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Integrated approach for the quality assessment of freshwater resources in a vineyard area (South Portugal)

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Abstract An integrated chemical and biological approach for the quality assessment of freshwater resources in a vineyard area of the 'Alentejo' region (South Portugal) is presented. This includes analysis to 11 pesticide compounds and whole toxicity testing on algae and crustaceans. Simazine, terbuthylazine, terbutryn, desethylatrazine and chlorpyrifos were the most frequently detected pesticides in water collected from wells and drainage channels. Mixtures of up to three compounds in different qualitative combinations were also found. The quality standards for individual pesticides (0.1 μg L^{-1}) and pesticides-total (0.5 μg L^{-1}) were exceeded in some samples. However, their maximum concentrations were lower than the WHO guidelines, the USEPA health advisory values and the environmental quality standards for priority substances applicable to surface water. In five samples, the herbicides terbuthylazine and terbutryn and the insecticide chlorpyrifos did not pass the toxicity exposure ratio (TER) trigger values specified for aquatic organisms (algae, *Daphnia* and fish). Maximum toxic effects on *Daphnia magna* (100%) and *Pseudokirchneriella subcapitata* (82.56%) were determined in groundwater samples, while in surface water, no toxicity was observed. Concerning effects on *Heterocypris incongruens* in sediment samples collected at the drainage channels, mortality and growth inhibition values were below 38%. Pro-active management of the use of pesticides is recommended for implementing at the farm and catchment level to reduce inputs into ground- and surface water.

Keywords Quality assessment · Whole toxicity testing · Water · Sediments · Pesticide mixtures · Vineyard

Introduction

Wine production exerts a considerable number of environmental pressures, notably in terms of soil degradation, intensive use of plant protection products (PPPs; in particular fungicides) and disposal of waste/by-products from vine-growing and wine-making. Increasing use of irrigation and the effects of excessive specialisation might also result in risks for the environment in certain regions

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(EC 2007). Particularly the use of PPPs and their potential impacts on human health and the environment should be evaluated and when necessary reduced.

Several legislative documents have been adopted by the European Union to protect water resources from pesticide stress. One of the most important pieces of legislation in this area is the Water Framework Directive (EC 2000), among others related to the specific uses of water and discharges of substances, like Council Directive 98/83/EC on the quality of water intended for human consumption (EC 1998) and Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration (EC 2006). The Directive 2008/105/EC of the European Parliament and of the Council lays down environmental quality standards for priority substances, which include some pesticides: alachlor, atrazine, chlorfenvinphos, chlorpyrifos, diuron, endosulfan, hexachlorobenzene, hexachlorocyclohexane, isoproturon, pentachlorophenol, simazine and trifluralin, and certain other pollutants, with the aim of achieving good surface water chemical status (EC 2008).

Worldwide, studies have detected the presence of agrochemicals in water, raising major health concerns (Barbash and Resek 1997; Fielding et al. 1991; Funari et al. 1995; Kolpin et al. 1998; Larson et al. 1997; Scheidleder et al. 1999). In Portugal, various studies have been carried out over the past decades to assess the impact of pesticides used in agricultural areas of the 'Baixo Sado', 'Mondego' and 'Ribatejo e Oeste' regions on the quality of groundwater (Batista et al. 2001, 2002; Batista 2003; Cerejeira 1993; Cerejeira et al. 1995a, b, 2000, 2003; Francés et al. 2001; Silva et al. 2006) and surface water bodies, including the evaluation of effects on aquatic biota (Cerejeira et al. 1998, 1999; Pereira et al. 2000a, b; Pereira 2003). However, no detailed studies have been performed so far in the Portuguese vineyard environment.

Vine-growing is an important socioeconomic crop in Portugal and depends to a large extent on the input of PPPs (Eurostat 2007). The present study aimed to assess the quality of freshwater resources in a vineyard area of the 'Alentejo' region (South Portugal) by (1) identifying and quantifying the pesticides of concern to the aquatic environment in ground- and surface water samples; (2) evaluating the toxicity of water and sediment samples to aquatic organisms, such as algae and crustaceans, using Toxkit microbiotests; (3) comparing the measured levels of pesticides with the parametric values for human consumption and the groundwater quality standards for individual pesticides and pesticides-total, and the environmental quality standards for priority substances applicable to surface water and (4) comparing the toxicity exposure ratios (TERs) with the critical thresholds specified for aquatic organisms (algae, *Daphnia* and fish).

