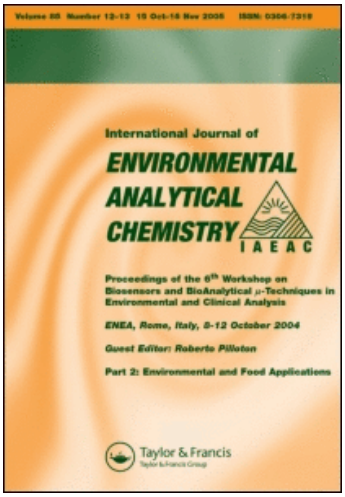

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Pesticides and nitrates in groundwater from oriziculture areas of the 'Baixo Sado' region (Portugal)

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The aim of this study was the groundwater evaluation to pesticide compounds and nitrates in oriziculture areas of the 'Baixo Sado' region (Portugal), based on their use, predictive approaches, and field and laboratory work. One or more of the pesticide compounds analysed (chlorfenvinphos, cycloxydim, 3,4-dichloroaniline, endosulphan, MCPA, molinate, oxadiazon, profoxydim, propanil) were detected in 62% of 171 water samples collected from 22 wells used for public supply, domestic supply, and irrigation, during 2002 and 2003. From the total samples, 6% presented maximum concentration levels of at least one of the compounds above $0.1\,\mu g\,L^{-1}$. Mixtures of pesticide compounds were observed in 25% of the total groundwater samples, with up to five substances being detected in each one. The concentration sum of all was above $0.5\,\mu g\,L^{-1}$ in four water samples. All the analysed compounds, with the exception of the insecticide chlorfenvinphos, occurred in groundwater. Molinate was the most frequently detected (55%), particularly with maximum concentration levels above $0.1\,\mu g\,L^{-1}$. Detection frequencies were higher in water samples collected from irrigation wells (78%). Groundwater exposure to total pesticides and nitrates was analysed. Maximum concentration levels were $59.6\,\mu g\,L^{-1}$ and $183\,mg\,L^{-1}$ NO_3^-, respectively. A seasonal variation pattern could be observed for both parameters in water samples collected from some wells. The results from this study show that sustainable use of pesticides and nitrogen fertilizers is required in order to achieve an overall contamination reduction from these compounds in the aquatic environment.

Keywords: Pesticides; Nitrates; Groundwater; Rice; Portugal

1. Introduction

In the last two decades, there have been increasing concerns about the possible contamination of water resources by agrochemical residues used on agricultural lands. These concerns stem from the awareness that maintaining the natural-resource base quality is crucial for sustaining agricultural productivity.

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In order to protect water quality and prevent risks to human health, some European Directives were implemented, as Council Directive 80/778/EEC of 15 July 1980 [1], replaced by Council Directive 98/83/EC of 3 November 1998 [2] on the quality of water intended for human consumption; Council Directive 91/676/EEC of 12 December of 1991 [3], concerning the protection of waters against pollution caused by nitrates from agricultural sources; and Directive 2000/60/EC of 23 October 2000 [4], establishing a framework for Community action in the field of water policy. Decision 2455/2001/EC of 20 November 2001 [5] established the list of priority substances in the field of water policy, e.g. some pesticides: alachlor, atrazine, chlorfenvinphos, chlorpyrifos, diuron, endosulphan, isoproturon, lindane, simazine and trifluralin. This supplements the water framework directive and becomes Annex X.

Although the Council Directive 91/414/EEC [6] does not directly relate to water-quality monitoring, it does have potentially significant implications for data interpretation on the environmental occurrence of pesticides in water. The Directive states that a 'plant protection product will only be authorized in the EC if it has no unacceptable influence on the environment, with regard to its fate and distribution and particularly water contamination including drinking water and groundwater'.

Worldwide studies have detected the presence of agrochemicals in water, raising major health concerns [7–13]. In Portugal, some studies performed in agricultural areas (maize, tomato, potato, horticulture, rice, vineyard, and orchards) indicated the occurrence of pesticides and nitrates in groundwater [14–24]. Other studies evaluated the impact of ricefield pesticides on the quality of freshwater resources, mainly their effects on aquatic biota in surface water bodies of the 'Baixo Sado' and 'Mondego' regions [25–27].

However, very few studies have been conducted to investigate the contamination levels of groundwater resources from pesticides used on ricefields [22, 24, 28, 29]. In this paper, a case study performed in the rice crop area of the 'Baixo Sado' region (Portugal) is reported based on pesticide use, predictive approaches, and field and laboratory work. Due to the close relation of this crop with water and intensive application of these compounds, mainly herbicides, but also fertilizers, groundwater exposure to pesticides, and nitrates, were evaluated.

2. Experimental

2.1 *Study area*

In Portugal, rice occupies about 25 000 ha [30], distributed by the Mondego, Tejo, and Sado river basins. This study was conducted in the 'Baixo Sado' region that falls in the Sado river basin, the largest located entirely in national territory, occupying an area of approximately 8093 km^2 [31]. Rice is the main irrigated crop, occupying 5798 ha, followed by maize, with 817 ha [32].

Hydrogeologically, the 'Baixo Sado' region belongs to the Tejo-Sado Basin/Left Border aquifer system, with porous characteristics, constituted by tertiary and quaternary age detrital formations, representing the most extensive aquifer system of the Iberian Peninsula [31]. This region presents low to intermediary

groundwater vulnerability indices, on a DRASTIC method basis developed for Portugal [33].

Concerning water supply in rice fields of the 'Baixo Sado' region, the majority of the farmers uses surface water sources, namely from two dams, streams or even from Sado River.

2.2 *Selection of pesticide compounds for analysis*

Selection of pesticides for analysis was based on several types of information, namely data from inquiries on rice growers in the Sado Valley. The results from these surveys indicated that the pesticides most often used and introduced in larger quantities by rice farmers in the 'Baixo Sado' region were the herbicides molinate (24 306 kg), propanil (22 109 kg), MCPA (2637 kg), dimethoate (1313 kg), quinclorac (595 kg), tricyclazole (381 kg), and chlorfenvinphos (215 kg). Although there are no available data, endosulphan is one of the most frequently applied insecticides in the region, namely to control red swamp crawfish.

Herbicides cycloxydim, profoxydim, and oxadiazon were used in lesser amounts to control *Echinochloa* spp., *Heteranthera* spp., and *Oryza sativa* L., with increasing importance in the last years.

The use of herbicides in rice fields of the 'Baixo Sado' region is usually carried out in one or two applications, before and/or after seeding date. The herbicides are applied by farm tractor or plane, without water in the plots, with the exception of molinate, which can also be applied under rice-flooded conditions. The presence of diseases or pests is the major criterion for the application of fungicides and insecticides during the rice-growing season.

