Pesticide residues and transformation products in groundwater from a Spanish agricultural region on the Mediterranean Coast

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ABSTRACT

An overview is given on the presence and changes over time of pesticide residues in groundwater from the Valencia region, one of the most important citrus cultivation sites of southern Europe. A multiresidue LC-MS/MS method was applied for the screening of around 50 pesticides in 75 water samples collected during 2000. The herbicides simazine, terbuthylazine, bromacil, terbumeton, and diuron were the most frequently detected compounds. On the basis of compounds detected in these samples, another method that focused on 30 herbicides and relevant transformation products (TPs) was developed and applied to around 80 water samples collected during 2003. Simazine was the most frequently found compound followed by several triazine metabolites. Data for this paper show the vulnerability of groundwater in this area to herbicide applications and illustrate the importance of including pesticide TPs in environmental monitoring programmes, as four out of the five compounds most frequently detected were pesticide TPs.

KEYWORDS

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1. INTRODUCTION

In spite of a general decrease in pesticide use and the appearance of a new generation of pesticides with lower impact on the environment, the development of monitoring programmes to provide more information on surface and groundwater pollution is still needed.

Pesticide concentration in groundwater depends on many factors such as crop and soil type, weather, season, degradation rates in the environment, physical and chemical characteristics of the pesticide, application rate and management practices [1]. There are generally fewer data available on the presence of pesticides in groundwater than for surface water. However, increasing interest in this topic in recent years, as well as the improvement in the methods of analysis, has produced more information about the quality of groundwater. In recent years several monitoring programmes have been applied in the USA [2-7] and in many European countries [8-15] to determine a large variety of pesticides and some transformation products (TPs) in groundwater. Although some data are available on pesticide residues in Spanish groundwater [16-20], there is a lack of monitoring programmes of sufficient scope to focus on the compounds that are most widely applied and, consequently, with the highest potential to contaminate groundwater. This is relevant, considering that Spain is one of the European countries with the highest pesticide usage (kg) per unit area (ha) [1]. Therefore, it seems very important that more data are obtained to provide better knowledge about European, and specifically Spanish, pollution of groundwater by pesticides.

Although herbicides are normally less toxic than other pesticide families, their inclusion in priority lists of monitoring programmes is of great importance to obtain more comprehensive knowledge of groundwater pollution, as these compounds have been the main pesticide contaminants in this type of water sample. Triazines, fenoxyacids, or phenylureas are among the most frequently detected due to their higher mobility in the soil-water environment [21,22].

Once the contact between pesticides and the soil-water environment is made, they can be degraded in different ways to a variety of TPs. Although TPs are usually less active and harmless than their parents, they can still have a certain degree of toxicity [23,24]. As a consequence of their polarity, they normally have a higher mobility in the soil-water environment and can reach groundwater more easily than their parent compounds. Therefore, the inclusion of relevant TPs in analytical methodology applied in water monitoring programmes is necessary to provide a realistic overview of pesticide pollution.

In contrast to food analysis where the analytes are determined by the residue definition given in the MRL regulation, the EU regulation of residues in drinking-water does not contain detailed residue definitions and only gives a general reference to pesticide-related compounds [25]. In this case, metabolites or degradation products (in general, pesticide TPs) may be unknown, and their detection and identification become an important part of the analytical task [26]. Pesticide TPs are considered particularly relevant within the group of so-called emerging contaminants [27,28], and their determination is an analytical challenge, as has been recently pointed out by our own research group [29].

Until recently, most available data on pesticides in water have been obtained by application of multiresidue methods based on solid-phase extraction (SPE), solid-phase microextraction (SPME) or solvent extraction (ELL), followed by gas chromatography coupled to mass spectrometry (GC-MS) [30,31]. However, when polar pesticides and TPs have to be included in the analytical methodology, the application of liquid chromatography coupled to mass spectrometry (LC-MS) is required, and it has become the preferred technique for polar pesticide residues determinations [26,32-36].

The use of LC in combination with tandem mass spectrometry (MS/MS) allows considerable improvement in sensitivity as well as selectivity, achieving limits of quantification (LOQ) low enough to satisfy the EU regulations in water, without even applying the common prior SPE step [37]. Although quantification requirements have been carefully considered in the scientific literature, not much attention has been paid to the confirmation of the identity of compounds detected in water, mainly when using LC-based methods. Thus, some methods have been based on the acquisition of only one transition, when using selected reaction monitoring (SRM) mode (LC-MS/MS methods), or even only on one ion measurement (LC-MS methods) for each target compound monitored. The acquisition of at least two MS^2 transitions and the measurement of their ion abundance ratio has been proposed to minimise the possibility of reporting false positives or false negatives [32,35], providing a more reliable confirmation process [38,39].

The main objective of this work is to provide a wider knowledge of pesticides and TPs present, and changes in concentration over time, in groundwater from the Valencia Mediterranean Region (eastern Spain), where there is intensive agricultural activity. For this purpose, the SPE-LC-MS/MS methodology developed at our laboratory has been applied to the determination of pesticides widely used in this area, mainly herbicides. Several wells, representing the different types of aquifers present in this area, were monitored during two sampling periods of

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a year (2000 and 2003). Around 50 pesticides and TPs were included in the 2000 monitoring, while 2003's analyses were focused on the most frequently detected compounds in the previous monitoring, mainly herbicides, and their TPs. Special attention has been paid to the reliable confirmation of the identity of compounds detected in samples, which was based on acquisition of two MS/MS transitions, and also by applying LC-QTOF MS to selected positive samples.

2. EXPERIMENTAL

2.1. Reagents and chemicals

Pesticides and TPs reference standards were purchased from Dr Ehrenstorfer (Augsburg, Germany), Riedel de Haën (Seelze, Germany) or Sigma (St. Louis, MO). Compounds investigated in this work are shown in Table 1.

HPLC-grade acetonitrile and methanol were purchased from ScharLab (Barcelona). LC-grade water was obtained by purification of demineralised water in a Nanopure II system (Barnstead Newton, MA). Formic and heptafluorobutyric acids were supplied by Fluka (Buchs, Switzerland).

