, ultimately affecting human health and environmental quality. In this work, PAHs levels in sediments of 5 lagoons /estuaries along the Atlantic and Mediterranean Portuguese coasts were evaluated. In each estuary, non-colonized sediment and one or more sediments colonized by different salt marsh plants (*Juncus maritimus, Phragmites australis, Triglochin striata, Halimione portulacoides, Scirpus maritimus, Spartina maritima* and *Sarcocornia fruticosa*) were collected. Some colonized sediments revealed higher capacity to sequester PAHs than non-colonized ones, probably due to their higher amounts of organic matter and thinner particulate fractions. So, some salt marsh plants may be potential agents for the containment of PAHs contamination in sediments.

Introduction

Phytoremediation is a low-cost and environmentally friendly technique for restoration of contaminated soils, sediments and waters [1]. Several works provided evidence that plants can increase the removal rates of a variety of organic (PAHs, pesticides, pharmaceuticals, etc.) and inorganic (Cu, Cd, Zn, Pb, etc.) contaminants [1,2]. Vegetation significantly alters the composition of sediments, increasing their organic matter content, promoting aeration and fostering microbial activity. Sun *et al.* [4] observed a decrease in phenanthrene and pyrene levels of 15.5 and 21.3%, respectively, in soils planted with ryegrass (*Lolium perenne*). These and other similar findings have been attributed to the promotion of microbial activity caused by root exudates, the modification of microbial communities and the possible induction of PAH degrading enzymes [3]. Indeed, plants exude several readily assimilated organic substances as amino acids, enzymes, simple sugars, etc. that support the growth of particular microbial communities important for pollutant abatement in the rhizosphere area [3]. The main purpose of this study was to assess the role of various salt marsh plants on the containment and remediation of sediments with different physico-chemical properties and levels of PAHs, under real environmental conditions.

Sediments sampling and analysis

The PAHs levels were analysed in estuarine sediments (both non-colonized and colonized) collected in the Atlantic (Lima, Cávado, Douro and Sado estuaries) and Mediterranean (Ria Formosa) coasts of Portugal. The colonizing plants are given in Table 1. The determinations were carried out by ultrasonic extraction (USE) followed by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS).

Results and Discussion

Table 1 presents the total PAHs concentrations measured in the sediments non-colonized and colonized by each of the selected salt marsh plants, as well as the accumulation factor (F_{Ac}- ratio conc. colonized/non-colonized). In some cases, plants favoured the accumulation of PAHs in their sediments with an accumulation factor higher than 1.0. This fact is correlated with a higher content of organic matter as well as a predominance of silt and clay fractions on these sediments relative to non-colonized ones. Exception is noted for *H. portulacoides* which apparently was less efficient on providing accumulation conditions on the rhizosphere or otherwise the biodegradation was more intense leading to lower PAHs levels in the sediments colonized by this plant.

***Table 1**. Total PAHs concentrations (sum of the 16 EPA priority PAHs, in μgkg^{-1}) in non-colonized and colonized sediments by salt marsh plants collected in different estuaries/lagoons. * (SD)*

Estuaries/lagoons	Sediment	*J. maritimus*	**F_{Ac}**	*P. australis*	**F_{Ac}**	*T. striata*	**F_{Ac}**	*S. maritimus*	**F_{Ac}**
Lima site 1	48 (8)*	89 (23)	**1.9**						
Lima site 2	134 (26)	88 (7)	**0.7**						
Lima site 3	249 (22)	318 (53)	**1.3**	355 (56)	**1.4**	309 (20)	**1.2**		
Lima site 4	21 (3)	99 (12)	**4.8**						
Douro site 1	1339 (255)	550 (150)	**0.4**						
Douro site 2	480 (84)	483 (56)	**1.0**					460 (97)	**1.0**
	Sediment	*H. portulacoides*	**F_{Ac}**	*S. maritima*	**F_{Ac}**	*S. fruticosa*	**F_{Ac}**		
Cávado	104 (10)	128 (16)	**1.2**						
Lisnave	500 (83)	291 (21)	**0.6**						
Comporta	117 (12)	108 (10)	**0.9**						
Ria Formosa	57 (11)			100 (10)	**1.8**	127 (15)	**2.2**		

It should be noted that point source contamination may have affected the most polluted sites (Douro 1 and Lisnave) since non-colonized sediments were about twice more contaminated than colonized sediments. Several human activities take place in Douro estuary (fishing, touristic, recreational) while Lisnave site is known to be affected by heavy shipping activities.

Conclusions

This study demonstrated that some salt marsh plants can contribute for the containment of PAHs contamination in sediments, which can then suffer biotic transformations or be available for artificial remediation procedures.

Acknowledgments

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CHANGES IN FARMERS' ATTITUDE TOWARDS SAFE HANDLING OF PESTICIDES AFTER AN EDUCATIONAL CAMPAIGN

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Summary

The aim of this study was to investigate the change in farmers' attitude towards pesticide management after an educational campaign. A self-reference questionnaire was distributed and answers related to occupational and environmental health risks were evaluated before and after the intervention. A significant change in attitudes towards use of personal protective equipment ($p=0.008$); safe disposal of pesticides ($p<0.001$) and symptom recognition after exposure ($p<0.001$) was observed after the intervention. The educational campaign also added awareness of environmental risks of pesticides, correct spraying methods and the prospect of organic farming.