The affinity for water and tendency to leach were calculated for each pesticide compound based on physical–chemical and partition coefficient properties and values selected from different studies (see table 1). The predicted environmental distribution (PED) for water was estimated through an evaluative model (fugacity model level I) applied in standard form [34]. Dimethoate, cycloxydim, MCPA, quinclorac, and tricyclazole show a high affinity for the water compartment, with a predicted environmental distribution higher than 80% (see table 2).

In order to evaluate the leachability of pesticide compounds being studied, Bacci and Gaggi [40] and GUS [41] leaching indexes were used. The compounds considered for analysis varied between 'leacher', 'transition', and 'improbable leacher', with the exception of three of them that are classed, according to both index calculations, as 'leacher' (3,4-dichloroaniline and quinclorac) and 'improbable leacher' (profoxydim) (see table 2).

All the aforementioned pesticide compounds were preliminary selected to be analysed, due to their common use (molinate, propanil, and MCPA) and particularly to control increasing numbers of certain rice weeds (cycloxydim, oxadiazon, and profoxydim), a high affinity for the water compartment and/or leachability (3,4-dichloroaniline, dimethoate, cycloxydim, MCPA, quinclorac and tricyclazole), and ecotoxicological relevance (chlorfenvinphos, endosulphan, molinate, and propanil). However, quinclorac and dimethoate were not analysed, due to unavailability of analytical methodology. Tricyclazole, used for control *Piricularia oryzae*, was not considered, since it is seldom applied.

Table 1. Physical–chemical and partition coefficients properties values selected from different authors for pesticide compounds.

Pesticide compound	Molecular weight ($g\,mol^{-1}$) [35]	Melting point (°C) [35]	Water solubility ($mg\,L^{-1}$) [35]	Vapour pressure (Pa) [35]	Log Kow [35]	Koc ($mL\,g^{-1}$ C.O.) [36]	pKa (or pKb) [35]	DT_{50soil} (d) [36]
Herbicide								
Cycloxydim	325.5	41	40	<0.01	1.36	96.5 [35]	4.17	6.25 [35]
3,4-Dichloroaniline[a]	162.02 [37]	272 [37]	336 [37]	1.84E−1 [37]	2.7 [37]	195 [37]	–	985 [37]
MCPA	200.6	119.75	273.9	2.3E−5	−0.71	112 [38]	3.07	20 [38]
Molinate	187.3	<25	970 [36, 38]	7.46E−1	2.88	190	–	21
Oxadiazon	345.2	87	1	1E−4	4.91	3200	–	60
Profoxydim	466	<25	5.31	1.7E−4	3.9	3257[b]	5.91	3 [35]
Propanil	218.1	91.5	130	2E−5	3.3	149	–	1
Quinclorac	242.1	274	0.065	<1E−5	−1.15	33.5 [39]	4.34	44 [39]
Insecticide								
Z-Chlorfenvinphos	359.6	−21	145	1E−3	3.85 [38]	295 [38]	–	<7 [38]
E-Chlorfenvinphos	359.6	−21	145	1E−3	4.22 [38]	295 [38]	–	<7 [38]
Dimethoate	229.3	49	23800	2.5E−4	0.704	34 [35]	–	3 [35]
α-Endosulphan	406.9	109.2	0.32	1.3E−3 [38]	4.74	2512 [38]	–	50 [35]
β-Endosulphan	406.9	213.3	0.33	6.1E−3 [38]	4.79	3162 [38]	–	50 [35]
Fungicide								
Tricyclazole	189.2	187.5	1600	2.7E−5	1.4	1200	1.6 [36]	21

[a] Propanil metabolite; [b] Koc = 0.41Kow; – no data.

Table 2. Predicted environmental distribution (PED) for water and leaching potential calculated for pesticide compounds.

Pesticide	PED for water (%)[a]	Bacci & Gaggi index[b]	GUS index[c]
Herbicide			
Cycloxydim	97.8	0.147 (L)	1.60 (IL)
3,4-Dichloroaniline	67.9	0.851 (L)	5.12 (L)
MCPA	99.9	0.325 (L)	2.54 (T)
Molinate	58.2	0.189 (L)	2.28 (T)
Oxadiazon	1.3	0.039 (T)	0.88 (IL)
Profoxydim	12.2	0.002 (IL)	0.23 (IL)
Propanil	35.6	0.014 (T)	0.00 (IL)
Quinclorac	99.9	0.747 (L)	4.07 (L)
Insecticide			
Z-Chlorfenvinphos	13.5	0.042 (T)	1.19 (IL)
E-Chlorfenvinphos	6.2	0.042 (T)	1.19 (IL)
Dimethoate	99.5	0.406 (L)	2.28 (T)
α-Endosulphan	2.0	0.041 (T)	1.02 (IL)
β-Endosulphan	1.7	0.031 (T)	0.85 (IL)
Fungicide			
Tricyclazole	97.8	0.049 (T)	1.22 (IL)

[a] Calculated according Mackay fugacity model ('Level I, version 3.00, 2004, Trentu University, Canada'); [b]if index $\geq$ 1E−1: leacher (L); if 1E−2 $\leq$ index $\leq$ 9E−2: transition (T); if 1E−4 $\leq$ index $\leq$ 9E−3: improbable leacher (IL) [42]; [c]if GUS > 2.8: leacher (L); if 1.8 < GUS < 2.8: transition (T); if GUS < 1.8: improbable leacher (IL) [41].

2.3 *Water sampling*

A total of 171 water samples were collected from 22 wells in the study area (see figure 1), including 11 for public supply (seven drilled wells, three fountains associated with drilled wells, and one spring), two for domestic supply (one drilled well and one spring), and nine for irrigation use (seven drilled wells and two dug wells), with depths varying between 9 m and 160 m. Sampling was carried out during April–October 2002 and May–November 2003, before, during, and after the rice-growing season. Samples were collected in 500 mL pre-cleaned amber glass bottles and acidified for MCPA with nitric or hydrochloric acid to pH 1–2. They were then kept cool in dry ice until arrival at the laboratory.

2.4 *Analytical methods for pesticide compounds*

2.4.1 Chemicals. Pesticide standards of 70% purity (cycloxydim) (BASF Aktiengesellschaft, Limburgerhof, Germany) or higher (Dr. Ehrenstorfer GmbH, Augsburg, Germany), pesti-grade solvents (J.T. Baker, Phillipsburg), analytical-grade reagents (Merck, Darmstadt, Germany), and sodium chloride (Panreac, Barcelona) were used throughout the analysis.

2.4.2 Analytical methods for chlorfenvinphos, endosulphan, molinate, oxadiazon, propanil, and the metabolite 3,4-dichloroaniline. Solid-phase microextraction (SPME) holder and fibre assemblies for manual sampling were obtained from Supelco (Bellefonte, PA). Carbowax divinylbenzene 65 μm (CW-DVB 65 μm) was used as stationary phase in SPME.

