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Determination of trace levels of herbicides in estuarine waters by gas and liquid chromatographic techniques

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ABSTRACT

A screening procedure based on a two-step liquid–liquid extraction with dichloromethane was used for the isolation of two fractions, one neutral containing molinate, trifluralin, atrazine, simazine, alachlor, metolachlor, isoproturon, chlortoluron and linuron and the other acidic containing bentazone, 2,4-D and 4-chloro-*o*-tolyloxyacetic acid. Recoveries varying from 60 to 100% with a relative standard deviation of 10% were achieved for most of the herbicides added to 1–4 ml water samples at levels varying from 2.5 to 25 μg/l. Exceptions were the acidic herbicides 2,4-D and MCPA, for which low recoveries up to 40% were obtained. Determinations were usually carried out by gas chromatography with nitrogen–phosphorus detection, allowing the determination of herbicides at the 5–10 ng/l level with further confirmation by gas chromatography–mass spectrometry with electron impact ionization. Liquid chromatography with diode-array detection permitted the characterization of herbicides at levels of 0.1–0.5 μg/l. Although liquid chromatography–diode-array detection was much less sensitive than gas chromatography–nitrogen–phosphorus detection determinations, its usefulness was demonstrated for direct characterization of acidic herbicides without derivatization. Illustrative examples of the determination of several herbicides in estuarine water samples from the Ebro Delta (Tarragona, Spain) are shown. Atrazine, simazine, molinate, alachlor, metolachlor and bentazone were the most common herbicides found with concentrations levels varying from 5 ng/l to 5 μg/l.

INTRODUCTION

There is a need for multi-residue analytical approaches for the trace level identification and determination of pesticides in water matrices, such as surface, estuarine, ground and sea water. Various preconcentration methods based on different physico-chemical principles are used for this purpose. Among them, liquid–liquid extraction (LLE) and off-line and on-line liquid–solid extraction (LSE) are commonly used and were recently described in a review [1].

Chlorotriazine herbicides have been extracted into dichloromethane [2–5], ethyl acetate [2] and mixtures of dichloromethane with ethyl acetate and ammonium formate [6]. For alachlor, dichloromethane has been recommended by the US EPA as an extraction solvent for waters [7]. Screening methods for different pesticide groups have been developed, generally using dichloromethane with further washing with NaOH [8,9], adding NaCl [10] or adjusting to neutral and acidic pH for the separation of two fractions [11,12]. Determinations of the different herbicides are generally carried out by gas chromatography with nitrogen–phosphorus detection (GC–NPD) [1,13,14] and by GC with mass spectrometric (MS) detection [13–19], both giving much lower detection limits than liquid chromatographic (LC) techniques. However, the use of LC facilitates the direct determination of acidic and thermally labile herbicides without the need for derivatization. This has been reported in combination with LLE [5–12], off-line LSE [8–10,20] and on-line LSE [21,22].

The aim of this work was to establish a method for the identification and confirmation of herbicides at low concentration levels and to determine the levels of molinate, trifluralin, atrazine, simazine, alachlor, metolachlor, isoproturon, chlortoluron, bentazone, 2,4-D and 4-chloro-*o*-tolyloxyacetic acid (MCPA) in estuarine water samples from the Ebro Delta (Tarragona, Spain). The choice of these her-

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bicides was agreed at a joint meeting held at the International Atomic Energy Agency laboratories in Monaco in October 1990 between the Food and Agriculture Organization of United Nations Environment Programme and representatives from France, Spain, Italy and Greece. On the basis of usage information, physico-chemical properties and persistency data, this list of priority herbicides was drawn up in order to carry out a pilot monitoring programme in estuarine areas of the mediterranean region. The analytical methodology used for achieving this purpose was decided to be liquid–liquid extraction followed by determinations using GC–NPD, GC–MS and LC–diode–array detection (DAD).