Materials and methods

Study area

The study was conducted in a vineyard farm located in 'Estremoz' ('Alentejo' region, South Portugal), producing high quality wines. Olive-tree and vine-growing are the main permanent crops in that council (RGA 2009).

Hydrogeologically, the study area belongs to the 'Estremoz-Cano' aquifer system, whose recharge is due to precipitation, run-off of some water lines, as well as from the paleozoic aquifer through the contact of the lacustrine calcareous with the paleozoic carbonate rocks (Almeida et al. 2000). The soil is mud-argillaceous derived from schist soils. Based on hydrogeological information from the DRASTIC method developed for Portugal, this region has a high groundwater contamination potential (Lobo Ferreira and Oliveira 1995). Vineyards are irrigated by drip systems with groundwater extracted from wells.

Environmental fate and ecotoxicological characterisation of the pesticides selected for the study

Alachlor, atrazine, chlorfenvinphos, chlorpyrifos, endosulfan, lindane, simazine and trifluralin were selected since they are indicated in the list of priority substances in the field of water policy (EC 2008). Terbuthylazine and terbutryn, herbicides belonging to the triazine chemical group and the metabolite desethylatrazine were also included in the study.

On the basis of a set of physicochemical and partition coefficient properties selected from different databases (FOOTPRINT 2009; Hornsby et al. 1996; Tomlin 2006), the environmental exposure was evaluated a priori through models, such as fugacity model level I (Mackay 2001) and the leaching indexes developed by Bacci and Gaggi (1993) and Gustafson (1989). Based on this calculation, Table 1 presents the Predicted Environmental Distribution (PED) for water of each pesticide, and its leachability considering the criteria set by the authors (Bacci 1994; Gustafson 1989).

Median effective/lethal concentration (EC_{50}/LC_{50}) values for algae, *Daphnia* and fish of the pesticides under study were selected from Tomlin (2006) and FOOTPRINT (2009), and classified according to the criteria referred in the literature (EC 2001) (Table 2).

Table 1 Predicted Environmental Distribution (PED) for water and leaching potential of the pesticides selected for the study

	PED for water (%)[a]	Bacci and Gaggi index[b]	GUS index[c]
Herbicide			
Alachlor	47.3	0.16 (L)	2.08 (T)
Atrazine	77.7	0.62 (L)	3.56 (L)
Simazine	89.8	0.56 (L)	3.35 (L)
Terbuthylazine	40.5	0.44 (L)	3.18 (L)
Terbutryn	19.8	4.43E-2 (T)	1.13 (IL)
Trifluralin	1.56	0.02 (T)	0.17 (IL)
Metabolite			
Desethylatrazine	–	0.62 (L)	3.54 (L)
Insecticide			
Z-Chlorfenvinphos	13.5	0.11 (L)	1.87 (T)
E-Chlorfenvinphos	6.23	0.11 (L)	1.87 (T)
Chlorpyrifos	2.15	1.08E-2 (T)	0.32 (IL)
α-Endosulfan	1.96	8.79E-3 (IL)	−0.16 (IL)
β-Endosulfan	1.75	8.79E-3 (IL)	−0.16 (IL)
Lindane	25.7	0.3 (L)	2.49 (T)

– no data

[a] Mackay fugacity model ('Level I, version 3.00, 2004, Trentu University, Canada')

[b] If index ≥ 1E-1: Leacher (L); if 1E-2 ≤ index ≤ 9E-2: Transition (T); if 1E-4 ≤ index ≤ 9E-3: Improbable Leacher (IL) (Bacci 1994)

[c] If GUS > 2.8: Leacher (L); if 1.8 < GUS < 2.8: Transition (T); if GUS < 1.8: Improbable Leacher (IL) (Gustafson 1989)

Water and sediment sampling

A total of 43 groundwater samples were collected from six wells: three drilled wells (DrW1, DrW2 and DrW3), two dugged wells (DuW1 and DuW2) and one spring (SpW). If possible, water was pumped for approximately 5 min from the well to purge the pipes in order to obtain a representative groundwater sample. Some wells did not have a pump so sampling had to be performed manually at approximately 1 m below the surface with a stainless steel/amber glass grab bottle. Nine surface water samples were collected after rain events from five spots (SW1–SW5) of two drainage channels. These surface water sampling points were located at the lower part of the vineyard fields receiving run-off and drainage water from them. Water samples were collected in 1 L single-use amber PET bottles and were then kept on dry ice until arrival at the laboratory for analysis of pesticides and toxicity to *Daphnia magna* and *Pseudokirchneriella subcapitata*. Seven surface sediment samples (0–5 cm) were collected with a spoon to 500 mL amber glass bottles for determination of effects on *Heterocypris incongruens*. Water sampling was carried out two to four times per year from 2004 to 2006, in periods between March and October. Sediment samples were only taken in 2005 and 2006 from four spots (SedW1–SedW4) because surface water was absent in the two drainage channels in 2004.