2.2 Description of the study area

The coastal strip of the Valencia community is formed by a succession of coastal alluvial plains filled with materials of the Plioquaternary Age consisting of conglomerates, gravel, sands, lime, and clays, with frequent lateral changes of facies. From north to the south, the plains of Vinaroz-Benicarló, Oropesa-Torreblanca, Castellón, Sagunto and Valencia are distinguishable (Figure 1).

From the hydrogeological point of view, each coastal plain constitutes a detritic unconfined aquifer, more or less complex, in which the natural recharge takes place by effective infiltration of rainwater (12%) and irrigation water (8%), as well as by lateral feeding from mesozoic aquifers. The natural discharge takes place by direct flow to the sea or through associated coastal wetland zones, some of them of great ecological interest.

The thickness of the unsaturated zone increases from the coastline towards the interior. The minimum thickness is found in well 2 (Burriana) where it does not surpass 2 m, while in the rest of the area it is normally between 8 and 20 m.

Table 1. Compounds monitored at 2000 (Method 1) and 2003 (Methods 2a and 2b) sampling periods

Compound	Use	Family	Method	Compound	Use	Family	Method
2-ABZ	-	Carbendazim TP	1,2a	2-HydroxylTbze	-	Triazine TP	2a
Azynphosmethyl	INS	OP	1	Imazalil	FG	Conazole	2a
6-Chloronicotinic acid	-	Diflubenzuron TP	1	Imidacloprid	INS	Nitroguanidine	1
	-	Imidaclorpid TP	1	Malathion	INS	OP	1
Bentazone	HB	Carbamate	2b	MCPA	HB	Phenoxyacetic	1,2b
Bromacil	HB	Uracil	1,2b	Mecarbam	INS	OP	1
Buprofezin	INS	Phenilthiadiazinone	1	Methidation	INS	OP	1,2a
Carbendazim	FG	Benzimidazole	1,2a	Methiocarb	INS	Carbamate	1
Carbofuran	INS	Benfuracarb TP	1	3-Me-p-Nitrophenol	-	Fenitrothion TP	1
CBF-3-OH	-	Benfuracarb TP	1	Methomyl	INS	Carbamate	1
CBF-7-phenol-3-keto	-	Benfuracarb TP	1	Molinate	HB	Carbamate	1,2b
Chorpyrifos	INS	OP	1	Oxamyl	INS	Carbamate	1,2b
Chorpyrifos-methyl	INS	OP	1	Pendimethalin	HB	Nitroaniline	1
Desethyl-2-OH-Tbze	-	Triazine TP	2a	Pirimiphos-methyl	INS	OP	1
Desethylterbumeton	-	Triazine TP	2a	Propanil	HB	Anilide	2a
DesethylTbze	-	Triazine TP	2a	Pyridaben	INS	Pyridazinone	1
DIA	-	Triazine TP	1,2a	Pyridaphenthion	INS	OP	1
2-OH-DIA	-	Triazine TP	2a	Pirimicarb	INS	Carbamate	1
Diazinon	INS	OP	1	Pyriproxyfen	INS	Phenoxypiridine	1
3,4-Dichloroaniline	-	Diuron TP	1,2a	Quinalphos	INS	OP	1
Dimethoate	INS	OP	1,2b	Simazine	HB	Triazine	1,2b
Diuron	HB	Phenylurea	1,2b	TCPY	-	Chlorpyrifos TP	1
Ethiofencarb	INS	Carbamate	1	Tebufenpyrad	INS	Pyrazole	1
Fluazipop free acid	HB	APP	1	Terbacil	HB	Uracil	1,2b
Fluroxypyr	HB	Pyridine	1,2b	Terbumeton	HB	Triazine	1,2b
Fomesafen	HB	Amide & NPE	1	Tbze	HB	Triazine	1,2b
Hexythiazox	AC	Carboxamide	1	Terbutryne	HB	Triazine	1,2a
2-Hydroxysimazine	-	Triazine TP	2a	Thiobencarb	HB	Carbamate	1,2a

Note: ABZ: aminobenzimidazol; CBF: carbofuran; Tbze: terbuthylazine; DIA: desisopropylatrazine; TCPY: 2,4,6-trichloro-3-pyridinol.INS: insecticide; HB: herbicide; FG: fungicide; AC: acaricide. OP: organophosphorate; APP: aryloxyphenoxypropionic; NPE: nitrophenyl ether.
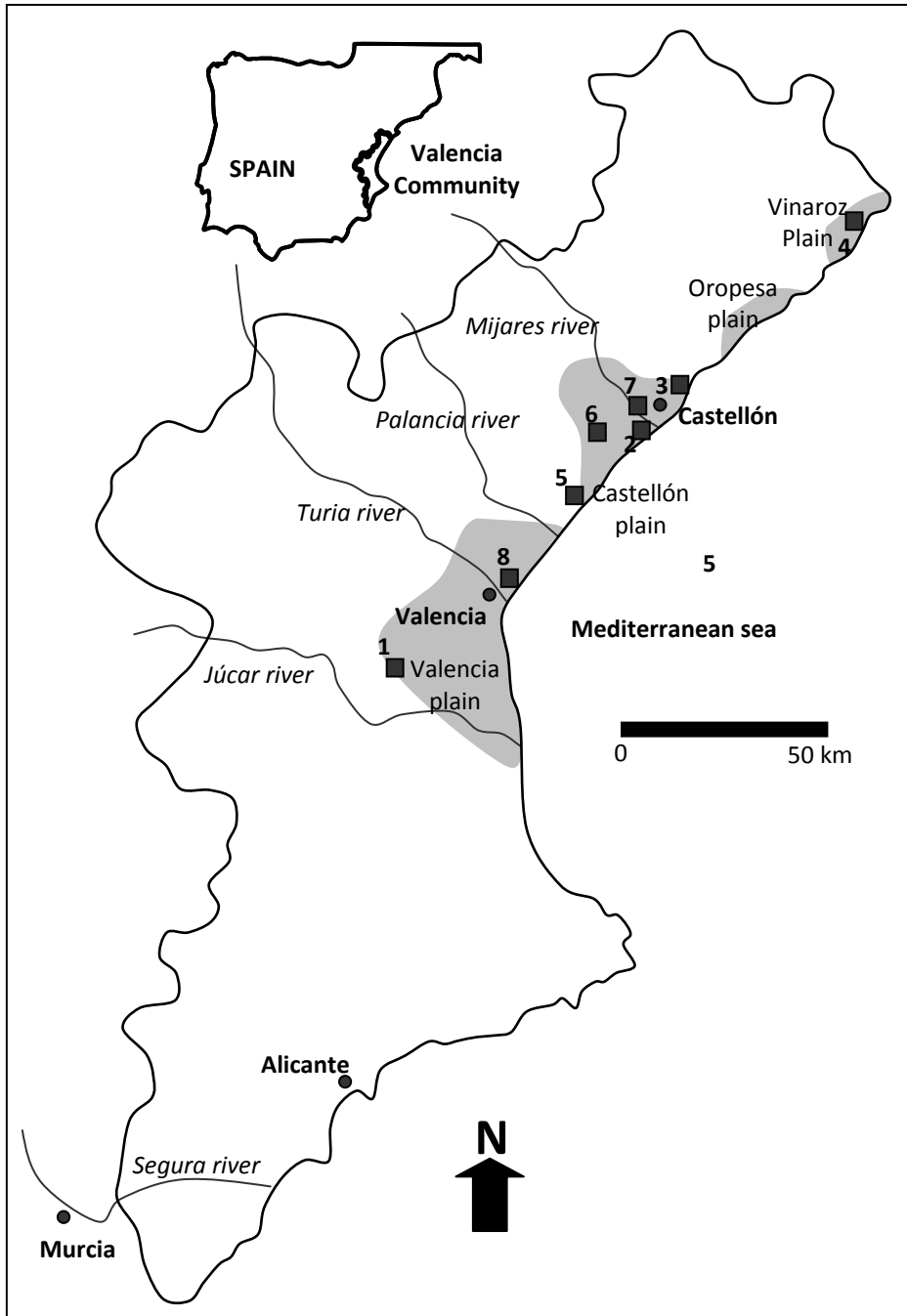
Figure 1. Map of the study area and sampling points. 1: Carcaixent; 2: Burriana; 3: Benicassim; 4: Benicarló; 5: Almenara; 6: Nules; 7: Castellon; 8: Alboraia.