Generally sufficiently volatile herbicides can be determined in water samples by GC–NPD and GC–MS at levels of 0.01 μg/l [15,17]. As a complementary technique, LC–DAD can be employed for the determination of the more polar herbicides at this concentration level only if there is sufficient quantitative enrichment, absorption in the UV range and the compound of interest exhibits high molar absorptivity [20].

The limits of detection (L.O.D.) were determined for each of the chromatographic techniques and recommendations for the analysis of water samples for the different herbicides are discussed. Applications to the characterization of trace levels of herbicide residues in polluted estuarine water samples are presented.

EXPERIMENTAL

Chemicals

Pesticide-grade ethyl acetate, *n*-hexane, diethyl ether and dichloromethane were obtained from Merck (Darmstadt, Germany). Molinate, alachlor, metolachlor, bentazone and MCPA were obtained from Promochem (Wesel, Germany), isoproturon and chlortoluron from Riedel-de Haën (Seelze-Hannover, Germany) and 2,4-D, trifluralin, atrazine and simazine from Polyscience (Niles, IL, USA).

Sample preparation

Estuarine water samples of 1–4 l were spiked with the different herbicides giving final concentrations of 2.5 μg/l. Preliminary experiments were performed by spiking with up to 25 μg/l of each herbicide. After agitation the solutions were extracted with 50–100 ml of dichloromethane and the extract was split into two portions and evaporated to dryness. One portion was dissolved in 100–200 μl of ethyl acetate for GC–NPD and GC–MS analysis and the other portion was dissolved in 100–200 μl of methanol for LC–DAD analysis. Sulphuric acid (pH < 2) and dichloromethane (50–100 ml) were added to the water sample, which was shaken again and the dichloromethane extract was carefully evaporated to dryness and the residue dissolved in 100–200 μl of methanol for LC–DAD analysis. Recoveries obtained for the different herbicides are given in Table I.

Chromatographic analysis

GC–NPD. Following Florisil clean-up, the extracts were injected on to the column of a GC 5300 Mega Series gas chromatograph (Carlo Erba, Milan, Italy) equipped with a nitrogen–phosphorus detector. A 15 m × 0.25 mm I.D. fused-silica capillary column coated with 0.15 μm film of chemically bonded cyanopropylphenyl DB 225 (J & W Scientific, Folsom, CA, USA) was used. Hydrogen was employed as the carrier gas at 60 kPa and helium as the make-up gas at 110 kPa. The temperatures of the injector and detector were held at 270°C. The column was programmed from 60 to 90°C at 10°C/min and from 90 to 220°C at 6°C/min.

GC–MS. A Hewlett-Packard (Palo Alto, CA, USA) Model 5995 instrument interfaced to a Model 59970C data system was used for GC–electron impact (EI) ionization MS. The same fused-silica column as described above was used and directly introduced into the ion source of the mass spectrometer. Helium was used as the carrier gas at 83 kPa. Other chromatographic conditions were identical with those described for GC–NPD. The ion source and the analyser were maintained at 200°C. EI mass spectra were obtained at 70 eV.

Table II gives the main ions together with their relative abundances and retention times obtained for the different herbicides in GC–MS.

LC–DAD. Eluent delivery was provided by two Model 64 high-pressure pumps (Knauer, Bad-Homburg, Germany) coupled with a Chrom-A-Scope rapid scanning UV–VIS detector (Barspec, Rehovot, Israel). Samples were injected via a 20-μl

loop (Rheodyne, Cotati, CA, USA). The neutral fraction of the herbicides was analysed using a Serva (Heidelberg, Germany) high-performance liquid chromatographic (HPLC) column packed with 4-μm octadecyl-Daltosil 100 (250 × 4.6 mm I.D.) Gradient elution was used from methanol–acetonitrile–water (20:20:60) to methanol–acetonitrile (50:50) in 40 min at a flow-rate of 1 ml/min.