Analysis of pesticides in water

Chemicals Pesticide standards of 99% purity (BASF Aktiengesellschaft, Limburgerhof, Germany) or higher (Dr. Ehrenstorfer GmbH, Augsburg, Germany), analytical-grade reagents (Merck, Darmstadt, Germany) and sodium chloride (Panreac Quimica, Barcelona, Espanha) were used throughout the analysis.

Multi-residue method A commercial autosampler (Combi PAL from CTC Analytics, AG, Switzerland) using a solid-phase microextraction (SPME) module, mounted on a

Table 2 Toxicity to algae, *Daphnia* and fish of the pesticides selected for the study

	EC_{50} (72 h) algae[a] mg L^{-1}	EC_{50} (48 h) *Daphnia*[a] mg L^{-1}	LC_{50} (96 h) fish[b] mg L^{-1}
Herbicide			
Alachlor	0.012 (VT)	13 (H)	2.1 (T)
Atrazine	0.043 (VT)	6.9 (T)	4.3 (T)
Simazine	0.042 (VT)	>100	49 (H)
Terbuthylazine	0.016 (VT)	21 (H)	3.8 (T)
Terbutryn	0.0024 (VT)	2.66 (T)	1.1 (T)
Trifluralin	0.0122 (VT)	0.245 (VT)	0.088 (VT)
Metabolite			
Desethylatrazine	0.1 (VT)	–	–
Insecticide			
Chlorfenvinphos	1.6 (T)	0.0003 (VT)	0.04 (VT)
Chlorpyrifos	0.48 (VT)	0.0017 (VT)	0.002 (VT)
Endosulfan	>0.56 (VT)	0.075 (VT)	0.002 (VT)
Lindane	0.78 (120 h)/2.5 (T)	1.6 (T)	0.022 (VT)

– no data

[a] $EC_{50} \leq 1$ mg L^{-1}: Very toxic (VT) (for algae and *Daphnia*); $1 < EC_{50} \leq 10$ mg L^{-1}: Toxic (T) (for algae and *Daphnia*); $10 < EC_{50} \leq 100$ mg L^{-1}: Harmul (H) (for algae and *Daphnia*)

[b] $LC_{50} \leq 1$ mg L^{-1}: Very toxic (VT) (for fish); $1 < LC_{50} \leq 10$ mg L^{-1}: Toxic (T) (for fish); $10 < LC_{50} \leq 100$ mg L^{-1}: Harmul (H) (for fish) (EC 2001)

gas chromatography–mass spectrometry system (GC–MS) was used. SPME fibres of 65 μm carbowax divinylbenzene (CW/DVB) were supplied by Supelco (Bellefonte, PA, USA). The SPME step was performed at the following conditions: water samples (10 mL) were placed in vials and extracted by immersion of the coated fibre for 60 min at room temperature; sample agitation was used at 600 rpm after the addition of 10% NaCl (w/v); no pH adjustment was needed. After extraction, the fibre was directly exposed to the hot injector of the GC system for analysis. Thermal desorption was carried out for 5 min.