Introduction

Plant protection products constitute an indispensable attribute of the agricultural economy, but also pose important occupational and environmental health risks [1]. Knowledge of the risks associated with pesticide management and handling is mandatory, in order to achieve the necessary safety standards and mitigate the impact of both professional or public exposure [2]. Educational campaigns have proven extremely useful in raising awareness among agricultural populations as regards these issues [3-5].

Materials and Methods

A self-administered questionnaire was distributed before and after an educational campaign to 103 farmers (70 males and 33 females). Out of them, 60 worked full-time (professionally) and 43 part-time. Mean age was 46.9±10.7 years. The campaign highlighted aspects of safe pesticide application, use of personal protective equipment, assessment of toxicity risks etc. Chi-square and McNemar tests were used to assess the impact of the educational campaign intervention. All statistical analyses were performed in SPSS (v15), where a p value of <0.05 was considered to be statistically significant.

Results and Discussion

Reported prevalence of occupational accidents during pesticide application was high (49.5%) and was characterized by the absence of personal protective equipment use (in 77.9% of the accidents), despite the fact that a large proportion (88.3%) of the population was aware of the impact of pesticide toxicity risks. The comparison of the questionnaire answers after the intervention showed significant improvement in many variables. More specifically, a significant improvement was observed in the use of personal protective equipment ($p=0.008$); safe disposal of pesticides ($p<0.001$) and symptom recognition after exposure ($p<0.001$). Farmers also positively responded to the idea of adopting organic agricultural practices ($p<0.001$).

Conclusions

The management and use of pesticides is an issue of concern for both occupational safety and environmental health. Educational seminars from health care providers can be beneficial to farmers. The reported intervention on pesticides use aimed to limit down overuse and achieve a greater compliance to occupational and environment safety guidelines. An increased awareness of the correct application of pesticides and of the benefits of organic farming was also attained.

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DYNAMICS OF TROPICAL FORESTS: DISCOVERING NEW PHARMACEUTICAL SUBSTANCES

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Introduction

Tropical forests cover about 70% of earth's surface. They extend from South and Central America to Africa, as well as from NE Australia to SE Asia. The greatest of all is located in South America and is crossed by river Amazon.

Materials and Methods

Material from international bibliography was collected and studied.

Results and Discussion

The potentiality of discovering new active pharmaceutical ingredients in tropical forests is quite impressive. Plants encountered in these areas are considered to be a "chemical storage", whose ingredients may turn out to be useful in modern medicine. About 50% of the 500,000 species of tall vegetation type are found in rainforests. Furthermore, over 80% of food supplies encountered in the developed world originate from these forest soils. Fruits, nuts and palm oil are imported after deforestation, causing devastating consequences to the local population. However, in the rainforests, local magicians and healers of the tribes have already discovered and continue to make use of these medical plants. The above information will be analyzed further in this study.

Curare, quinine, clonidine, Vinca alkaloids and ipecac constitute only some of the phytochemicals that are worth mentioning.

The U.S. National Cancer Institute acknowledged more than 3,000 plants that contain phytochemicals acting against cancer cells; 70% of these are found in rainforests.

Conclusions

It is considered likely that future therapies (for serious diseases afflicting humanity) will be found in tropical forests.

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PESTICIDES AND MENTAL HEALTH

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Introduction

This paper's object is the investigation of safe water pumping from a potentially polluted, by pesticides, aquifer. For the completion of this problem we used a mathematical model: GMS (Groundwater Modeling System)

Materials and Methods

Some parameters of the problem,uch as hydraulic conductivity and feedback, are particularly prone to uncertainty. A method for dealing with this is to use a stochastic modeling approach. The model (MODFLOW) is used to make predictions. Thus, a series of models were constructed where each model who considered equally likely. After developing multiple MODFLOW simulations using sampling method Latin hypercube, the transport of pollutants generated from each flow model is simulated using MT3DMS. With the model MODPATH (part of GMS) we simulate the motion of particles to analyze the long-term effects of contamination from any pollution area. So, by doing reverse tracking of the movement of particles from a drilling, we can see if the zone of influence of it outweighs the danger area.

Results and Discussion

Conclusively the measurement results show that a similar problem can be treated on two broad areas. First, deepening in the field est performing various drills, lowering standard deviation of k, therefore having a safe evaluation of the permeability factor satisfying with this water supply needs of residents. Surely this means greater initial costs. Second, non deepening in the field resulting in minimalizing initial cost arising to a safe provision value correlated to a lesser extent with permeability factor lower to above. Shortly we observe that there is greater benefit from the second solution which provides small initial cost. On the other hand term there is no use as water supply needs of the population are less satisfied. Ultimately first solution is more appropriate as short term can charge a higher cost however over time benefits are bigger as water supply needs are met to a greater extent.

Conclusions

Pesticides may the potentiality to cause acute and delayed health effects to exposed populations. Pesticide exposure pose a variety of adverse health effects; from simple irritation of the skin and eyes to more severe health impacts, concerning the nervous system as well as the endocrine and the reproductive system. Furthermore, according to systematic reviews, positive association has been highlighted between pesticide exposure and mutagenesis (and finally carcinogenesis), such as non-Hodgkin lymphoma and leukaemia. Moreover, exposure to pesticides has been implicated for teratogenic effects, a few of which might concern neurodevelopmental disorders whereas others may result in fetal death.