2.3 Sampling

Groundwater samples were collected during 2000 and 2003. In each monitoring period, about 80 groundwater samples were collected from eight representative wells (Figure 1). Sampling points were chosen on the basis of agricultural relevance of the area and aquifer type. Sampling was carried out from April to December, at 2 week intervals from June to October, and monthly in the other months. Samples were collected in duplicate in high-density polyethylene containers (*ca.* 60 mL) and were stored in the dark at a temperature below -18°C until analysis.

2.4 Instrumentation

An HPLC system, based on a 233XL Gilson autosampler and two pumps, an Agilent 1100 binary pump used to condition and wash the SPE on-line cartridge, and a Waters Alliance 2690 quaternary pump used for the chromatographic separation, was interfaced to a Quattro LC triple quadrupole (QqQ) mass spectrometer with an orthogonal Z-spray-electrospray interface (Waters). The experimental setup can be found elsewhere [34,36].

2.5 Analytical procedure

Water samples, previously centrifuged at 3500 rpm for 10 min if suspended particulate matter was present, were analysed by LC tandem MS. For samples collected in 2000, an SPE-LC-MS/MS method [36] was applied to determine 37 pesticides widely used in the study area and 10 TPs (see Method 1, Table 1). In these analyses, only one MS/MS transition was acquired. An additional injection, to acquire another transition per compound, was applied to confirm positive findings at concentrations higher than 0.1 µg L^{-1}. For samples collected in 2003, on the basis of the first sampling period results, the monitoring was focused on 18 pesticides and nine TPs (belonging mainly to the triazine family). Two complementary SPE-LC-MS/MS methods [34] were applied to every water sample: one for acidic and neutral pesticides (Method 2b), and the other for basic and the remaining neutral analytes (Method 2a; see Table 1). For these analyses, two transitions were acquired for each compound, which allowed the simultaneous quantification and confirmation of positives in only one analytical run.

All methods applied were previously validated and fulfilled the analytical characteristics typically required in the field of pesticide residue analysis.

3.RESULTS AND DISCUSSION

3.1 2000 Monitoring programme

The groundwater samples collected in the study area (75) were analysed by applying a method based on on-line trace enrichment coupled to tandem MS (SPE-LC-MS/MS) [36]. Figure 2A shows the detection frequency for the compounds found (25 out of 47 analysed). Herbicides were by far the most commonly detected compounds, making up about 90% of positive detections. As an example, among the 15 most frequently found compounds, 11 were herbicides, two were herbicide TPs, one was a fungicide, and one was an insecticide. Desisopropylatrazine (DIA), a triazine TP, was the most frequently found compound (72% of samples analysed), while the triazines simazine and terbuthylazine and the fungicide carbendazime were detected in around 50% of the samples.

Several target compounds were often found at concentrations higher than 0.1 µg L^{-1} (close to 40% of the compounds detected were quantified over this level). In the case of bromacil, MCPA and DIA, concentrations were over 0.1 µg L^{-1} in more than 50% of their positive findings (Figure 2A), with maximum concentrations of 4.5 µg L^{-1} for bromacil, 0.4 µg L^{-1} for DIA, and 0.8 µg L^{-1} for MCPA (Table 2). Although less frequently, polar insecticides such as ethiofencarb, carbofuran, or methidation were also found in some samples but represented less than 5% of detections and always at levels below 0.1 µg L^{-1}.