GC–MS analyses were performed with a Varian ChromPack CP-3800 gas chromatograph coupled to a Saturn 2000 GC/MS (Varian, Walnut Creek, CA, USA) equipped with an ion-trap detector. A J&W DB-5MS 30 m × 0.25 mm Low Bleed/MS column was used (J&W Scientific, Folsom, CA, USA). Helium was employed as carrier gas at 83 kPa (12 psi). Injector, interface, and mass-spectrometric detector temperatures were 270°C, 260°C and 190°C, respectively. Column temperature was programmed from 50°C to 170°C at 10°C min^{-1}, from 170°C to 180°C at 1°C min^{-1}, from 180°C to 200°C at 5°C min^{-1}, maintained 6 min at this temperature and then from 220°C to 240°C at 15°C min^{-1}, with final time of 4 min. The ionization mode was electronic impact. Ions used for identification and quantification were as follows (in parentheses): alachlor (160 + 188), atrazine (200 + 215), *E*-chlorfenvinphos (267 + 323), *Z*-chlorfenvinphos (267 + 323), chlorpyrifos (197 + 314 + 316), desethylatrazine (172 + 187), α-endosulfan (195 + 241 + 339), β-endosulfan (195 + 241 + 339), lindane (181 + 183 + 219), simazine (173 + 186 + 201), terbuthylazine (173 + 214 + 229), terbutryn (170 + 185 + 226 + 241) and trifluralin (264 + 306). Detection limits varied between 0.1 ng L^{-1} (trifluralin) and 35 ng L^{-1} (desethylatrazine).

The analytical method used was capable of measuring concentrations equal to the parametric value (0.1 μg L^{-1}) with a trueness of 25% and a precision of 25%. Pesticide detection limits were determined by the equation $3.27 \times s$, where s is the standard deviation of 10 blanks fortified with the lowest calibration curve concentration level (EC 2002).

Bioassays for toxicity testing

Toxkit microbiotests (MicroBioTests, Mariakerke, Gent, Belgium) were used for toxicity testing of water and sediments. The Algaltoxkit F™ is a 72-h growth inhibition test (at 25°C with 24 h

Table 3 Occurrence of the individual pesticides detected in ground- and surface water samples in relation to the parametric value for human consumption and the groundwater quality standard, the WHO guidelines and the USEPA health advisory values

Water type	Number of samples (N) with concentrations above the										Maximum concentration μg L^{-1}		
	DL			0.1 μg L^{-1a}			WHO guidelines[b]		USEPA health advisory values[c]				
	GW ($N = 43$)	SW ($N = 9$)	Total ($N = 52$)	GW ($N = 43$)	SW ($N = 9$)	Total ($N = 52$)	GW ($N = 43$)	SW ($N = 9$)	GW ($N = 43$)	SW ($N = 9$)	GW	SW	Total
Herbicide													
Simazine	21	5	26	3	4	7	0	0	0	0	0.78 (DrW2)	0.82 (SW1)	0.82 (SW1)
Terbuthylazine	8	4	12	2	4	6	0	0	–	–	2.27 (DuW2)	0.83 (SW5)	2.27 (DuW2)
Terbutryn	3	0	3	2	0	2	–	–	–	–	4.22 (DrW2)	<DL	4.22 (DrW2)
Metabolite													
Desethylatrazine	2	1	3	1	0	1	–	–	–	–	0.1 (DuW2)	0.07 (SW3)	0.1 (DuW2)
Insecticide													
Chlorpyrifos	2	0	2	0	0	0	0	0	0	0	<0.05 (DuW1, DuW2)	<DL	<0.05 (DuW1, DuW2)

DL detection limit; *GW* groundwater; *SW* surface water, – no data

[a] Parametric value for human consumption and groundwater quality standard for individual pesticides (EC 1998, 2006)

[b] 2, 7 and 30 μg L^{-1} for simazine, terbuthylazine and chlorpyrifos, respectively (WHO 2008)

[c] 4 and 20 μg L^{-1} for simazine and chlorpyrifos, respectively (U.S. EPA 2006)

of light), based on the green algae *P. subcapitata* (SOP 2004a). Algal growth was determined by optical density measurements in a Hitachi U-2000 spectrophotometer UV–Vis (Hitachi, Ltd., Tokyo, Japan). The Daphtoxkit F™ magna is a 24–48-h acute toxicity test (exposure in darkness at 20°C), based on immobility or mortality of the cladoceran crustacean *D. magna* (SOP 2003). These microbiotests adhere to 'International Organization for Standardization' guidelines (ISO 1996, 2004). The Ostracodtoxkit F™ is a 6-day chronic toxicity test (at 25°C in darkness) with the ostracod *H. incongruens* based on two distinct effect criteria: mortality of the test organism or growth inhibition, resulting from the direct contact with (non-diluted) sediment (SOP 2004b).