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ARE WE INHALING …. DISEASES?

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Introduction

Enviromental pollution will make humanity live a tremendous experience. Radioactivity, gasses and all kinds of chemicals infect us even before our birth.

Materials and Methods

Material from international bibliography was collected and studied.

Results and Discussion

Continuous epidemiological research proves that we live in a world where even the air we inhale is harmful. As an example we can mention the enviromental cancirogen elements that have increased infant cancer. Substances like toluene or antiseptics cause bronchial asthma. Large quantities of arsenic, nickel and lead end up in our lungs.

Conclusions

In this report we will mention as many substances as possible so that there will be precautions in work areas and in the enviroment generally.

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MICROALGAE FOR REMOVAL OF INORGANIC COMPOUNDS AND PESTICIDES FROM WATER

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Summary

The work was carried out in order to assess whether two species of microalgae, Chlorella vulgaris and Scedesmus quadricauda, could be used for the removal of agricultural chemicals (nitrates, phosphates and pesticides) from water. The high efficiency exhibited by the two algal species suggests that an algae-based decontamination process could be propose to reduce the problems of pollution of agricultural origin.

Introduction

Algae are a large variety of photosynthetic species capable of growing in a variety of aquatic environments. Recent studies have demonstrated the ability of some microalgae to develop on non-conventional substrates, suggesting the possibility to treat nitrate and phosphates contaminated waste water.

At present, there are not data relating to the possibility of using microalgae for the purification of residual water of hydroponics and water containing agrochemicals. These two aspects of reality agriculture, are major problems in economic and environmental terms.

In the present work we assessed the possibility of growing the microalgae Chlorella vulgaris and Scenedesmus quadricauda in wastewater from a tomato hydroponics cultivation with the intention of obtaining the purification of the water from nitrate, nitrites and phosphates.

Taking into account the fact that some agricultural practices (washing of equipment, residual solutions, etc) are source of pesticide polluted water, the same algae were tested for their capacity to decontaminate a water solution of the fungicides metalaxyl, pyrimethanil and fenexamide.

Materials and methods

For the tests with the wastewater, the rearing system consisted of a Plexiglas tank divided into eight compartments equipped with tubes to allow the insufflation of air. The efficiency of the water decontamination was assessed by measuring the main water quality parameters at the beginning and at the end of experiment. In parallel, the growth of microalgae was measured on a standard substrate (BG11) as control.

The evaluation of the dissipation of agrochemicals was carried out using Erlenmeyer flasks, properly ventilated, containing the substrate BG11 added with each pesticide, inoculated and not inoculated with the microalgae.

Results

After 56 days of microalgae inoculum application, the concentration of nitrates, nitrites and phosphates in the waste water decreased to 99%, 98% and 89%, respectively. The evaluation of the biomass yield and the carbohydrate content of microalgae in both species, showed a slightly lower growth in the waste water but a higher carbohydrate content with respect to the control.

The algae grown in the presence of the fungicides did not exhibit any phytotoxicity phenomena. Moreover, the concentration of the tested active ingredients dropped to not detectable levels in a much shorter time than in the control without algae.

Conclusions

It has been shown that the microalgae C. vulgaris and S. quadricauda are able to grow into an agricultural wastewater leading to a significant reduction of nitrates, nitrites, and phosphates. Moreover, in these conditions, the capacity of the two species to produce a high percentage of carbohydrates suggests that they are promising materials as source of biomass for the production of bioethanol.

The two microalgae have also demonstrated a remarkable ability to decontaminate the water from the three tested fungicides, representing an interesting operational perspective to solve the problem of disposing of water containing pesticide residues.

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CLONING AND CHARACTERIZATION OF HERBICIDE-DEGRADING GLUTATHIONE TRANSFERASES FROM CICER ARIETINUM

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Summary

Glutathione transferases (GSTs) from tau class are unique to plants and play important roles in stress tolerance and the detoxification of herbicides in crops and weeds. Here we report the cloning, kinetic and structural characterization of three members of the GST family from *Cicer arietinum* leaves (*Ca*GSTs). These GSTs were identified by *in silico* transcriptome analysis and their full length cDNAs with complete open reading frame were isolated using RT-PCR. Analysis of the cDNA clones showed that the deduced amino acid sequences share high homology with GSTs that belong to tau classes. The GST isoenzymes were expressed in *E. coli*, purified by affinity and ion exchange chromatography and their substrate specificity was determined towards nineteen different electrophilic substrates including herbicides. Comparative molecular modelling was used to identify key structural characteristics and to provide insights into the substrate specificity and the catalytic mechanism of these enzymes. The results provide new insights into catalytic and structural diversity of GSTs and the detoxifying mechanism used by *Cicer arietinum*.