For the analysis of the acidic fraction containing bentazone, 2,4-D and MCPA, a LiChroCART cartridge column (125 × 4.0 mm I.D.) packed with 4-μm LiChrospher 100 RP-18 (Merck, Darmstadt, Germany) was used. Isocratic elution with methanol–water (60:40) containing 1% of acetic acid at a flow-rate of 1 ml/min was used.

Determination by LC–DAD was performed using UV absorption at 220 nm.

RESULTS AND DISCUSSION

LC–DAD

Most of the neutral herbicides gave recoveries up to 70%, similarly to results reported previously [2–5,8,9,15]. The phenoxy acid herbicides 2,4-D and MCPA were exceptions, with recoveries lower than 34% (see Table I), which are much lower than those reported using a similar method [11]. Such a low extraction efficiency can be explained by the low level of spiking here (2.5 μg/l) compared with that in the literature [11], so evident difficulties in determining 2,4-D and MCPA occur. In contrast, bentazone, the other acidic herbicide which exhibits a much shorter retention time (3 min) with a better peak shape under the analytical conditions, gave a much better recovery.

The best separation of the neutral fraction containing the different herbicides was achieved with a commercially available 250 × 4.6 mm I.D. HPLC column with a 4-μm particle size. The separation is shown in Fig. 1A. The only problem was the co-elution of peaks 7 and 8 (alachlor and metolachlor), which could not be overcome. However, these two compounds have very similar structures that only differ only in a CH_2 group, and would be expected to behave similarly under the conditions of analysis. It should be mentioned that the best separation reported for a variety of herbicides [8] was achieved using a 250-mm laboratory-packed HPLC column with a 3-μm particle size. As this column is not commercially available, we used that mentioned above, which gave an excellent performance. In any case, alachlor and metolachlor could be perfectly separated using GC (see Table II).

TABLE I

MEAN RECOVERIES OF HERBICIDES IN ESTUARINE WATER SAMPLES USING LIQUID–LIQUID EXTRACTION WITH DICHLOROMETHANE

Spiking level: 2.5 μg/l ($n = 6$).

Herbicide	Mean recovery (%)	Relative standard deviation (%)
Atrazine	100	9
Simazine	93	9
Alachlor	90	10
Metolachlor	90	12
Molinate	70	17
Trifluralin	85	8
Chlortoluron	100	7
Isoproturon	100	8
Linuron	100	7
Bentazone[a]	81	7
2,4-D[a]	20	20
MCPA[a]	34	20

[a] These herbicides were extracted with the same solvent after acidification to pH < 2.

In Fig. 1B, the LC–DAD trace for a water extract containing low levels of herbicides (below 0.1 μg/l) is shown. This represents the L.O.D. of the LC–DAD method. Of the different compounds examined, only atrazine (peak 3) could be unequivocally identified by its UV spectrum (see Fig. 1B). The other herbicides, such as molinate, the UV spectrum of which also shown, and alachlor–metolachlor, could not be positively identified as the UV spectra gave an absorption band that overlapped the UV spectrum of the LC eluent. This can also be observed in the UV spectrum of atrazine (peak 3), but as atrazine has its absorption maximum at 220 nm, we can still see its maximum and the characteristic spectrum, but in the other instances, with maximum absorbance at 200 nm, there is a clear interference of the spectrum of the LC eluent when working at the L.O.D. of the method. It was impossible to identify positively simazine (peak 1) as it co-eluted with a highly interfering compound from the water matrix.

Fig. 1C and D show the chromatograms of a standard sample containing the three phenoxy acid

herbicides and a positive identification of bentazone at a concentration level of 5.5 μg/l. In this instance there was no problem in the identification of this herbicide in the real sample of Ebro Delta water as the concentration found was sufficient to achieve a good UV spectrum for confirmation.

GC–NPD and GC–MS

Fig. 2 shows (A) the GC–NPD and (B) the GC–MS traces for the same extract of Ebro Delta estuarine waters containing herbicides at levels varying from 0.005 to 0.050 μg/l. The different EI mass spectra of the positively identified herbicides are shown in Fig. 3 and the main fragments obtained for each herbicide are given in Table II.