Results and discussion

Pesticide concentrations in ground- and surface water

In all ground- and surface water samples, the most frequently detected pesticides were simazine > terbuthylazine > terbutryn = desethylatrazine > chlorpyrifos. However, the herbicide terbutryn and the insecticide chlorpyrifos were not detected in surface water. The total number of water samples with concentrations above the parametric value for human consumption, and the groundwater quality standard for individual pesticides (0.1 μg L^{-1}) was highest for the herbicide simazine, followed by terbuthylazine, terbutryn and the metabolite desethylatrazine. However, they did not exceed the WHO guidelines (2, 7 and 30 μg L^{-1} for simazine, terbuthylazine and chlorpyrifos, respectively) (WHO 2008) and the USEPA health advisory values (4 and 20 μg L^{-1} for simazine and chlorpyrifos, respectively) (U.S. EPA 2006) established for these compounds (Table 3).

Maximum concentrations for each pesticide were, in decreasing order, 4.22 μg L^{-1} (terbutryn), 2.27 μg L^{-1} (terbuthylazine), 0.82 μg L^{-1} (simazine), 0.1 μg L^{-1} (desethylatrazine) and <0.05 μg L^{-1} (chlorpyrifos). The highest value (i.e. 4.22 μg L^{-1} terbutryn) was quantified in a water sample collected at the drilled well DrW2 that supplies water for domestic use and the wine making cellar (Table 3).

The concentrations of chlorpyrifos and simazine were below the maximum allowable

Table 4 Occurrence of the individual pesticides detected in ground- and surface water samples in relation to the environmental quality standards applicable to surface water and the TER trigger values specified for aquatic organisms (algae, *Daphnia* and fish)

Water type	Number of samples (N) with							
	TER[a] for algae < 10		TER[b] for *Daphnia* < 100		TER[c] for fish < 100		Concentrations above the MAC-EQS[d] Inland and other surface waters	
	GW ($N = 43$)	SW ($N = 9$)	GW ($N = 43$)	SW ($N = 9$)	GW ($N = 43$)	SW ($N = 9$)	GW ($N = 43$)	SW ($N = 9$)
Herbicide								
Simazine	0	0	0	0	0	0	0	0
Terbuthylazine	1	0	0	0	0	0	–	–
Terbutryn	2	–	0	–	0	–	–	–
Metabolite								
Desethylatrazine	0	0	–	–	–	–	–	–
Insecticide								
Chlorpyrifos	0	–	2	–	2	–	0	0

GW Groundwater; *SW* Surface water, –no data

[a] EC50 (72 h) algae/exposure ratio

[b] EC50 (48 h) *Daphnia*/exposure ratio

[c] LC50 (96 h) fish/exposure ratio (EC 1997)

[d] Maximum allowable concentration-Environmental quality standard 4 and 0.1 μg L^{-1} for simazine and chlorpyrifos, respectively (EC 2008)

concentration-environmental quality standards (0.1 and 4 μg L^{-1}, respectively) for priority substances in the field of water policy (EC 2008) (Table 4). Due to the lack of environmental quality standards for all pesticides, the lower-tier risk assessment procedure for PPPs was used based on ecotoxicological data produced on standard organisms assumed as representative of the aquatic environment (e.g. algae, *Daphnia* and fish). Taking into account the ratio between the effect measures (see Table 2) and the quantified concentrations, the herbicides terbuthylazine and terbutryn were above the TER trigger value specified for algae in one and two groundwater samples, as well as the insecticide chlorpyrifos for *Daphnia* and fish in two groundwater samples (Table 4). Although these biological elements are not representative of this resource natural, the results are particularly important for groundwater dependent ecosystems.

The detection of the herbicide terbuthylazine was expected *a priori* by the authors because it was registered for use in vine-growing at the time of the study, it presents an average affinity for the water compartment and it is classified as leacher (see Table 1). Although also used in vine-growing, the insecticide chlorpyrifos have lesser potential to contaminate surface and groundwater (see Table 1); so the low concentration detected was probably due to a point contamination. The two former pesticides were also detected in two wells used for irrigation in other agricultural areas of Portugal, with a maximum concentration of <0.05 μg L^{-1} (Batista 2003).