The average pesticide concentrations at each sampling point are compared in Figure 3A. It can be observed that herbicides, especially triazines, were the group of pesticides most commonly detected. These results are in accordance with data previously reported in other areas [6,8,11,15,22]. The highest pesticide levels were found in sampling points 1 and 2. Well 1, located at Carcaixent, belongs to a karstic aquifer, and its piezometric level is situated at 60 m. The agricultural soil is predominantly sandy, with a thickness no greater than 80 cm and directly overlying high karstified limestones. Well 2, located at Burriana, belong to a coastal aquifer with an unsaturated zone thickness less than 2 m, having a high hydraulic conductivity, due to the prevalence of gravel and sands. Both wells 1 and 2 are subject to extensive citrus crop activity. The sum of average concentrations of triazines plus DIA, and the sum for the rest of the herbicides (mainly bromacil), in wells 1 and 2 were higher than 0.5 µg L^{-1}. For the rest of the wells monitored, the average concentration levels normally did not exceed 0.1 µg L^{-1}. Positives referred to as 'other pesticides' were mainly attributed to carbendazim and its TP, 2-aminobenzimidazole. No relevant variation of pesticides concentrations were observed during sampling performed in 2000.
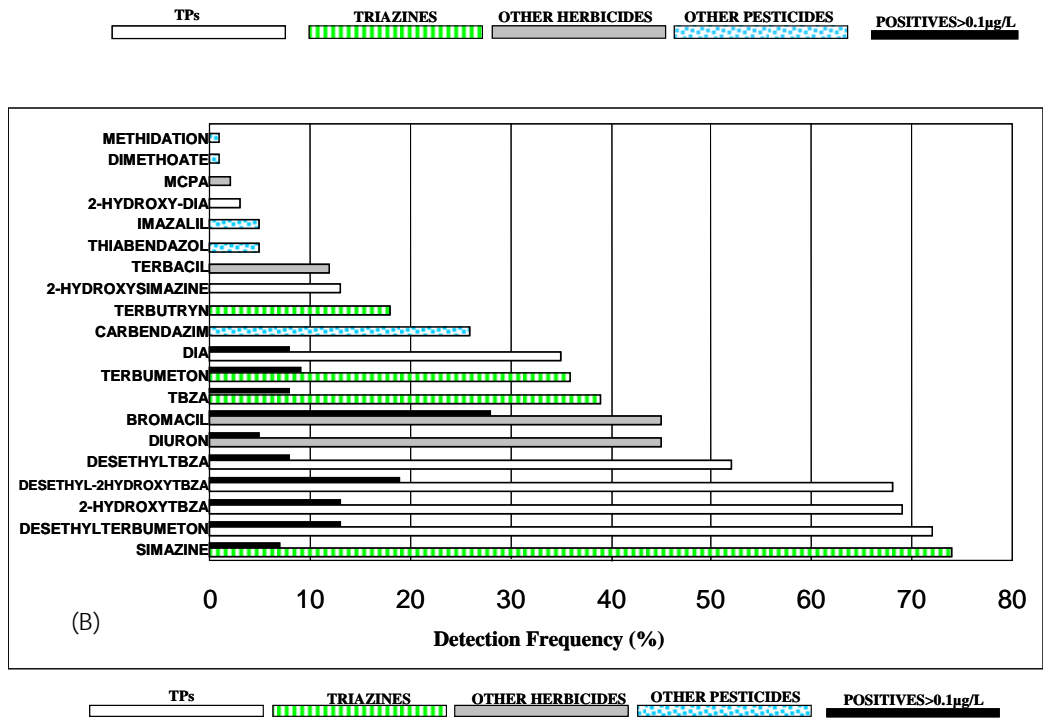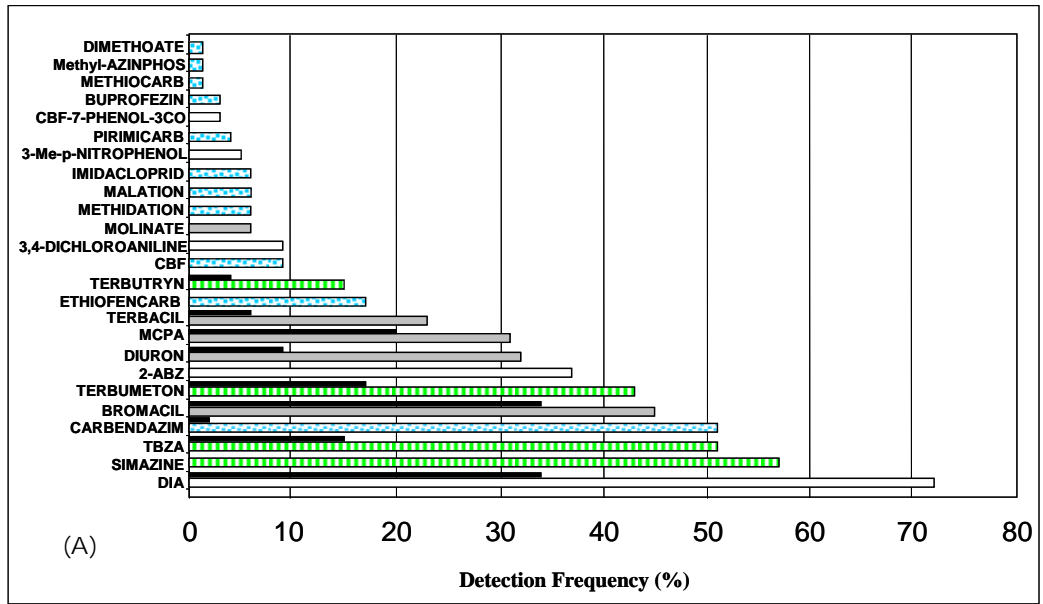
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Figure 2. Detection frequency (%) of pesticides and transformation products (TPs) in groundwater samples collected at (A) 2000 and (B) 2003 monitoring periods.