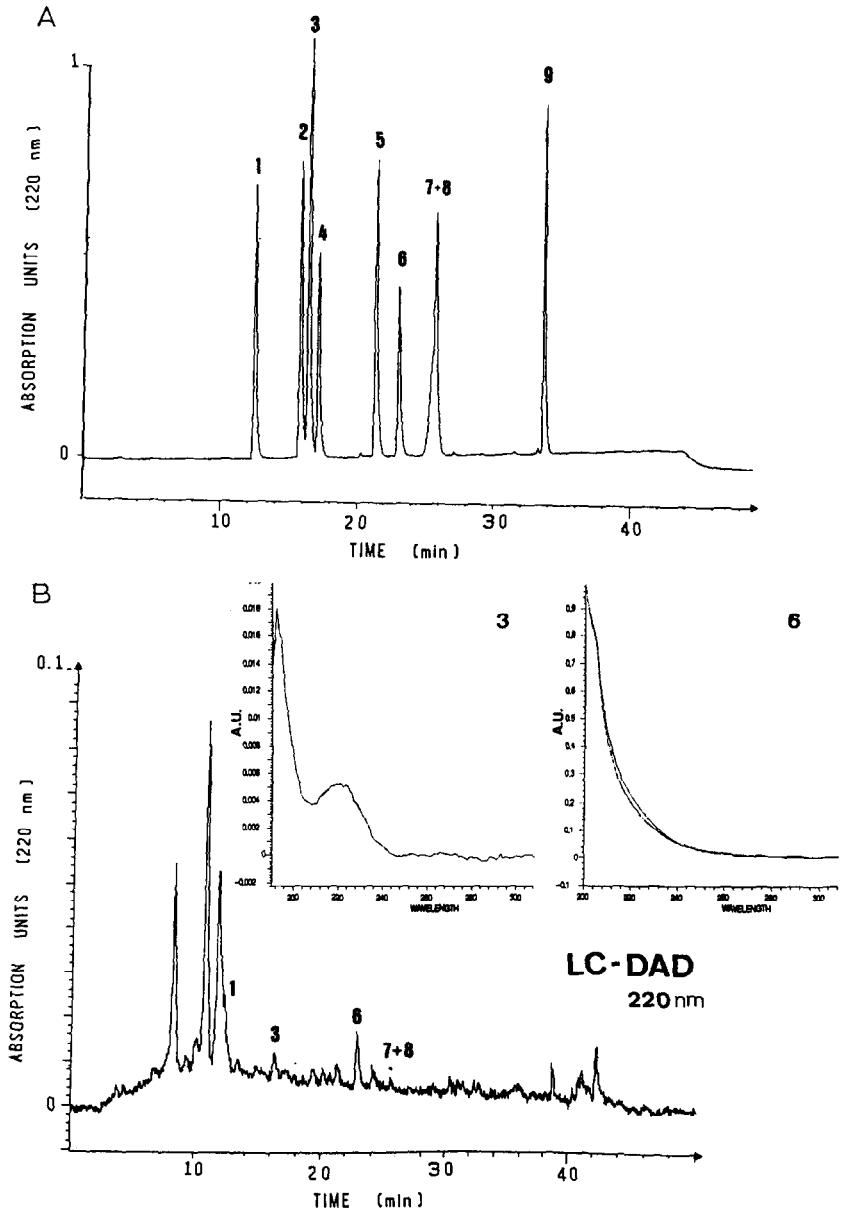

Fig. 1.