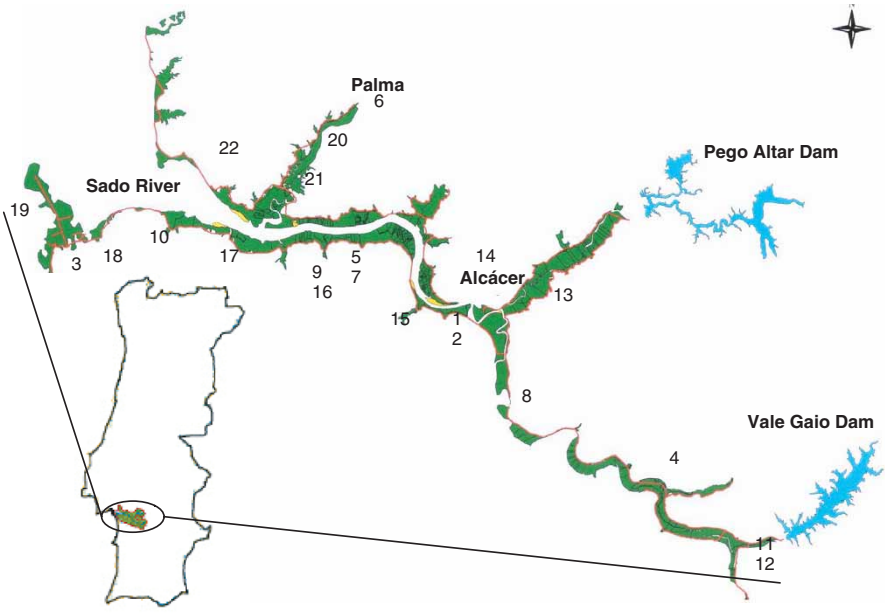

Figure 1. Schematic localization of 22 wells sampled in the 'Baixo Sado' region (Portugal). Drilled wells for public supply: 1–7; fountains for public supply: 8–11; drilled wells, dug wells, and spring for domestic supply and irrigation use: 12–22.

Water samples (10 mL) were placed into 10 mL vials and stirred after the addition of 10% NaCl (w/v). The fibre was added to the aqueous phase in 4 mL vials for an appropriate time period of 60 min and stirred at 600 rpm at room temperature. 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.

Gas chromatography–mass spectrometry analyses were performed in a Varian ChromPack CP-3800 gas chromatograph coupled to a Saturn 2000 GC/MS equipped with an ion-trap detector (Varian, Walnut Creek, CA). A J&W DB-5MS 30 m × 0.25 mm Low Bleed/MS column was used (J&W Scientific, Folsom). Helium was employed as carrier gas at 83 kPa (12 psi). The injector, interface, and mass-spectrometric detector temperatures were, respectively, 270, 260, and 190°C. The column temperature was programmed from 50 to 170°C at 10°C min^{-1}, from 170 to 180°C at 1°C min^{-1}, from 180 to 200°C at 5°C min^{-1}, maintained for 6 min at this temperature and then programmed from 220 to 240°C at 15°C min^{-1}, with a final time of 4 min. The ionization mode was by electronic impact (EI). The ions used for identification and quantification were as follows (in parentheses): *Z*-chlorfenvinphos (267 + 323), *E*-chlorfenvinphos (267 + 323), 3,4-dichloroaniline (126 + 161 + 163), α-endosulphan (195 + 241), β-endosulphan (195 + 241), molinate (98 + 126), oxadiazon (175 + 258), and propanil (161 + 163 + 217). Detection limits varied between 0.015 µg L^{-1} (3,4-dichloroaniline) and 0.054 µg L^{-1} (β-endosulphan).

2.4.3 Analytical methods for MCPA. Solid-phase extraction (SPE) was performed with SDB-XC-47 mm disks from Supelco 3M Empore™ (St. Paul, MN). Disks were

sequentially washed with 5 mL of dichloromethane, 5 mL of methanol, and 5 mL of aqueous solution with formic acid (2%). A 500 mL sample aliquot was passed through the disk, followed by elution with 2×5 mL of methanol. Solvent evaporation to 0.3 mL was performed under a nitrogen stream. Finally, this extract was diluted to 1 mL by adding formic acid aqueous solution (2%).

Liquid chromatography–mass spectrometry analyses were carried out with an Agilent Series HP1100 (Palo Alto, CA) equipped with an electrospray ionization interface. Chromatographic separation was achieved with a Hypersil ODS 5 μm, 2.1×100 mm CIM (Agilent, Palo Alto, CA), with the mobile phase being composed of methanol and formic acid aqueous solution (2%; 40:60). Chromatograms were recorded under scanning and selected ion monitoring (SIM) conditions in negative-ion operation mode. The MCPA ions used for identification and quantification were $199 + 201$. The detection limit for MCPA was $0.006\ \mu g\ L^{-1}$.

2.4.4 Analytical methods for cycloxydim and profoxydim. Automated SPE was performed with the ASPEC XL-GILSON (Villiers-le-Bel, France) system. Versatile Oasis®-60 mg cartridges (Waters, Milford, MA) were sequentially washed with 1 mL of dichloromethane, 1 mL of methanol, and 1 mL of water. A 500 mL sample aliquot was passed through the cartridges, followed by elution carried out with 2×2.5 mL of methanol. Solvent evaporation was performed under a nitrogen stream to a final volume of 0.5 mL.

Liquid chromatography–mass spectrometry analyses were performed using an Agilent Series HP1100 Series (Palo Alto, CA) equipped with an electrospray ionization interface. Chromatographic separation was achieved using a ZORBAX 300SB-C18 5 μm, 4.6×150 mm (Agilent Technologies, Palo Alto, CA), with the mobile phase being composed by methanol and water (80:20). Chromatograms were recorded under SCAN and SIM conditions in negative-ion operation mode. The cycloxydim and profoxydim ions used for identification and quantification were $324 + 325$ and $464 + 466$, respectively. The detection limits for cycloxydim and profoxydim were $0.014\ \mu g\ L^{-1}$ and $0.008\ \mu g\ L^{-1}$, respectively.

For the previous pesticides, the used analysis methods were capable of measuring concentrations equal to the parametric value ($0.1\ \mu g\ L^{-1}$) 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.

2.5 *Analytical method for nitrogen (nitrate)*

2.5.1 Chemicals. Analytical-grade reagents used throughout the analysis were obtained from Merck (Darmstadt, Germany).

2.5.2 Analytical method for nitrogen (nitrate). Nitrogen (nitrate) was analysed by the automated cadmium reduction method with an auto-sampler continuous-flow analytical instrument Skalar San Plus System using *FlowAccess* (Windows software for Skalar SAN plus systems) (Breda, Netherlands). NO_3^- is reduced almost quantitatively to nitrite (NO_2^-) in the presence of cadmium (Cd). This method uses

commercially available Cd granules treated with copper sulphate ($CuSO_4$) and packed in a glass column. The produced NO_2^- was determined by diazotizing with sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye that is measured colorimetrically. A correction may be made for any NO_2^- present in sample by analysing without the reduction step. Absorbance was measured at 540 nm and related to nitrate ion concentration. The detection limit was 0.36 mg L^{-1}.