Table 2. Summary of the results obtained during 2000 pesticide monitoring in groundwater (total number of samples analyzed 75)

| COMPOUND	% POSITIVE SAMPLES	% SAMPLES >0.1µg L^{-1}	MAXIMUM LEVEL (µg L^{-1})
Azinphos-methyl	1	0	<0.025
Bromacil	45	35	4.5
Buprofezin	3	0	<0.025
Carbendazim	51	3	0.12
Carbofuran	8	0	<0.025
Chlorpyrifos	0	0	n.d.
Chlorpyrifos-methyl	0	0	n.d.
Diazinon	0	0	n.d.
Dimethoate	1	0	0.04
Diuron	32	8	0.24
Ethiofencarb	17	0	0.07
Fluazifop	0	0	n.d.
Fluroxypyr	0	0	n.d.
Fomesafen	0	0	n.d.
Hexythiazox	0	0	n.d.
Imidacloprid	7	0	0.04
Malathion	7	0	0.06
MCPA	31	20	0.84
Mecarbam	0	0	n.d.
Methidation	7	0	<0.025
Methiocarb	1	0	<0.025
Methomyl	0	0	n.d.
Molinate	7	0	0.03
Oxamyl	0	0	n.d.
Pendimetalin	0	0	n.d.
Pirimiphos-methyl	0	0	n.d.
Pyridaben	0	0	n.d.
Pyridaphenthion	0	0	n.d.
Pyrimicarb	4	0	<0.025
Pyriproxyfen	0	0	n.d.
Quinalphos	0	0	n.d.
Simazine	57	0	0.09
Tebufenpyrad	0	0	n.d.
Terbacil	24	7	0.72
Terbumeton	43	17	1.54
Terbuthylazine	51	15	0.49
Terbutryn	15	4	0.31
Thiobencarb	0	0	n.d.
2-Aminobenzimidazole [a]	37	0	0.03
CBF-3-hydroxy [a]	0	0	n.d.
CBF-7-PhOH-3CO [a]	3	0	0.06
4-Chloroaniline [a]	0	0	n.d.
6-Chloronicotinic acid [a]	0	0	n.d.
DIA [a]	72	35	0.36
3,4-dicloroaniline [a]	8	0	<0.025
3-Me-p-NO_2PhOH [a]	5	0	0.05
TCPY [a]	0	0	n.d.

[a] Transformation products
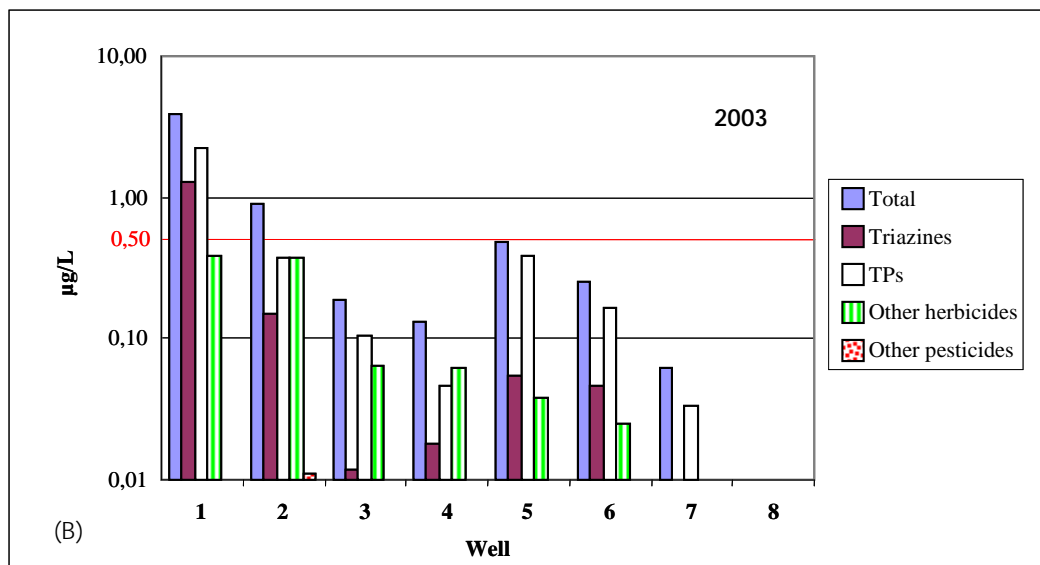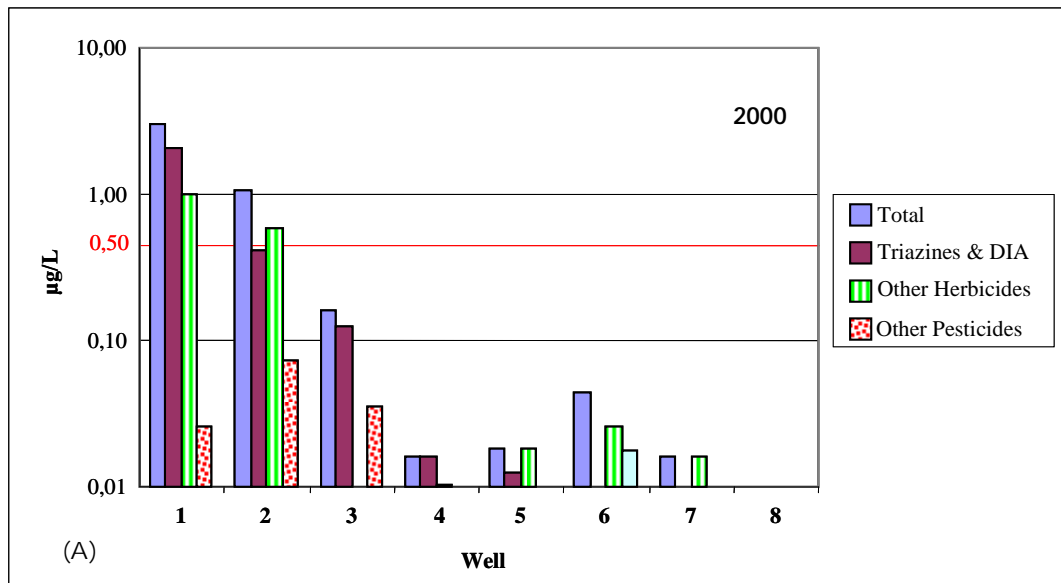
Figure 3. Comparison of pesticide concentrations found at every sampling point during (A) 2000 and (B) 2003 (data correspond to the sum of the average concentration of each pesticide during the monitoring period).

3.2 2003 monitoring programme

Results obtained during the first monitoring period were taken into account when selecting the list of target pesticides for the 2003 monitoring period (Table 1). Thus, seldom detected, less-polar analytes (mainly insecticides) were removed from the list, and emphasis was placed on polar analytes, mostly herbicides and their TPs, and other compounds, such as carbendazime and its TP 2-aminobenzimidazole, that were also widely detected in the previous monitoring.