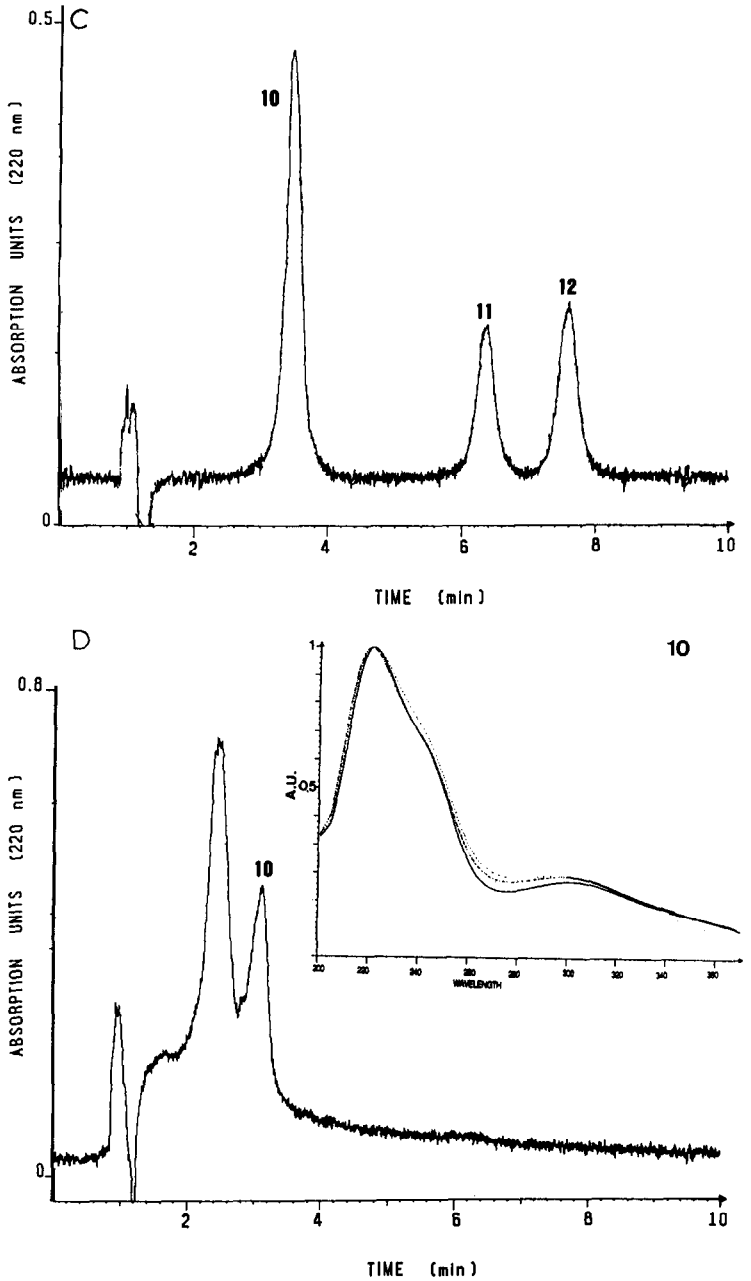

Fig. 1. (A) LC–DAD of a standard sample containing the neutral fraction. Peaks: 1 = simazine; 2 = chlortoluron; 3 = atrazine; 4 = isoproturon; 5 = linuron; 6 = molinate; 7 = alachlor; 8 = metolachlor; 9 = trifluralin. Amount of each herbicide injected: 2 μg. Serva HPLC column (250 × 4.6 mm I.D.) packed with 4-μm octadecyl-Daltosil 100. Gradient elution from methanol–acetonitrile–water (20:20:60) to methanol–acetonitrile (50:50) in 40 min at a flow-rate of 1 ml/min. (B) LC–DAD of an extract of a real water sample from the Ebro Delta containing simazine (0.040 μg/l), atrazine (0.010 μg/l) molinate (0.080 μg/l) and alachlor (0.025 μg/l). Peak purity of atrazine indicates its positive identification. The other herbicides were not positively identified owing to the low concentrations and absorption maxima at lower wavelength (200 nm), so they were determined by GC–NPD and confirmed by GC–MS. LC conditions as in (A). (C) LC–DAD of a standard sample containing the herbicides (10) bentazone, (11) 2,4-D and (12) MCPA. Amount of each herbicide injected: 2 μg. LiChroCART cartridge column (125 × 4.6 mm I.D.) packed with 4-μm LiChrospher 100 RP-18. Isocratic elution with methanol–water (60:40) containing 1% acetic acid at a flow-rate of 1 ml/min. (D) LC–DAD corresponding to an extract of a real water sample from the Erbo Delta containing 5.5 μg/l of bentazone. Peak purity of bentazone indicates its positive identification. LC conditions as in (C).