Introduction

GSTs are multifunctional enzymes that catalyze the conjugation of glutathione (GSH) to reactive electrophiles. These electrophiles are diverse and include important endogenous compounds, as well as xenobiotic chemicals, therefore GSTs play an important role in stress tolerance and herbicide detoxification. GSTs are usually active as a dimer of 24–29 kDa subunits. Each monomer of dimeric GSTs contains a G-site, at the N-terminal, capable of binding the GSH substrate and an H-site, at the C-terminal, that has xenobiotic compound-binding capabilities [1-4].

There has been a particular interest in plant GSTs with regard to herbicide selectivity and environmental safety. Different classes of herbicides such as triazines, thiocarbamates, chloroacetanilides, diphenylethers, and aryloxyphenoxypropionates can be metabolized by GSTs. Herbicide tolerance in plants is based primarily on the differential ability of plant species to detoxify a herbicide, with the formation of a herbicide-GSH conjugate in the resistant but not in the susceptible species [1,3]. The plant-specific phi and tau GSTs are primarily responsible for herbicide detoxification, showing class specificity in substrate preference [3].

The study of herbicide stress mechanism and detoxification systems in plants is of academic interest and practical importance. The widespread use of herbicides has led to an increasing number of resistant weed species. Herbicide resistance has the potential to cause not only large economic losses in agriculture, but also serious problems on the environment and human health, as a result of rising herbicide application rates. The lack of a basic understanding of the molecular mechanisms underlying herbicide detoxification remains the greatest obstacle to the use of eco-friendly approaches to deal with this problem [1-4].

Results and Discussion

The results showed that the glutathione transferases from *C. arietinum* (*Ca*GSTs) catalyze a broad range of reactions and exhibit quite varied substrate specificity. In general, of the several halogenated aromatic compounds that were tested, 1-chloro-2,4-dinitrobenzene (CDNB), and its analogues: 1-bromo-2,4-dinitrobenzene (BDNB), 1-fluoro-2,4-dinitrobenzene (FDNB), 1-iodo-2,4-dinitrobenzene (IDNB), and p-nitrobenzyl chloride (pNBC), were acceptable substrates for *Ca*GSTs, although large differences in specific activities were observed [4]. Herbicides that belong to chloroacetanilides, diphenylethers and aryloxyphenoxypropionates classes were also acceptable substrates. *Ca*GSTs were also examined for glutathione-dependent peroxidase activity (GPOX) using cumene hydroperoxide, tert-butyl hydroperoxide and benzoyl peroxides as substrates. Among all peroxides tested cumene hydroperoxide seems to be the best substrate, whereas the bulkier substrate benzoyl peroxide is not acceptable substrate for *Ca*GSTs. Molecular modelling showed that the isoenzymes shares the same structural organization with other plant cytosolic GSTs and some differences at the level of C-terminal domain. In particular, major variations were identified in helices H4 and H9 that affect xenobiotic substrate recognition and catalytic mechanism.

Conclusions

In the present work, we describe the characterization of three GST isoenzymes from *Cicer arietinum* leaves. The results showed that *Ca*GSTs are capable of catalysing several different reactions and substrates, including herbicides, and exhibit wide substrate specificity. The methodology reported in the present study for the discovery of novel enzymes that are involved in herbicide stress response may find application to other plant/stress systems.

Acknowledgments

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PP63
ACT1116

SEASONAL INFLUENCE IN THE REMOVAL EFFICIENCIES OF ANTI-INFLAMMATORIES AND/OR ANALGESICS IN WWTPs

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Summary

Our aim was to assess the removal efficiencies of three anti-inflammatories and/or analgesics (diclofenac, ibuprofen and paracetamol) in two different seasons in 15 wastewater treatment plants (WWTPs) in Portugal. Although higher average removal efficiencies were registered in the dry season, no statistical significance was observed. However, between the three compounds removal efficiencies, statistical significance was established.

Introduction

Non-steroidal anti-inflammatories and analgesics are amongst the most prescribed pharmaceuticals throughout the world. In Portugal the highest prescription and consumption regard, among others, ibuprofen, diclofenac and paracetamol [1]. After intake, these highly active compounds undergo metabolic transformations, with subsequent excretion of significant fractions of the unmetabolized or of active metabolites to raw sewage and wastewater treatment plants (WWTPs). Scientific studies have already demonstrated their incomplete removal by WWTPs, being these facilities considered as the major environmental source since their effluents are discharged to the surrounding water bodies [2].

Many factors including solids retention time, organic load, microbial community, raw sewage temperature and pH were shown to have pronounced effects on the efficiency of activated sludge treatments. As such, seasonal variations may also affect the treatment efficiency of WWTPs.

Material and methods

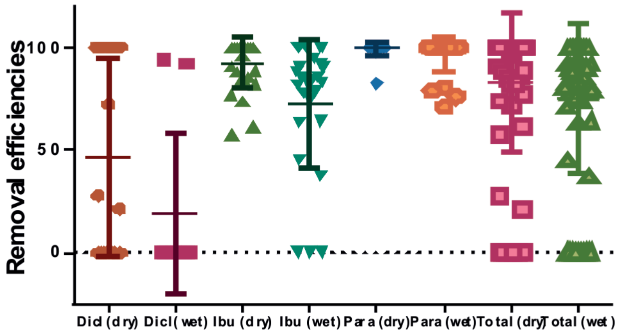
24-h composite samples of wastewater influents (WWIs) and effluents (WWEs) of 15 different WWTPs were collected during 14 May/14 August 2013 (dry season) and 1 October/13 February 2014 (wet season) (two samples by season). The population equivalent treated ranges between 6850 and 756000, representing 26.1% of national population (10526700 in 2012). WWTPs average loads ranges from 349 to 140000 m^3/day and have their discharge points in the main Portuguese rivers and Atlantic Ocean. The method used for identification and quantification of these pharmaceuticals was based on the LC-MS/MS methodology reported by Sousa et al. (2011).