Table 3. Summary of the results obtained during 2003 pesticide monitoring in groundwater (total number of samples analyzed 85)

COMPOUND	% POSITIVE SAMPLES	% SAMPLES >0.1µg L^{-1}	MAXIMUM LEVEL (µg L^{-1})
Bentazone	0	0	n.d.
Bromacil	45	28	0.57
Carbendazim	26	1	0.37
Dimethoate	1	0	<0.025
Diuron	45	5	0.37
Fluroxypyr	0	0	n.d.
Imazalil	5	0	0.09
MCPA	2	0	0.03
Methidathion	0	0	0.03
Molinate	0	0	n.d.
Oxamyl	0	0	n.d.
Propanil	0	0	n.d.
Simazine	74	7	0.52
Terbacil	12	1	0.13
Terbumeton	36	9	1.07
Terbuthylazine	39	8	0.46
Terbutryn	18	0	<0.025
Thiabendazole	5	0	0.03
Thiobencarb	0	0	n.d.
2-Aminobenzimidazole [a]	0	0	n.d.
Desethyl-2-hydroxyterbuthylazine [a]	68	19	0.21
Desethylterbumeton [a]	72	13	1.62
Desethylterbuthylazine [a]	52	8	1.42
Desisopropylatrazine [a]	35	8	0.25
Desisopropyl-2-hydroxyatrazine [a]	5	0	0.04
3,4-Dichloroaniline [a]	0	0	n.d.
2-hydroxysimazine [a]	13	1	0.15
2-hydroxyterbuthylazine [a]	69	13	0.15

[a] Transformation products

A total of 85 groundwater samples were analysed by applying two complementary SPE-LC-MS/MS methods [34], with the result that 20 out of the 28 compounds investigated were detected. Triazine herbicides were widely detected (Figure 2B), with simazine being the compound most frequently found in both sampling periods. The maximum triazine levels were 0.5 µg L^{-1} for simazine and terbuthylazine, and 1.1 µg L^{-1} for terbumeton (Table 3).

The results obtained in this work support the interest for including TPs in monitoring programmes. Thus, DIA was the compound most commonly detected in 2000, and when the number of target TPs was increased in 2003, the result was that four out of the five compounds most frequently detected were desethylterbumeton, 2-hydroxyterbuthylazine (2-hydroxyTbze), desethyl-2-hydroxyterbuthylazine (desethyl-2-OH-Tbze) and desethylterbuthylazine (desethylTbze), all of them triazine TPs (Figure 2B). It should be emphasised that these compounds were also the most frequently found at concentrations higher than 0.1 µg L^{-1}, reaching maximum values of 1.6 µg L^{-1} for desethylterbumeton, 0.7 µg L^{-1} for desethylTbze, and 0.2 µg L^{-1} for 2-hydroxyTbze and desethyl-2-OH-Tbze (Table 3).

The relevance of TPs inclusion is also proven when comparing the average concentration of a parent compound and its TPs. The case of terbuthylazine is illustrated in Figure 4, where it can be seen that TP concentrations were generally higher than that of the parent compound. TPs levels exceeded 0.1 µg L^{-1} in several wells, even in those where the terbuthylazine average concentration was lower than 0.025 µg L^{-1}, as in sampling points 2 and 5. In some cases, TPs were detected in spite of the fact that parent terbuthylazine was absent (wells 3 and 4).

A similar pattern was observed for other triazines (simazine, terbumeton) and their TPs (2-hydroxysimazine and desisopropyl-2hydroxyatrazine; DIA and desethylterbumeton, respectively). Thus, average simazine concentration in well 1 was 0.07 µg L^{-1}, while for DIA it was 0.14 µg L^{-1}. With regard to terbumeton, its degradation product desethylterbumeton was always found at higher concentration than the parent compound. Also, this TP was detected in wells 3 and 4 where terbumeton was not found.

In relation to other compounds also detected in 2000, DIA and bromacil were also found at relevant concentrations during 2003, while MCPA detections were minimised. Bromacil, due to its high water solubility and propensity to leaching, can be found in groundwater overtime [40]. Our data show that positive findings of bromacil exceeded 0.1 µg L^{-1} in around 30% of samples, in both 2000 and 2003, with maximum values of 4.5 µg L^{-1} and 0.6 µg L^{-1}, respectively (Table 2).
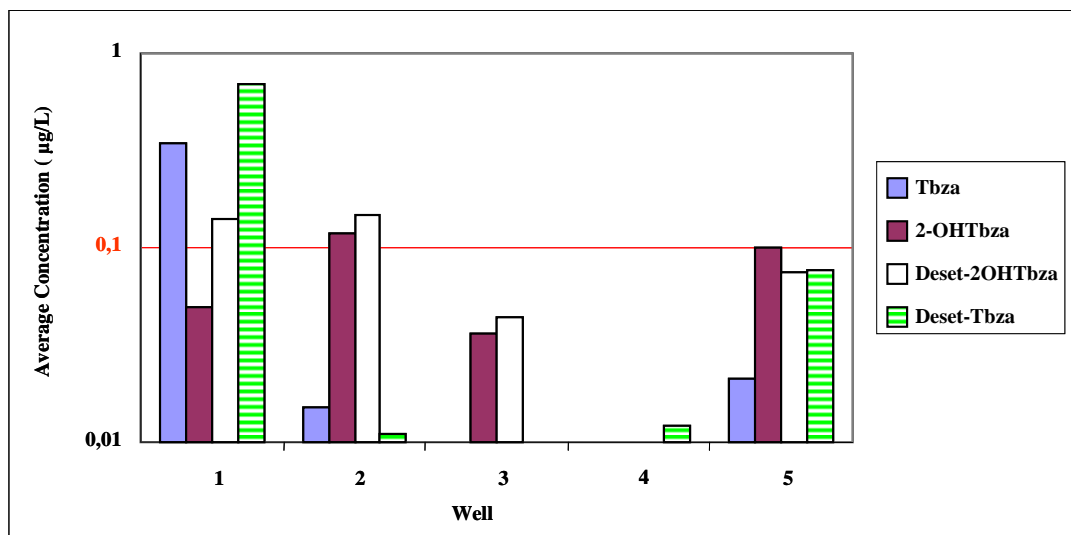
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Figure 4. Average concentrations of terbuthylazine and some of its transformation products at every sampling points during 2003.