TABLE II

RETENTION TIMES AND MAIN IONS OF HERBICIDES ANALYSED BY GC–MS

Herbicide	Retention time (min)	*m/z* (relative intensity, %)
Molinate	10.5	126 (100), 187 (35)
Trifluralin	13.7	264 (80), 306 (100)
Atrazine	17.7	200 (100), 215 (60)
Simazine	18.2	186 (60), 201 (100)
Alachlor	18.5	160 (100), 188 (100)
Metolachlor	19.3	162 (100), 238 (60)

The GC–MS characterization of the chlorotriazine herbicides atrazine and simazine has been discussed previously [14]. The base peaks at *m/z* 200 and 201 corresponded to $[M - CH_3]^+$ and $[M]^{+\cdot}$ for atrazine and simazine, respectively. The second most abundant ions were obtained with *m/z* 215 and 58 and 173 and 186, corresponding to $[M]^{+\cdot}$ and $[C_3H_7NH]^+$ and $[M - C_2H_4]^+$ and $[M - CH_3]^+$ for atrazine and simazine, respectively. Molinate exhibited two main ions corresponding to the molecular mass and to the loss of $[SCH_2CH_3]^+$ at *m/z* 187 and 126, respectively. Diagnostic ions for trifluralin were at *m/z* 306 and 264, corresponding to $[M - CH_2CH_3]^+$ and $[M - (CH_2)_4CH_3]^+$, respectively. For alachlor, the two diagnostic ions at *m/z* 188 and 160 corresponded to $[M - CH_2OCH_3 - HCl]^+$ and $[M - CH_3OH - OCCH_2Cl]^+$, respectively. Other ions at *m/z* 269 and 237 corresponding to the molecular mass and $[M - CH_3OH]^+$, respectively, can also be observed in the EI mass spectrum in Fig. 3 (peak 7). Metolachlor exhibited a similar fragmentation pattern to alachlor, as these herbicides have similar structures. The main ions were at *m/z* 238 and 162, assigned to $[M - CH_2OCH_3]^+$ and $[M - CH_2OCH_3 - OCCHCl]^+$, respectively.

The various ions found for these herbicides agree with previous results obtained using EI [15,17,23]. The different ions indicated in Table II can be used as diagnostic ions for screening purposes, as they correspond to higher *m/z* values. Alachlor and metolachlor exhibit an intense peak in the EI mass spectrum corresponding to $[CH_2OCH_3]^+$ at *m/z* 45 [23], but it is not recommended as a diagnostic ion as main ions at higher *m/z* values are obtained.