All values were expressed as means ± SD and were analyzed by GraphPad Prism version 6.00 for Windows. Regarding ANOVA assumptions, three different tests were performed to assess the normality of the data. Kruskal–Wallis ANOVA by ranks, followed by post-hoc multiple comparisons of mean ranks were used to determine whether removal efficiencies were significantly different among seasons.

Results and Discussion

Regarding all anti-inflammatories (diclofenac (Dic), ibuprofen (Ibu) and paracetamol (Para)), lower mean removal efficiencies were observed in the wet season 75.6%±36.4 (18.5%±39.2, 72.9%±31.1 and 96.9%±8.2 respectively) than in dry season 82.6%±34.2 (46.4%±48.3, 92.6%±12.6 and 99.4%±3.2 respectively). Probably because in the wet season the microbial activity and biological reactions are reduced due to lower temperatures and dilution effects, leading to a lower removal efficiency Fig. 1 [3]. However, no statistical significance was found in these data. Nevertheless, between the three pharmaceuticals, statistical significance between their removal efficiencies was established.

Fig. 1 – Removal efficiencies of anti-inflammatories by season



Conclusions

Results showed that significant differences were present, for the removal efficiencies of the three different anti-inflammatories. Although the higher mean removal efficiencies for all anti-inflammatories found in the dry season, no statistical significance was observed.

Acknowledgments

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PP64
ACT1077

NON-TARGETED UHPLC FINGERPRINTING AND MULTIVARIATE STATISTICAL ANALYIS OF BIOLOGICAL SAMPLES FOR DISEASE DIAGNOSIS

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Summary

The present work is a part of a study that aims to the determination of metabolomic profiling of cervicovaginal secretions as a diagnostic tool of cervical carcinogenesis. Cervicovaginal secretions were collected easily in a non-invasive and repetitive way while three groups of women were studied: a) women with cervical precancer, b) women after therapy and c) normal individuals in order to assess how these differ and probably influence the risk of cervical carcinogenesis.

Introduction

Metabolites, are low molecular weight molecules, part of primary and intermediate metabolism, and estimated to be represented by more than 2,000 compounds in the human metabolome. Metabolomics is the study of these low-molecular weight molecules found in biological fluids and tissues of different individuals, whether normal or afflicted with disease, which reflect changes in biological functions [1]. Metabolomics is an interdisciplinary approach, which combines analytical chemistry, mathematics, statistics, and bioinformatics for data interpretation in a system biology perspective. After identification of disease-specific biomarkers; biomarker concentration levels are closely related to biochemical, physiological, environmental, and genetic status of an organism and are regarded as the ultimate outcome of cellular regulation, resulting in the visible phenotypes.

Samples and Sample preparation

Cervico- vaginal secretions sampling was performed with low volume syringes. Immediately upon collecting the samples the metabolic activity (e.g enzymatic processes) needs to be ceased until the time of analysis so the samples were stored in -80 °C [2]. Sample preparation consisted of a workflow based on US assisted liquid–liquid extraction for cleanup and preconcentration of the target analytes prior to chromatographic analysis by tandem mass spectrometry detection. An aliquot of cervical secretions was placed in 1 mL Eppedorf tube and four times the sample volume of an organic solvent was added to the tube. The solution was vortexed and then placed in ultrasound bath. After ultrasound the solution was kept in ice and then this procecure was repeated once more. Centrifugation was followed and the extract was evaporated with a gentle stream of nitrogen until a final volume of 50 μL.

Results and Discussion

Optimization of sample-preparation protocol is extremely important in metabolomic studies because it affects both metabolite profile and quality data, leading to possible erroneous conclusions. To accomplish a non-targeted metabolic profiling LLE with different extraction solvents and sonication extraction were applied. LC/MS data were acquired using LTQ Orbitrap instrument (Thermo Scientific). Analysis was performed in positive and negative ion modes of detection with the mass scanning range being 100-1000 m/z. Reversed phase (RP), Hilic and Hilic amide separations were applied and the results were evaluated with the aid of SIEVE™ 2.1 software (trial version).