Wells 1 (Carcaixent) and 2 (Burriana) were again the most polluted sites (Figure 3B). The sum of triazine average concentrations in well 1 was higher than 0.5 µg L^{-1}. Positives referred to as 'other herbicides' corresponded to bromacil findings in both wells, close to 0.5 µg L^{-1}. As in 2000, no pesticides were detected in well 8, and the sum of parent pesticides concentrations did not exceed 0.1 µg L^{-1} in the rest of the sampling points. The relevance of the TP findings is illustrated in Figure 3B, where it can be seen that the 'total pesticide concentration' came mainly from pesticide TPs. No significant variation of pesticide and TP concentrations in any well was observed during the 2003 sampling period.

A more detailed study of data obtained for the 18 common compounds included in both monitoring periods for wells 1 and 2 (the most polluted) shows that the herbicides simazine, terbumeton, terbutryn, diuron, terbacil, and bromacil, and a triazine TP (DIA) were the most commonly detected compounds. In both wells, all these herbicides, except simazine, were found at lower concentrations in 2003. In some cases, a significantly lower average concentration level was observed in 2003, for example from 0.26 µg L^{-1} in 2000 to <0.025 µg L^{-1} in 2003 for terbutryn (well 2), or from 0.12 µg L^{-1} to 0.04 µg L^{-1} for terbacil (well 1). A similar result occurred for bromacil (both wells), terbuthylazine, terbumeton, and DIA (well 1), where considerable concentration decreases were observed, although levels higher than 0.1 µg L^{-1} were still found. Average

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simazine and diuron concentrations were always lower than 0.1 µg L^{-1} in both wells in both years. A summary is shown in Figure 5 where the concentration decrease over time is observed for both wells, especially well 2, where most of compounds were found at levels lower than 0.1 µg L^{-1} in 2003. This decrease in pesticide pollution might be explained by a reduction in the application of these pesticides, an improvement in agricultural practices, or pesticide degradation to non-target metabolites.
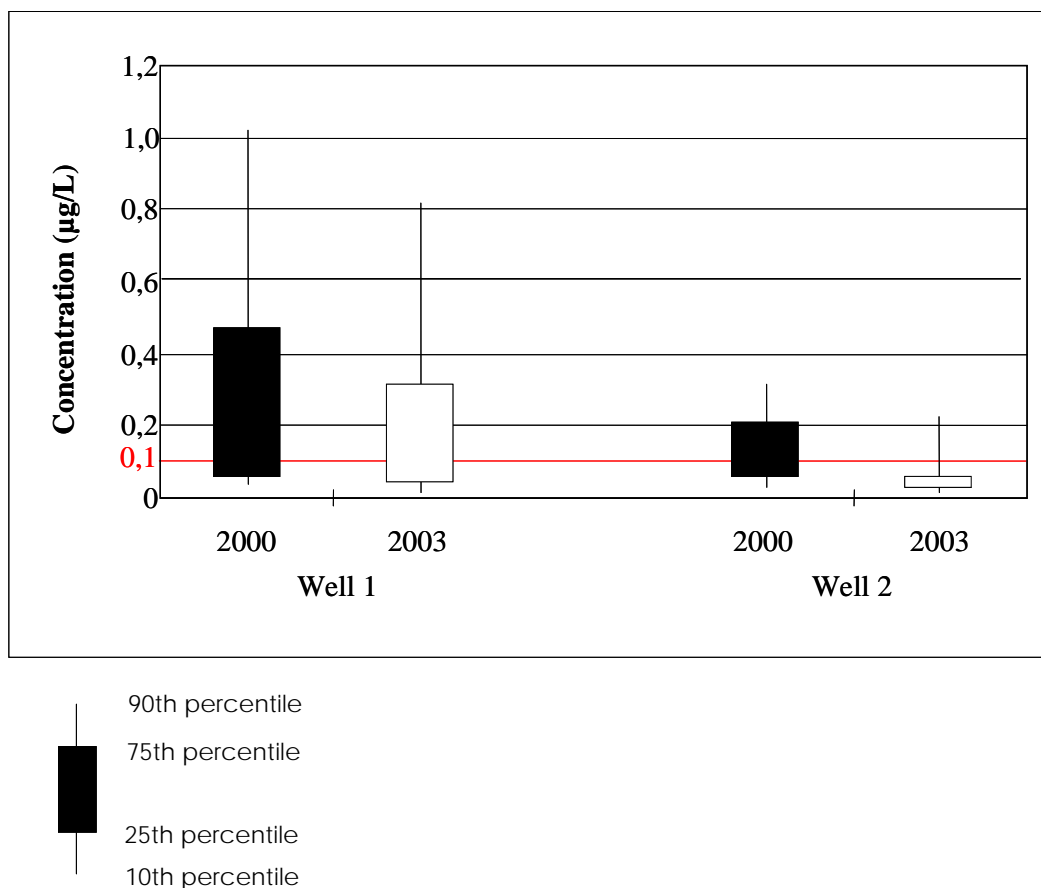

Figure 5. Evolution, over a 3-year period, of the total pesticide concentration in the two most polluted sites. Data correspond to the 18 common compounds monitored in both 2000 and 2003.

Data presented in this paper are limited, as only eight sampling points have been included in monitoring, which obviously do not represent the whole area understudy. However, data obtained from the analysis of around 150 samples illustrate how some pesticides and TPs can reach groundwater in areas of intensive agricultural activity. A more complete monitoring

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selecting a higher number of wells would be required to have a full overview of the Valencian area.

3.3 Confirmation

In the analysis of samples collected in 2000, only one transition was acquired for both quantification and qualification. The acquisition of an additional transition for confirmation purposes would have required the use of a higher number of channels, requiring longer chromatographic runs to decrease the data points available per peak and considerably decreasing the sample throughput. Confirmation of positives was performed only in those cases where analyte concentration exceeded 0.1 µg L^{-1} by re-injecting the samples into the LC-MS/MS system and acquiring one additional transition per analyte.