For nitrate, the analysis method used was capable of measuring concentrations equal to the parametric value (50 mg L^{-1}) with a trueness of 10% and a precision of 10%. The nitrate detection limit was determined using the equation $\bar{x} + 3 \cdot s$, where $\bar{x}$ and s are, respectively, the mean and standard deviation of blank measures.

3. Results and discussion

3.1 *Overall detection frequencies for pesticide compounds*

One or more of the analysed pesticide compounds were detected in 62% of 171 groundwater samples. From the total samples, 6% presented maximum concentration levels of at least one of the compounds above 0.1 μg L^{-1} (see table 3), the parametric value in water for human consumption [2].

Concerning groundwater exposure to pesticide compounds and taking into account the well type and its use, positive detection was observed in 54% of 84 water samples for public supply. Pesticides were detected with maximum concentration levels >0.1–1 μg L^{-1} in one and two water samples collected, respectively, from a fountain

Table 3. Detection frequencies observed for pesticide compounds analysed in water samples collected from 22 wells of the 'Baixo Sado' region, in 2002 and 2003, in relation to well type and use.

	Samples										
				Maximum concentration levels (μg L^{-1})							
		Positive detection		<0.05		0.05–0.1		>0.1–1		>1	
Well use/well type	Analysed	No.	%	No.	%	No.	%	No.	%	No.	%
Public supply											
Drilled wells	52	28	54	16	31	10	19	2	4	0	0
Fountain associated with a spring	11	4	36	1	9	2	18	1	9	0	0
Fountains associated with drilled wells	21	13	62	11	52	2	10	0	0	0	0
Total	84	45	54	28	33	14	17	3	4	0	0
Domestic supply											
Drilled wells	6	0	0	0	0	0	0	0	0	0	0
Spring	4	2	50	2	50	0	0	0	0	0	0
Total	10	2	20	2	20	0	0	0	0	0	0
Irrigation											
Drilled wells	56	41	73	24	43	13	23	3	5	1	2
Dug wells	21	19	90	8	38	7	33	3	14	1	5
Total	77	60	78	32	41	20	26	6	8	2	3
Total	171	107	62	62	36	34	20	9	5	2	1

associated with a spring and drilled wells, with concentration levels not being detected above $1\,\mu g\,L^{-1}$. From a total of 10 water samples for domestic supply, two collected from a spring showed a maximum concentration level $<0.05\,\mu g\,L^{-1}$ (see table 3).

Relative to 77 water samples used for irrigation, pesticide compounds with maximum concentration levels >0.1–$1\,\mu g\,L^{-1}$ were found in six samples. Pesticides with maximum concentration levels above $1\,\mu g\,L^{-1}$ were detected in only two water samples collected from a drilled and a dug well. Overall, positive detection was observed in 78% of water samples (see table 3).

These results show that pesticides applied in rice fields of the 'Baixo Sado' region are affecting the groundwater beneath those oriziculture ecosystems. Figure 2 shows box-whiskers of maximum concentration levels observed for pesticide compounds analysed in water samples collected from wells for public/domestic supply and irrigation use. The medians obtained varied across the box plots, with maximum concentration levels between <LD and $0.05\,\mu g\,L^{-1}$. The highest observed values were found in the irrigation wells, particularly in the dug wells. As shown by several studies reviewed by Barbash and Resek [10], this type of well has shallow water-table depths, leading to less groundwater isolation from surface contaminant sources. Furthermore, irrigation wells tend to be located closer to agricultural areas, where pesticides are most commonly applied.

Concerning the active substances analysed, the following detection frequencies were observed: 55% (molinate), 18% (propanil), 8% (oxadiazon), 4% (cycloxydim), 2% (profoxydim), and 1% (endosulphan and MCPA). The detection frequencies with concentration levels above $0.1\,\mu g\,L^{-1}$ were: 6% (molinate), 3% (propanil), and 1% (endosulphan and oxadiazon) (see table 4).

The maximum concentration levels observed for each pesticide compound were, in decreasing order, $59.46\,\mu g\,L^{-1}$ (molinate), $1.86\,\mu g\,L^{-1}$ (propanil), $0.71\,\mu g\,L^{-1}$ (3,4-dichloroaniline), $0.2\,\mu g\,L^{-1}$ (endosulphan), $0.13\,\mu g\,L^{-1}$ (oxadiazon), $0.09\,\mu g\,L^{-1}$ (MCPA), $0.07\,\mu g\,L^{-1}$ (profoxydim), and $0.03\,\mu g\,L^{-1}$ (cycloxydim) (see table 4).

The concentration of molinate herbicide exceeded its WHO guideline value of $6\,\mu g\,L^{-1}$ [43] in only one sample collected in a dug well for irrigation use (see table 4). As previously mentioned, molinate is the pesticide most used in the 'Baixo Sado' region, is regularly applied under flooded conditions [25], and has a high water solubility (see table 1), conditions favourable to its leaching. In a review by Funari *et al.* [9] on herbicide occurrence in groundwater, molinate was reported to be present in this compartment, reaching maximum levels of 6.3–$154\,\mu g\,L^{-1}$. In agricultural areas, including oriziculture areas, of the 'Beira Litoral' region (Portugal), this compound was also detected in 5% of 79 wells analysed, with a maximum concentration of $16.3\,\mu g\,L^{-1}$ [22, 24]. As groundwater it is needed not only for human consumption but also to maintain ecosystems, it is important to note that this molecule was classified as very toxic for aquatic organisms, capable causing long-term adverse effects in the aquatic environment [44].