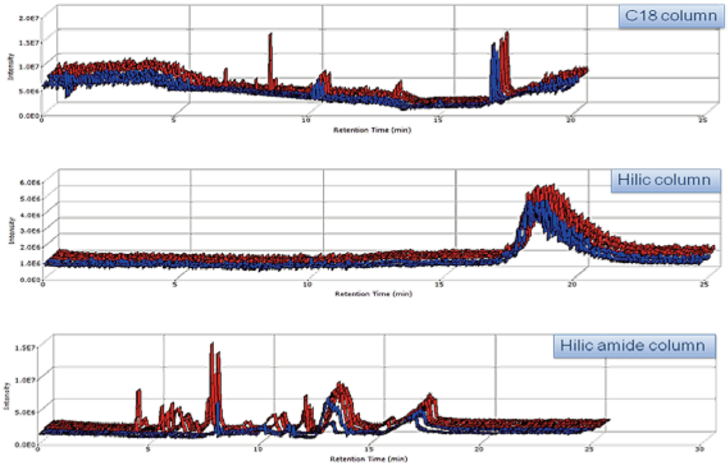

Figure 1. RP, Hilic and Hilic Amide UHPLC LTQ-Orbitrap, cervical mucus profiles. The blue one represents an individual person while the red one represents a patient. The chromatographic performance of Hilic amide mode as shown above according to the signal intensity is higher and the separation is more efficient.

Conclusions

LLE combined with ultrasound and Hilic amide separation showed better efficiency in the extraction of metabolites in untargeted analysis. Identification of biomarkers could reveal phenotypic properties in a biological system making metabolomics capable to study cancer phenotype appropriately.

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PP65
ACT1122

REMOVAL OF PHARMACEUTICAL RESIDUES FROM WATER BY ADSORPTION ONTO ACTIVATED AND LOW COST CARBON MATERIALS

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Summary

The aim of this work was to study the adsorption of four pharmaceuticals as emerging water contaminants onto activated and low cost carbon materials. The pharmaceuticals studied namely, triclosan, sulfamethoxazole, gemfibrozil and carbamazepine, were selected due to their widespread usage and the frequency with which they have been identified in water systems. The influence of solution pH level, adsorbent dose, initial concentration and contact time, were investigated on the adsorption of the selected compounds.

Introduction

While the point-source emissions of pollutants from manufacturing waste streams have long been monitored and subject to controls, the environmental impact of the public's (i.e., the individual's) activities regarding the use of chemicals is more difficult to assess. Of particular question is the widespread release to sewage and surface/ground waters of pharmaceuticals and personal care products after their ingestion, external application, or disposal. Certain pharmaceutically active compounds have been known for over 20 years to enter the environment, especially in populated geographic locales, by a variety of routes — primarily via treated and untreated sewage effluent. A larger picture, however, has emerged only more recently, where it is evident that numerous personal care products and drugs from a wide spectrum of therapeutic classes can occur in the environment, especially in natural waters receiving sewage [1].

Recent studies have shown that pharmaceuticals residues are not completely removed during conventional wastewater treatment and they are able to enter natural and surface water, groundwater as well as drinking water [2, 3]. Thus, it appears imperative to explore, develop, and improve technologies for the elimination of such compounds to ensure a sustainable healthy natural environment. Adsorption process has numerous advantages, including: applicable at very low concentrations, being suitable both for batch and continuous processes, ease of operation, possibility of regeneration and reuse and low capital cost [4].

Taking into account all the above, the main objective of this research work was to evaluate the adsorption potential of various carbon adsorbents for four pharmaceutical compounds, namely triclosan, sulfamethoxazole, gemfibrozil and carbamazepine. The adsorption of the pharmaceutical products was studied, while considering the kinetic and thermodynamic aspects of the retention process.

Adsorption studies

The batch adsorption study was conducted in 15 ml polypropylene centrifuge tubes. For this, 10 mL of pharmaceutical aqueous solution with different initial concentrations, predetermined adsorbent dose and different pH values were placed in above mentioned tube. The optimum dose (0.01 g) of adsorbent was selected from preliminary experiments. The mixture was then shaken at predetermined fixed contact time for specific compounds onto different adsorbents obtained from kinetic study. At the end of the shaking the samples were centrifuged at 4200 rpm for 5 min and the supernatant was collected with a pipette. The initial and the equilibrium concentrations of the four drugs were analyzed using a 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 removal % was calculated as

$$Removal\ \% = 100 \times \frac{(C_0 - C_e)}{C_0}$$

Where C_0 and C_e are the initial and the equilibrium concentrations of specific compounds in the solutions in mgL^{-1} respectively.

Results and Discussion

The experimental results showed that the studied low cost materials can be used as effective adsorbents for the removal of triclosan, sulfamethoxazole, gemfibrozil and carbamazepine from aqueous phase, depending on pH, adsorbent dose and concentration of the compounds. The studied compounds removal from water, achieved in this study, was 86–97% for all pharmaceuticals for an equilibrium time ~90 min.

Acknowledgments

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ACT1124

MULTI-RESIDUE DETERMINATION OF PESTICIDES AND METABOLITES IN OLIVE OIL USING MODIFIED QUECHERS EXTRACTION AND GC-MS

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Summary

A fast multiresidue screening method for the determination of 104 pesticides and metabolites, most with endocrine disruptive activity that combines a modified QuEChERS extraction methodology for sample preparation of virgin olive oil and GC-MS detection is presented. Seven QuEChERS-based sample preparation procedures were evaluated where different combinations of PSA , C_{18} and GCB sorbents as well as initial sample amounts were studied in an effort to find out the most suitable approach. Best results in terms of recoveries were obtained using 3g oil and 7g water (at 4° Celcius), 10ml acetonitrile for the extraction and 25 mg PSA, 150 mg $MgSO_4$, 50 mg C18 and 5 mg GCB, per ml of the extract for the clean up. The method provided good precision and sensitivity. The recoveries at three fortification levels between 30 and 1000 μgKg^{-1} were 60-120%, RSD<20%(inter-day and intra-day) for all analytes. LODs <10μgKg^{-1} for 95% of the analytes. Matrix-matched calibration standards were used with good linearity (R^2 >0.996). The method was applied to the analysis of twenty one (21) virgin olive oil samples from the greek market. Twelve (12) of the samples contained detectable residues. The proposed methodology is rapid, simple, sensitive and low cost.