The herbicides simazine and terbutryn were also found in concentrations above 0.1 μg L^{-1} in water samples. This may be explained by the fact that they were registered for several uses and have a moderate persistence in soil. In vineyard and orchard areas of the 'Ribatejo e Oeste' and 'Beira Litoral' regions (Portugal), simazine was also detected in 31% of 241 wells analysed, with a maximum concentration of 2.39 μg L^{-1}. The metabolite desethylatrazine is mainly related to the use of the herbicide atrazine and was previously detected in 60% of 103 wells analysed in 1999 and 2000 (Batista et al. 2002; Batista 2003). These compounds were also detected in waters of the Ebro river basin (Spain) during the years 2001–2004

Table 5 Occurrence of the pesticides detected in ground- and surface water samples in relation to the parametric value for human consumption and the groundwater quality standard, and their qualitative combinations

Water type	Number of samples (*N*) with									Maximum concentration μg L^{-1}
	Concentrations above the		One pesticide compound	Two pesticide compounds				Three pesticide compounds		
	DL	0.5 μg L^{-1a}		Simazine + terbuthylazine	Simazine + terbutryn	Simazine + DEA	Simazine + chlorpyrifos	Simazine + terbuthylazine + terbutryn	Simazine + terbuthylazine + chlorpyrifos	
GW ($N = 43$)	26	6	18	2	1	2	1	1	1	4.22 (DrW2)
SW ($N = 9$)	9	5	8	0	0	1	0	0	0	0.84 (SW3)
Total ($N = 52$)	35	11	26	2	1	3	1	1	1	4.22 (DrW2)

GW groundwater; *SW* surface water

[a] Parametric value for human consumption and groundwater quality standard for pesticides-total (EC 1998, 2006)

(Claver et al. 2006). They were included in the study because of their use on several crops present in the basin, among which vine-growing. In a review on herbicide occurrence in groundwater by Funari et al. (1995), simazine, terbuthylazine and the metabolite deethylatrazine were reported to be present in this compartment, reaching maximum levels from 0.07 to 35 μg L^{-1}. In a study conducted in shallow groundwater of the USA during 1993–1995, simazine detection was mainly related with wheat and orchards/vineyards, with frequencies of 43% and 31.7%, respectively, of 1,034 sites sampled (Kolpin et al. 1998).

The frequency of detection of the individual pesticides, as well as the total concentration of pesticides above 0.5 μg L^{-1} (parametric value for human consumption and groundwater quality standard for pesticides-total) are higher for surface water than for groundwater samples. This was in line with the expectations since the surface water receives run-offs drained directly from the vineyard fields where the applications were performed, whereas in groundwater the compounds are more diluted. However, the maximum concentration for pesticides-total (4.22 μg L^{-1}) was detected in a groundwater sample due to one single compound (terbutryn; Table 5). Although there are more samples with only one pesticide, mixtures of up to three compounds in different qualitative combinations were also found, as shown in Table 5.

The evolution of the pesticide concentrations in ground- and surface water samples collected at the dug well DuW2 and drainage channel point SW2