Samples collected in 2003 were analysed by applying two different SPE-LC-MS/MS methods, in this case acquiring two transitions per compound, which allowed simultaneous quantification and reliable identification of positive findings. The most sensitive transition was used for quantification (Q) and the additional one for confirmation (q). This methodology was feasible due to the reduction in the number of compounds monitored. As an example, Figure 6 shows the SRM chromatograms corresponding to two water samples where two parent herbicides and several of their TPs were detected. All findings were confirmed with compliance of both retention time and Q/q ratio, with those of a reference standard.

In order to compare and confirm several selected findings, around 20 positive samples collected in 2003, with analyte concentrations higher than 0.1 µg L^{-1}, were re-analysed using an SPE system coupled on-line with an LC-QTOF instrument. This additional confirmation was carried out by recording the product ion spectra of the selected compound. All positives detected and identified by QqQ-MS were also confirmed with QTOF, in this way verifying that the use of two specific transitions in QqQ was a satisfactory approach for reliable identification. The lower sensitivity of our QTOF instrument compared with triple quadrupole working in SRM mode made the confirmation of positives samples at lower concentration levels difficult.
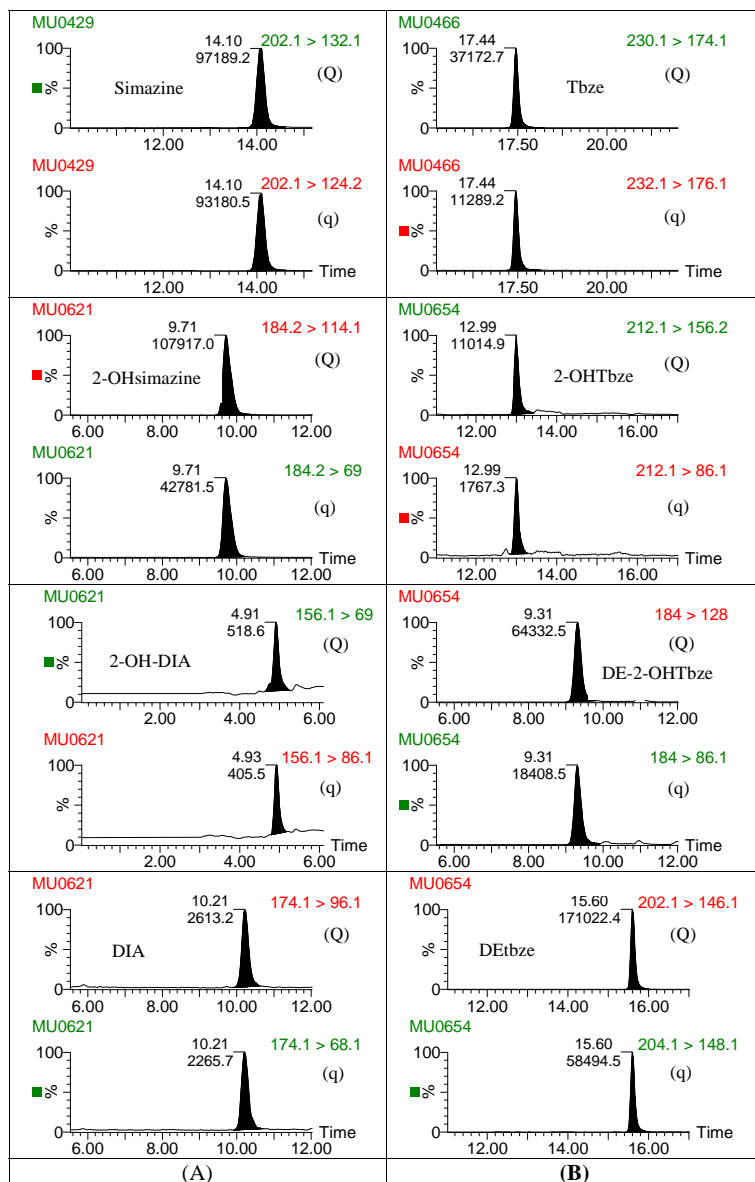
Figure 6. Selected LC-MS/MS chromatograms for positive groundwater samples. (A) Sample collected on 28 October 2003 at well 2 containing simazine at 0.52 µg L^{-1}, 2-hydroxysimazine (2-OHsimazine) at 0.15 µg L^{-1}, desisopropyl-2-hydroxyatrazine (2-OH-DIA) at 0.04 µg L^{-1} and desisopropylatrazine (DIA) at 0.19 µg L^{-1}. (B) Sample collected on 17 December 2003 at well 1 containing terbuthylazine (Tbze) at 0.12 µg L^{-1}, 2-hydroxyterbuthylazine (2-OHtbze) at 0.03 µg L^{-1}, desethyl-2-hydroxyterbuthylazine (DE-2-OHtbze) at 0.12 µg L^{-1} and desethylterbuthylazine (DEtbze) at 0.43 µg L^{-1}. (Q) quantification transition; (q) confirmation transition.

4.CONCLUSIONS

Several herbicides have been widely detected in groundwater from the Spanish Mediterranean Region, an important agricultural area with a predominance of citrus crops. Triazines, such as simazine, terbuthylazine, terbumeton, and terbutryn, and other herbicides such as bromacil, diuron, or MCPA were frequently found at concentrations around 0.1 μg L^{-1} or higher in most of the samples collected during the year (both 2000 and 2003).

The relevance of pesticide TPs inclusion in groundwater monitoring programmes has been demonstrated, as several TPs were found at concentrations higher than that of their parent compounds. Hence, in future works the inclusion of other TPs of widely detected pesticides, such as bromacil, will also be considered.

Although a decrease in pesticide concentrations has been observed from 2000 to 2003, groundwater pollution in the wells tested is still notable. Therefore, good agricultural practices that are sustainable and that respect the environment are required together with intensive monitoring programs that increase our understanding of pesticide interactions in the environment and as a means of regulation. Furthermore, special attention must be paid to those areas where the most contaminated wells are located. A detailed hydrogeological study should be carried out to better understand the high vulnerability of these aquifers to agricultural practices.

Both accurate quantification at low analyte levels and reliable identification of positives findings have been carried out using LC with tandem mass spectrometry and the acquisition of two specific MS/MS transitions. The use of LC-QTOF allowed an additional, unequivocal confirmation of positives in those cases where the analyte concentration exceeded 0.1 μg L^{-1}.

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