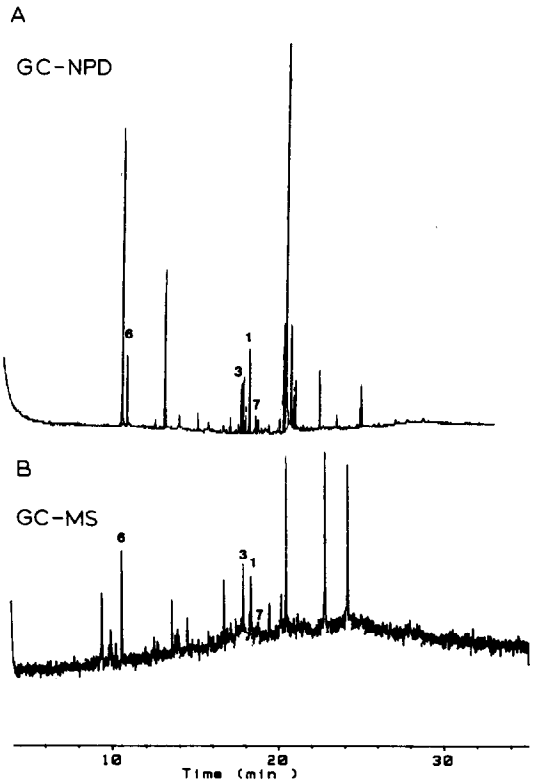

Fig. 2. (A) GC-NPD and (B) GC–MS of an extract of a real water sample from the Ebro Delta containing (6) molinate (0.050 μg/l), (3) atrazine (0.010 μg/l), (1) simazine (0.012 μg/l) and (7) alachlor (0.005 μg/l). 15 m × 0.25 mm I.D. fused-silica capillary GC column coated with chemically bonded cyanopropylphenyl DB 225, programmed from 60 to 90°C at 10°C/min and from 90 to 220°C at 6°C/min.

Performance of the analytical system

The repeatabilities of the GC–NPD and LC–DAD systems were determined after analysis of the dichloromethane extracts of the samples containing atrazine, simazine, alachlor and metolachlor at the level of 0.1 μg/l. The relative standard deviation was 2–3% ($n = 6$). The reproducibility of the same extracts was higher and varied between 3 and 5% ($n = 6$). Calibration graphs for atrazine, simazine, alachlor, metolachlor and molinate were constructed and were linear over the concentration ranges investigated, from 1 ng/l to 1 μg/l for GC–NPD. In LC–DAD, the concentration ranged from 50 ng/l to