Introduction

Olive oil is an important component of the Mediterranean diet. "Virgin olive oil" is obtained from the fruit of the olive tree (Olea Europaea), exclusively by mechanical and/or physical means without any subsequent treatment. The nutritional health benefits of this oil has increased the demand for this product worldwide [1]. Pesticide residue determination in olive oil is a very demanding task considering the inherent complexity of the matrix due to its high fat content. Methods applied to determine pesticide residues in fatty food often require a lot of steps and are very time-consuming. Also, the different classes and physicochemical properties involved make it difficult to develop methodologies that cover all of the analytes under study. The QuEChERS method has been efficiently used for the extraction of pesticides in fruits and vegetables [2], rice [3] and cereals [4] and with some modification in fatty matrices like olives and olive oil [5].The aim of the present study was to evaluate a QuEChERS based method combined with GC-MS for the determination of 104 pesticides and metabolites in olive oil. The analytes include organochlorines, organophosphates, pyrethrins, carbamates, azoles, triazines, strobylureas, dicarboximides, pyrimidines, chloroacetamides, triazols, dinitroanilides, and amides.

Methods and materials

Pesticide analytical standards >98% purity, solvents of pesticide grade, reagents of analytical grade PSA (Varian), GCB and C18(Supelco) were used. A Shimadzu 2010 GC-MS system equipped with an auto sampler and a SLB - 5MS (30 m x0.25 mm x 0.25 μm) capillary column and He as carrier gas (1.7 $mLmin^{-1}$). Injector at 220°C. Detector at 280°C. Column temperature program: 50°C (1 min), to 180°C (at 20°C min^{-1}), to 190°C (at 10 °C min^{-1}), to 240°C (at 3°C min^{-1}), to 300°C (at 10°C min^{-1}) held for 5 min. Pesticide determination was performed in selected ion monitoring (SIM) mode at 70eV. One quantification and two identification ions were monitored for each compound. 2,4,5,6 tetrachloro-m-xylene was added as internal standard. The samples were extracted according to a QuEChеRS based procedure selected after evaluation among seven different combinations of sample amount, and clean up sorbents. 3g of olive oil , 7 g of water (at low temperature, under 4°C) and 10ml of acetonitrile were added in a 50 ml PP tube and they were shaken by hand for 1min. Then 4g of $MgSO_4$, 1g NaCl, 1g trisodium citrate dihydrate and 0.5g disodium hydrogencitrate sesquihydrate were added and were shaken vigorously for 1min. The extract was centrifuged at 4000 rpm for 5min. 5ml of the acetonitrile phase were transferred to a 15ml centrifuge tube containing 125mg PSA, 250mg C18, 25mg GCB sorbents and 750mg $MgSO_4$. They were centrifuged (4000 rpm for 5min) and 3 ml of the extract were evaporated to 1 ml in a nitrogen steam.

Results and Discussion

Average recoveries for the herbicides ranged from 71% (pendimethalin) to 115% (metazachlor) and for the fungicides were from 64% (quintozene) to 116% (triadimenol). For the organophosphorus insecticides ranged from 70% (methamidophos) to 119% (malaoxon) and for the pyrethroid insecticides varied from 60% (bifenthrin) to 113% (fenvalerate II). The recoveries of the organochlorine insecticides ranged from 61% (4,4′-DDD) to 118% (endosulfan sulfate). Intra-day and inter-day RSD values for all the studied pesticides were below 20%. Matrix matched standards at five concentration levels (30 μgKg^{-1}-1000μgKg^{-1}) gave good the linearity (R^2 >0.996). LODs were below 20μgKg^{-1} for all the selected compounds and lower than maximum residue limits for pesticide residues in olive oil (EU and Codex Allimentarius). LODs of the organochlorines ranged from 0.51 μgKg^{-1} (γ-chlordane) to 7.67 μgKg^{-1} (endosulfan I). For the pyrethroid and the organophosphorus insecticides were bellow 11 μgKg^{-1} (10.7 μgKg^{-1} for resmethrin I) and 15 μgKg^{-1} (14.2 μgKg^{-1} for methamidophos) respectively.For the herbicides and fungicides they ranged from 0.27 μgKg^{-1}(s-metolachlor) to 6.85 μgKg^{-1}(propiconazole). The method was applied to the analysis of twenty one (21) virgin olive oil samples from the greek market. Twelve (12) of the samples contained detectable residues. Fenthion from 4.1 to 45.9 μgKg^{-1}in 50% of positive samples, chlorpyrifos from 3.4 to 21.1 μgKg^{-1}in 33% of samples pirimiphos methyl below 0.92 μgKg^{-1} in 42% of samples. One pesticide was found in four samples, two pesticides in seven samples and three pesticides in one sample .