The herbicides propanil and MCPA, with their importance use in the study region, showed detection frequencies lower than molinate (see table 4), probably due to their minor water solubility (see table 1) and application under dry conditions. Besides, propanil presents the lowest half-life value in soil among pesticide compounds studied (see table 1). In fact, the maximum concentration levels observed for these compounds in several studies were lower than those for molinate, although MCPA has been reported in other studies [8, 9]. In a study conducted in the first 20 National Water-Quality Assessment Study Units of the USA during 1993–1995, propanil detection was

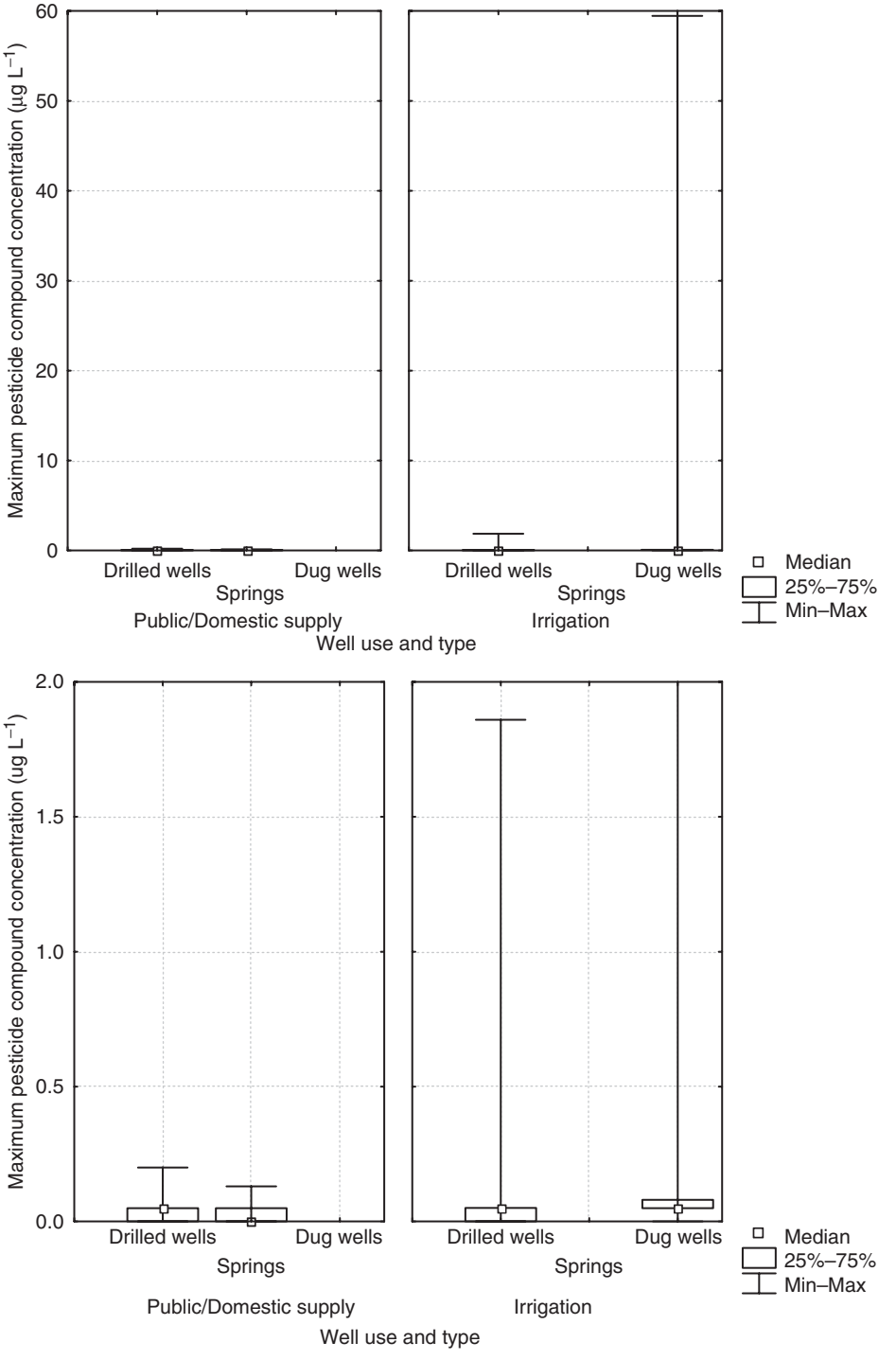

Figure 2. Box-whiskers of maximum concentration levels observed for pesticide compounds analysed in water samples collected from wells for public/domestic supply and irrigation use for the 'Baixo Sado' region, in 2002 and 2003.

Table 4. Detection frequencies observed for each pesticide compound analysed in water samples collected from 22 wells of the 'Baixo Sado' region, in 2002 and 2003.

	Samples											
				With concentration levels ($\mu g\,L^{-1}$)								
		With positive detection		<0.05		0.05–0.1		>0.1–1		>1		Max. conc. level ($\mu g\,L^{-1}$)
Pesticide compound	Analysed	No.	%	No.	%	No.	%	No.	%	No.	%	
Herbicide												
Cycloxydim	102	4	4	4	4	0	0	0	0	0	0	0.03
3,4-Dichloroaniline	171	27	16	16	9	10	6	1	1	0	0	0.71
MCPA	133	1	1	0	0	1	1	0	0	0	0	0.09
Molinate	171	94	56	63	37	22	13	8	5	1	1	59.46
Oxadiazon	171	13	8	9	5	3	2	1	1	0	0	0.13
Profoxydim	102	2	2	1	1	1	1	0	0	0	0	0.07
Propanil	171	31	19	15	9	12	7	3	2	1	1	1.86
Insecticide												
Chlorfenvinphos	171	0	0	0	0	0	0	0	0	0	0	–
Endosulphan	171	1	1	0	0	0	0	1	1	0	0	0.2

also related beneath other land-use settings, as wheat, wheat & alfalfa, and urban, with frequencies of 4.6%, 1.2%, and 0.4%, respectively, of 1034 sites sampled [8].

Other herbicides analysed are seldom detected worldwide, once they are rapidly degraded (cycloxydim and profoxydim) and show a lack of movement from the target location, due to an average degree of soil binding (oxadiazon and profoxydim) (see table 1). Besides these desirable features that define them as potential environmentally friendly pesticides, cycloxydim is still classified as harmful to aquatic organisms, being able to cause long-term adverse effects in the aquatic environment [44].

From the insecticides analysed, only endosulphan was found with a positive detection $>0.1\,\mu g\,L^{-1}$ (see table 4). Bouman *et al.* [28] also detected this compound (max. $1.9\,\mu g\,L^{-1}$) in shallow groundwater under rice-based cropping systems in Luzon (Philippines), from 1989 to 2000. In other agricultural areas (maize, tomato, potato, horticulture, rice, vineyards, and orchards) of Portugal, endosulphan isomers (α and β) were detected in 6% of 79 wells analysed, with maximum concentration levels of $1.43\,\mu g\,L^{-1}$ and $0.69\,\mu g\,L^{-1}$ [22, 24]. Although this insecticide has a low solubility in water, it can be carried by water adsorbed to suspended solids and sediments (see table 1).