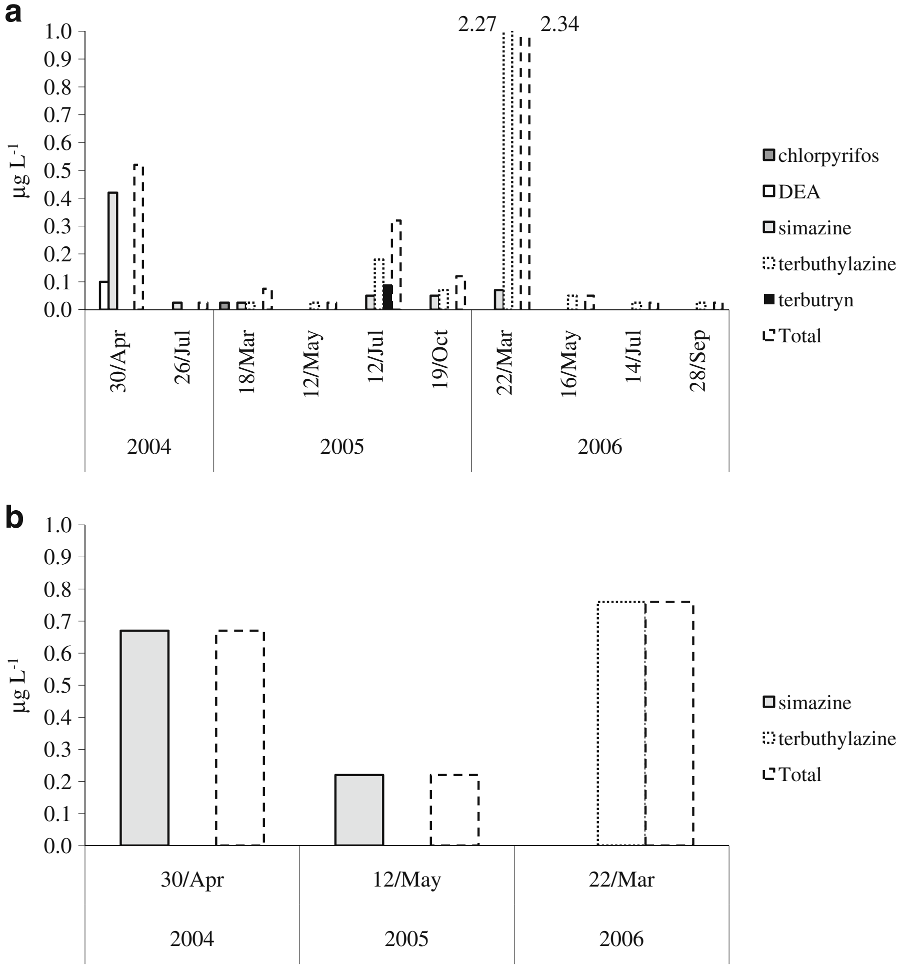

Fig. 1 Evolution of the pesticide concentrations in **a** groundwater samples collected at the dug well DuW2; **b** surface water samples collected at the drainage channel point SW2 in the vineyard farm ('Alentejo' region) from 2004 to 2006

Table 6 Occurrence of effects on algae *Pseudokirchneriella subcapitata* and crustacean *Daphnia magna* in ground- and surface water samples

Water type	*Pseudokirchneriella subcapitata*			*Daphnia magna*		
	Number of samples (N) with		Maximum effects (%)	Number of samples (N) with		Maximum effect (%)
	No effects	Effects above 50%		No effects	Effects above 50%	
GW ($N = 43$)	23	2	82.56 (DuW1)	2	10	100 (DrW2)
SW ($N = 9$)	5	0	19.45 (SW2)	1	0	10 (SW1, SW2, SW4)
Total ($N = 52$)	28	2	82.56 (DuW1)	3	10	100 (DrW2)

GW groundwater; *SW* surface water

is graphically presented in Fig. 1. These dynamics may have been influenced by management practices across vineyard seasons, such as application of pesticides and irrigation, as well as by rain events.

Effects on aquatic organisms in water and sediments

The number of groundwater samples with toxic effects above 50% was greater for the crustacean *D. magna* than for the green algae *P. subcapitata*. A 100% immobility of *D. magna* was noted in two groundwater samples collected at the drilled well DrW2, whereas the maximum growth inhibition for *P. subcapitata* (82.56%) was registered at the dug well DuW1 (Table 6).

The maximum concentration of pesticides-total in groundwater samples with toxic effects above 50% was 0.07 μg L^{-1}, suggesting that the effects observed on aquatic organisms (*P. subcapitata* and *D. magna*) can be caused by a number of chemical, physical and biological factors than can act independently or jointly.

No toxic effects above 50% were observed in surface water samples on *P. subcapitata* and *D. magna* (Table 6). With regard to effects on *H. incongruens* in sediment samples, mortality and growth inhibition values were below or equal to 25% in 2005; a mortality of 38% was noted in one sample in 2006.

Conclusions

This study revealed ground- and surface water contamination by herbicides simazine, terbuthylazine, terbutryn, metabolite desethylatrazine and the insecticide chlorpyrifos in a vineyard area of the 'Alentejo' region (South Portugal) in the period 2004–2006. All these pesticides were presented in concentrations higher than the parametric value for human consumption and the groundwater quality standard for individual pesticides (0.1 μg L^{-1}), and/or did not pass the TER triggers specified for algae, *Daphnia* and fish. The quality standard for pesticides-total (0.5 μg L^{-1}) was also exceeded in a number of samples. Toxic effects greater than 50% on *P. subcapitata* and *D. magna* were only found in groundwater samples.

Future investigations in the scope of the management of river basins are needed to identify the trends of pesticide concentrations in groundwater. Other compounds than those analysed in the present study should also be evaluated in relation to their use, potential impact on human health and the environment. It is also important to understand the complex effect of combined multiple stressors, with particular emphasis on the combination effects resulting from the joint occurrence of pesticide mixtures.

The sustainable use of pesticides and their substitution by compounds with more favourable physico-chemical properties and lower toxicity, taking into account both abiotic and biotic factors, must be considered as important mitigation measures to reduce inputs into ground- and surface water at the farm and catchment scales.

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