Conclusions

The proposed method is simple, rapid, accurate, sensitive and low cost and can be applied for routine monitoring of the target analytes in olive oil.

Acknowledgments

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PP67
ACT1099

DETERMINATION AND RISK ASSESSMENT OF ZEARALENONE IN BEERS, BY DISPERSIVE SUSPENDED MICRO-EXTRACTION IN COMBINATION WITH LC-MS

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Summary

In the present study, a new liquid phase micro-extraction method has been developed, based on magnetic stirring and solvent dispersion, for the determination of selected mycotoxin residues in beer samples. The analytical methodology was validated according to the Commission Decision 2006/401EC, using liquid chromatography mass spectrometry (LC-MS).

Introduction

Beer is the oldest alcoholic beverage and a cereal-based product worldwide consumed. This invention has been argued to be responsible for humanity's ability to develop technology Focus on natural contaminants, mycotoxins has been wide reported in cereals [1]. Mycotoxins are naturally occurring secondary metabolites of fungi and can be produced on a wide range of agricultural commodities. Cereals used in beer production, particularly barley, wheat and maize, can be contaminated by different mycotoxins such as Zearalenone (*Fig.1*).

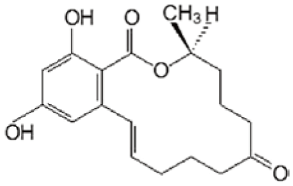

Fig 1 Chemical structure of zearalenone

Zearalenone (ZON) is a mycotoxin produced by several fungi, including Fusarium graminearum, which can infect and proliferate on various agricultural commodities in the field and/or during storage. Specific limits for ZON have been regulated in 2007 by the European Union, ranging from 20 μg /kg for infants, small children foods and cereal sub products to 75 μg /kg for maize flour (EU Commission Regulation (EC) No 1126/, 2007). ZON was classified as group 3 carcinogen in 1993 and 1999 by IARC [2]. In the course of beer fermentation, ZON is mainly converted to a-zearalenol (8%) and also to ß-zearalenol (69%), which has lower estrogenic activity than that of ZON (*Fig 2*) [3]. An endocrine disruptor (ED) is any compound that interacts with and disturbs the endocrine system. This occurs through various mechanisms, such as mimicking natural hormones or blocking their receptors [4]. Exposure to EDs may lead to adverse health effects. The structure of Zearalenone allows its binding to mammalian oestrogen receptors, though it is quite similar to estradiol. The direct role of 17β-estradiol, the most potent naturally occurring estrogen, is the growth and development of the reproductive tract and female secondary sex characteristics [5]. The implication of estrogens in the development or progression of numerous diseases, such as cancer, osteoporosis and cardiovascular disease, has also been reviewed [6].

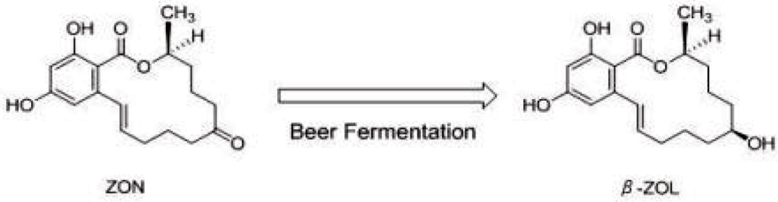

Fig 2. The conversion of zearalenone to β-ZOL during beer fermentation

The proposed method provides a simple, convenient, rapid, sensitive, and cost-effective process, for the determination of ZON, in beers. Varieties of experiment factors were optimized, such as: sample volume, extraction solvent volume, extraction and restoration speed, extraction and restoration time, pH and salinity. Under the optimum conditions, limit of detection (LOD) was 0.44 μg /kg and the limit of quantification 1.45 μg /kg. The relative standard deviation was calculated at two concentration levels and was found (RSD, n =7) for inter-day precision to 5 and 7 % and for intra-day precision at 4 and 5%. The linearity was obtained by seven points in the concentration range of 2.5-45 μg/kg with a correlation coefficient of (R^2) 0.997. The recovery was calculated to 86%.

Sampling and Analysis

A total of 100 bottled commercial beer samples were purchased from different stores, from the region of Epirus, and kept at 4 °C in dark and dry place. They were separated into three basic categories: a) blonde, b) black and c) red beers and them, into 5 subcategories: a) Ale b) Lager c) Alcohol free, d) Beer with tequila and e) Beer with spices. Each bottled beer sample was gently shaken and approximately 5 ml was degassed by ultra-sonication. 1 mL of beer sample was diluted to 5 mL buffer solution. A 500-μL aliquot of toluene was added as the extraction solvent to the surface of the sample. A magnetic micro stirring bar was placed in the bottom of the vial. It was placed on a magnetic stirrer to stir the sample. The DSME procedure first involved an extraction step, followed by a restoration step. After the extraction procedure, 20 μL of the organic droplet were injected into the LC-MS for further analysis.

It is also presenting a multi-mycotoxin analysis based on liquid-liquid extraction with full spectral Orbitrap MS detection in a single analysis using electro spray source ionization (ESI) and a comparison between LLE and DSME. The data presented in this study will be useful in facilitating improved mycotoxin risk assessment in Greece.

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