The major propanil metabolite, 3,4-dichloroaniline, was detected in 16% of the total groundwater samples, with a detection frequency of 1% in concentration levels above $0.1\,\mu g\,L^{-1}$ (see table 4). These results show the importance of analysing degradation products of pesticides, particularly those that have a high potential for groundwater contamination. This metabolite results from propanil biological degradation, particularly in soil, moving readily in this compartment [45]. In agricultural areas, including oriziculture areas, of the 'Beira Litoral' region (Portugal), this compound was detected at a lower detection frequency (5%) but reached a higher maximum concentration level ($3.75\,\mu g\,L^{-1}$) [22, 24]. In a study reported by Funari *et al.* [9], 3,4-dichloroaniline and its parent compound (propanil) were both detected in shallow

groundwater, reaching maximum concentrations levels of 0.3 and 1.23 $\mu g\,L^{-1}$, respectively.

Box-whiskers were used to compare concentration levels observed for each pesticide compound in the groundwater samples analysed. All distributions medians were <LD, except for molinate with a value <0.05 $\mu g\,L^{-1}$ (see figure 3).

The highest concentration levels observed for molinate and propanil are probably associated with various types of accidental releases, such as spills, back-siphonage accidents, and entry (or 'run-in') of contaminated surface waters into open or improperly sealed wells.

3.2 *Mixtures of pesticide compounds*

Mixtures of the analysed pesticide compounds were observed in 25% of the total groundwater samples, with up to five substances being detected in each one. The concentration sum of all pesticide compounds was above 0.5 $\mu g\,L^{-1}$ (parametric value for total pesticides in water for human consumption) in four water samples, with a maximum concentration level of 59.6 $\mu g\,L^{-1}$ being detected.

Figure 4 illustrates the seasonal patterns observed for total pesticides in water samples collected from several wells of the 'Baixo Sado' region, in 2002 and 2003.

The highest concentration levels of total pesticides were detected in the spring and summer of 2002 and 2003, being verified lower levels in the following months. In the irrigation wells, namely in the dug well, pesticide compounds were detected in high concentrations on one occasion, but not after subsequent sampling, which may be due to a transient contamination point source (see figure 4).

Seasonal variations in pesticide occurrence in groundwater are largely attributed to the application of pesticides during the spring, but these variations are also influenced by seasonal changes in temperature and precipitation, coupled with the timing of agricultural practices such as irrigation and, perhaps, tillage [10].

3.3 *Groundwater exposure to total pesticides vs. nitrates*

The simultaneous occurrence of total pesticides and nitrates was analysed for total groundwater samples. As illustrated in figure 5, and observed in several other studies carried out in groundwater from agricultural areas [10, 46], no positive correlation between the concentration levels of both parameters was verified. Total pesticides concentration levels >0.5 $\mu g\,L^{-1}$ were detected in four water samples with nitrate values $\leq$10 $mg\,L^{-1}$ NO_3^-, while 11 water samples with nitrate concentration levels >50 $mg\,L^{-1}$ NO_3^- (parametric value for nitrate in water for human consumption) (max. 183 $mg\,L^{-1}$) showed, simultaneosly, total pesticides concentration levels $\leq$0.5 $\mu g\,L^{-1}$ (see figure 5).

Figure 6 illustrates the seasonal patterns observed for total pesticides (TP) and nitrates (N) in water samples collected from several wells of the 'Baixo Sado' region in 2002 and 2003.

The lack of any correlation between total pesticides and nitrate concentration levels, as can be deduced from figure 5, can be explained by the substantial contrasts in environmental behaviour of nitrates and many pesticides, with respect to both their susceptibility to retardation and their persistence under different redox conditions.

In a study performed under rice-based cropping systems in Luzon (Philippines), from 1989 to 2000, it was concluded that leaching of nitrates and pesticides into shallow

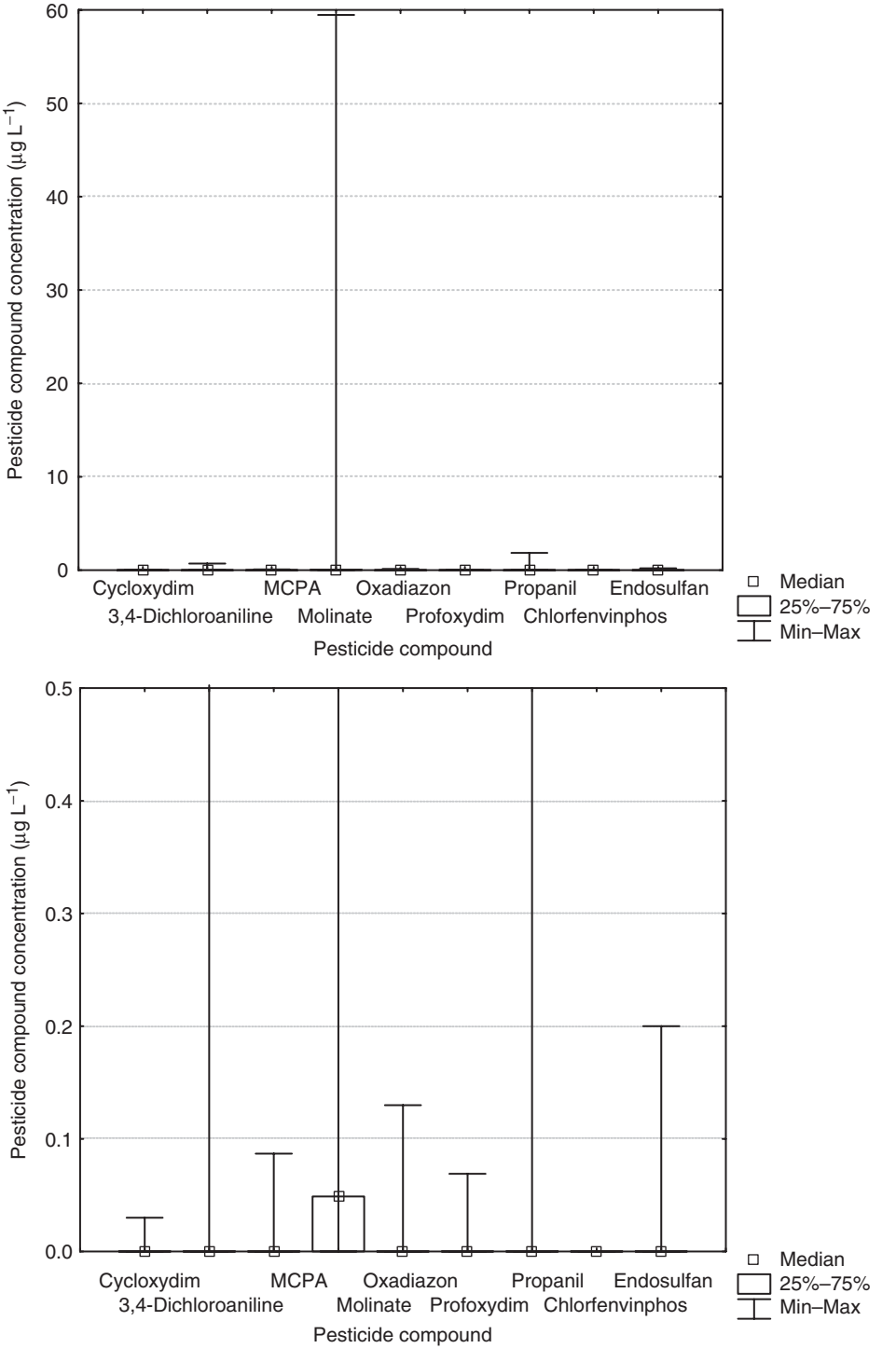

Figure 3. Box-whiskers of concentration levels observed for each pesticide compound in water samples collected from 22 wells of the 'Baixo Sado' region, in 2002 and 2003.

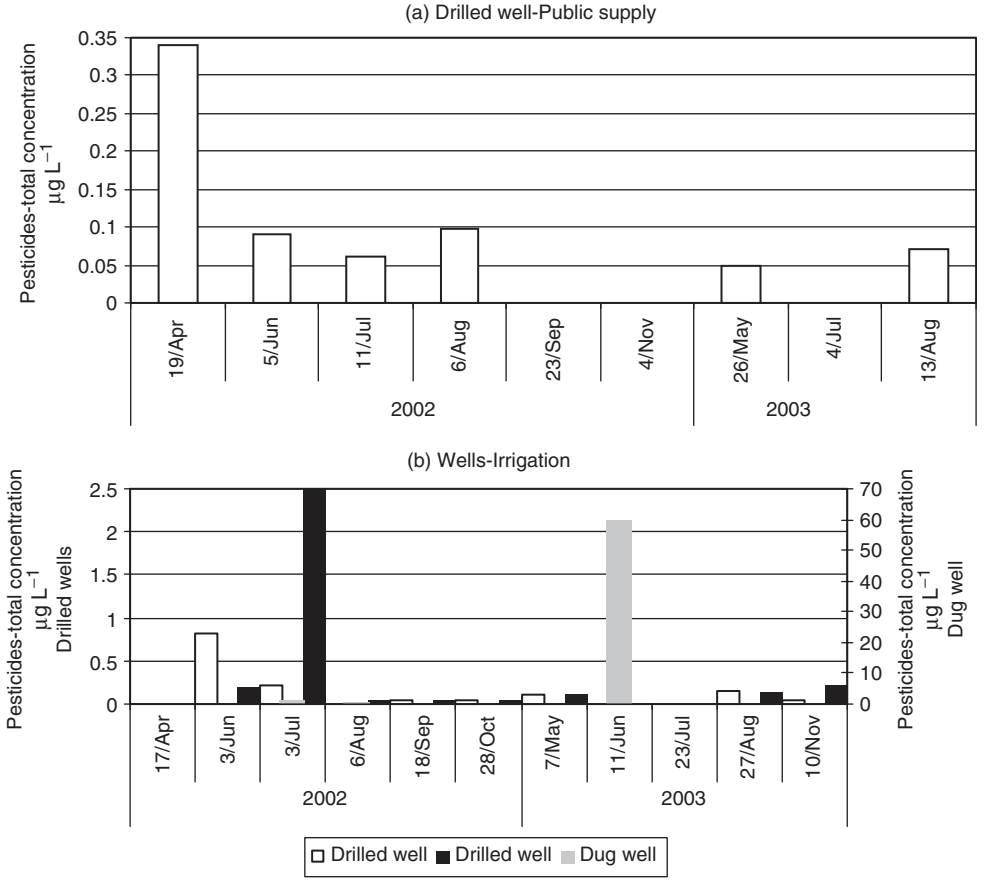

Figure 4. Seasonal patterns observed for total pesticides in water samples collected from (a) a drilled well for public supply and (b) two drilled wells and a dug well for irrigation use of the 'Baixo Sado' region, in 2002 and 2003.

groundwater under rice may be minimal because of the relatively large volatilization losses and fast chemical and microbial degradation under anaerobic conditions in the tropics [28].

Otherwise, in other agricultural areas of Portugal, particularly in the 'Ribatejo e Oeste' and 'Beira Litoral' regions, nitrate concentration levels $>50\,mg\,L^{-1}$ NO_3^- were observed in 32% of 303 wells analysed, as well as a positive relation between pesticide detection frequency and nitrate concentration level, although no positive relation between both concentration levels was observed [22].

4. Conclusions

The results obtained in this study show that pesticides applied in rice fields of the 'Baixo Sado' region are affecting groundwater beneath those important oriziculture

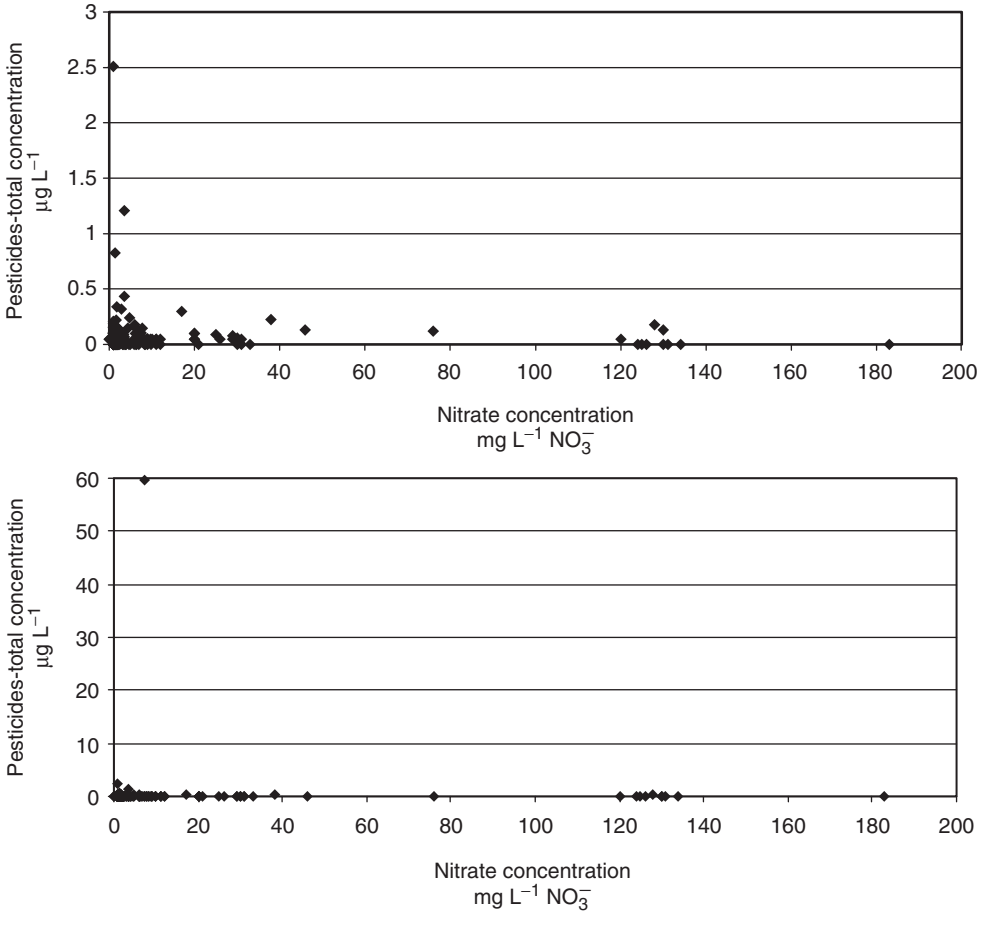

Figure 5. Concentration levels of total pesticides *vs.* nitrates observed in water samples collected from 22 wells of the 'Baixo Sado' region, in 2002 and 2003.

ecosystems of Portugal. One or more of the analysed pesticide compounds (chlorfenvinphos, cycloxydim, 3,4-dichloroaniline, endosulphan, MCPA, molinate, oxadiazon, profoxydim, propanil) were detected in 62% of 171 water samples collected from 22 wells used for public supply, domestic supply and irrigation use, during 2002 and 2003. Of the total number of samples, 6% presented maximum concentration levels of at least one of the compounds above $0.1\,\mu g\,L^{-1}$, the parametric value in water for human consumption.

Mixtures of pesticide compounds were observed in 25% of the total groundwater samples, with up to five substances being detected in each one. The concentration sum of all pesticide compounds was above $0.5\,\mu g\,L^{-1}$ (the parametric value for total pesticides in water for human consumption) in four water samples.

All the analysed compounds, with the exception of the insecticide chlorfenvinphos, were detected in groundwater samples. Molinate was the most frequently detected (55%), particularly with maximum concentration levels above $0.1\,\mu g\,L^{-1}$ (6%), probably due to its high use in the 'Baixo Sado' region, physical–chemical

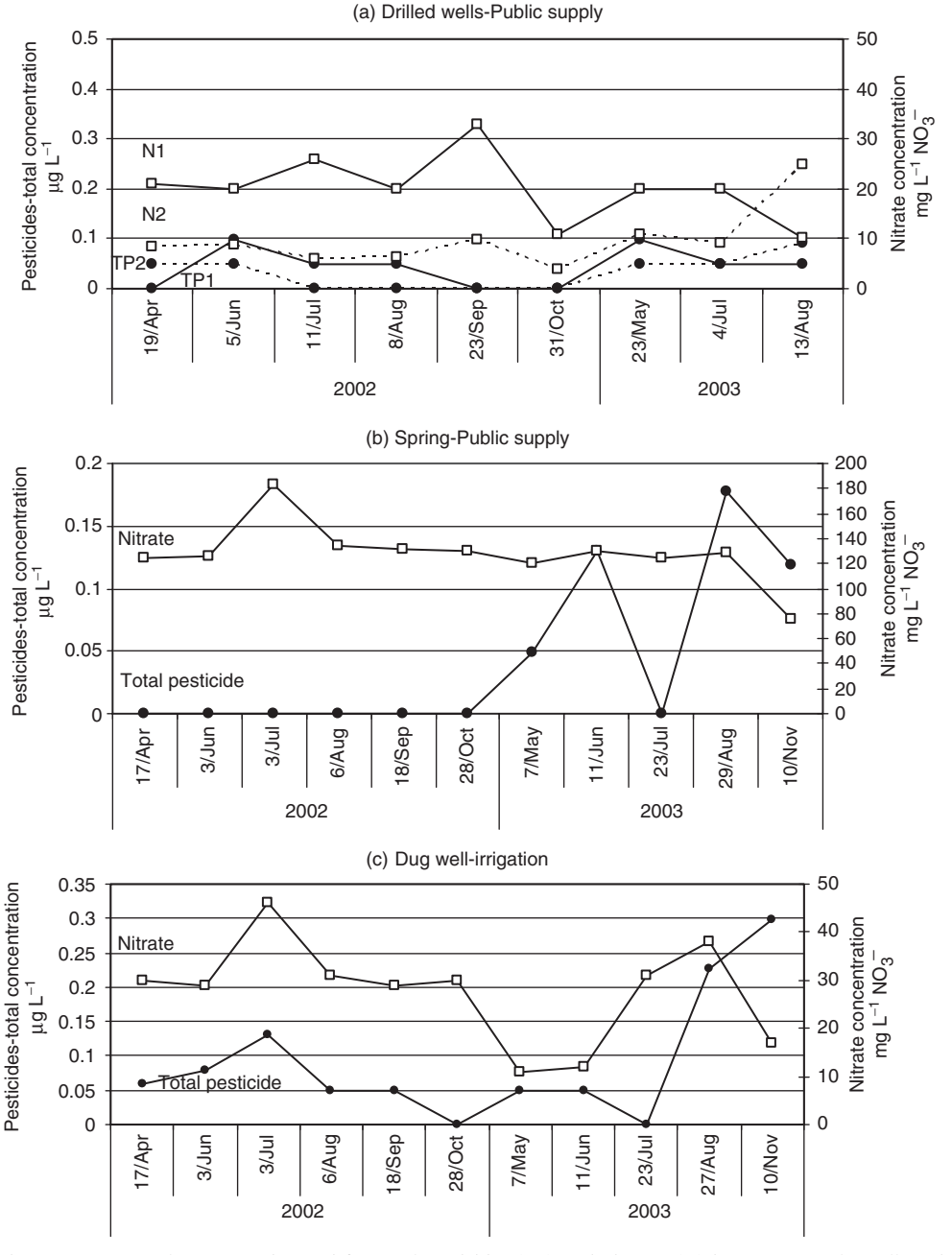

Figure 6. Seasonal patterns observed for total pesticides (TP) and nitrates (N) in water samples collected from (a) two drilled wells for public supply, (b) a spring for public supply and (c) a dug well for irrigation use of the 'Baixo Sado' region, in 2002 and 2003.

properties (high solubility in water), and particular method of application (under flooded conditions).

The detection frequencies observed for the analysed pesticide compounds were higher in water samples collected from irrigation wells (78%) that provide much less groundwater isolation from surface contaminant sources.

The simultaneous occurrence of total pesticides and nitrates was analysed for total groundwater samples, with no positive correlation being verified between them. The maximum concentration levels detected for both parameters were $59.6\,\mu g\,L^{-1}$ and $183\,mg\,L^{-1}$ NO_3^-, respectively. A seasonal variation pattern was observed for both parameters in water samples collected from some wells.

Finally, the impact of agrochemicals on groundwater resources should be minimized. Some pesticide management measures, including best management practices, need to be adopted in order to improve the environmental quality of rice ecosystems.

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