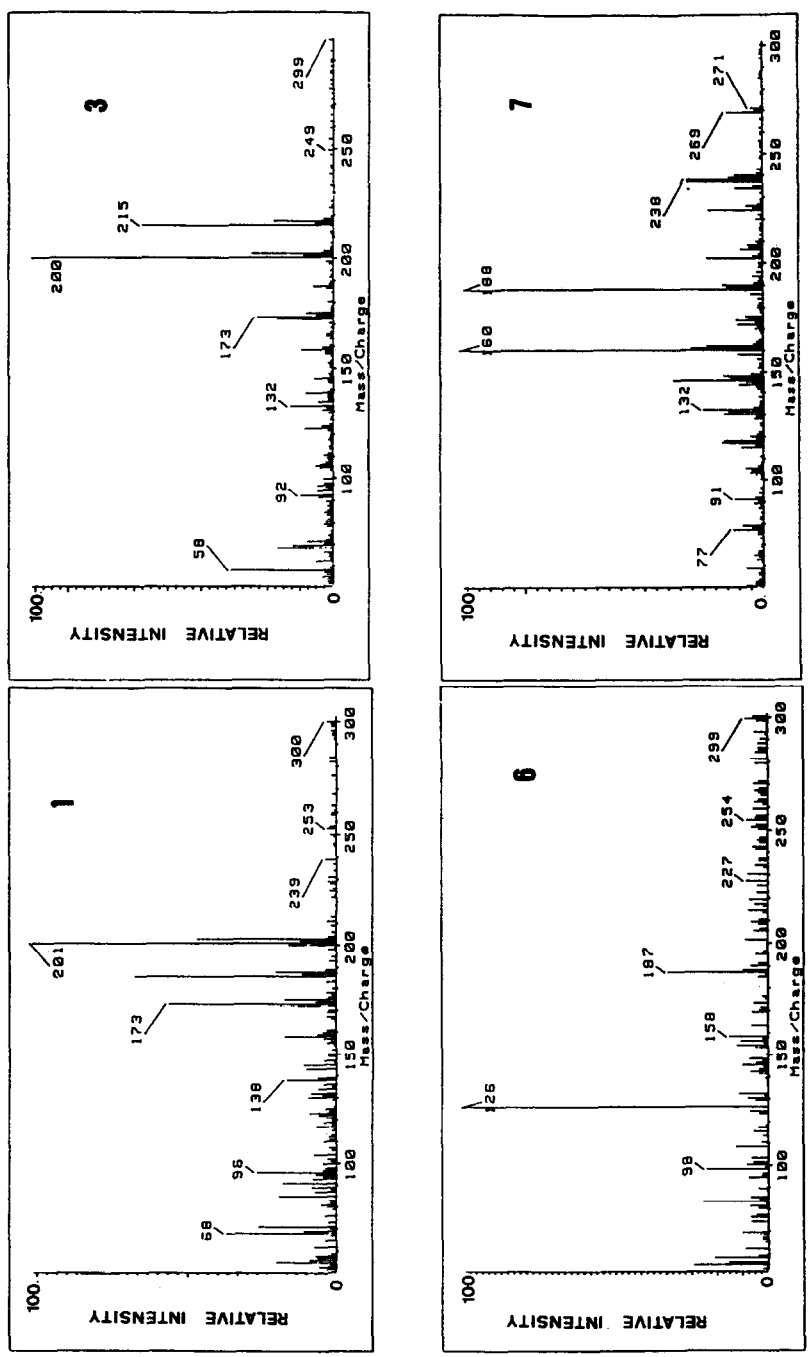

Fig. 3. EI mass spectra of the herbicides positively identified in Fig. 2. Numbers on the spectra correspond to the peaks in Fig. 2.

10 μg/l for atrazine, simazine and bentazone and from 500 ng/l to 10 μg/l for molinate, alachlor and metolachlor.

The limits of detection at a signal-to-noise ratio of 3 for the different herbicides were calculated for both GC–NPD and LC–DAD using the analytical protocol described under Experimental. For GC–NPD, the L.O.D. was calculated to be at the 0.1 ng/l level, thus allowing the application of the method to the analysis of relatively "clean" water samples. Indeed, GC–NPD will be the method of choice recommended for the determination of herbicides at trace levels, if they are sufficiently volatile. For confirmation purposes, the use of GC–MS under full-scan conditions is recommended. Quantification by GC–MS using selected ion monitoring also permits determinations at the 0.1 ng/l level [15,17].

The L.O.D. for LC–DAD was, as usual, worse than for GC–NPD. The L.O.D. was calculated to be 10 ng/l for herbicides exhibiting absorption maxima above 210 nm, such as atrazine, simazine, chlortoluron, isoproturon and bentazone, whereas for molinate, alachlor, metolachlor and trifluralin, which exhibit UV maxima below 210 nm, the L.O.D. was ten times higher, 100 ng/l. For the two chlorinated phenoxy acids 2,4-D and MCPA, although they exhibit absorption maxima at 220 nm, the L.O.D. is *ca.* 500 ng/l owing to their poor extraction efficiency and LC properties.

Environmental levels

A pilot monitoring programme was carried out in the Ebro Delta area to investigate the levels of the different herbicides. The Ebro Delta area is used mainly for rice growing but also contains other cultivations such as lettuce and corn. Of the different herbicides used in this area, molinate and bentazone, which are typical in rice cultivation, accounted 56 and 9 tons of active ingredient, respectively, during 1991, whereas the other herbicides such as alachlor, metolachlor, atrazine and simazine were used to lesser extents, *ca.* 1 ton of active compound, during the same period. Fig. 4 shows the levels of the most common herbicides found in the area at one of the stations located on the Ebro River, located betweeen the rice-growing fields and the corn fields. The concentrations of herbicides here are much lower than those in the drainage canals, which contained *ca.* ten times higher concentrations. The most common herbicides detected at almost all the stations at all periods were atrazine, simazine, alachlor and metolachlor. Most of the levels of the different herbicides were in the range 5–550 ng/l, with some exceptions, such as molinate and bentazone, which reached levels up to 3 and 5 μg/l, respectively, in one of the drainage canals.

CONCLUSIONS

A combination of LC–DAD and GC–NPD with

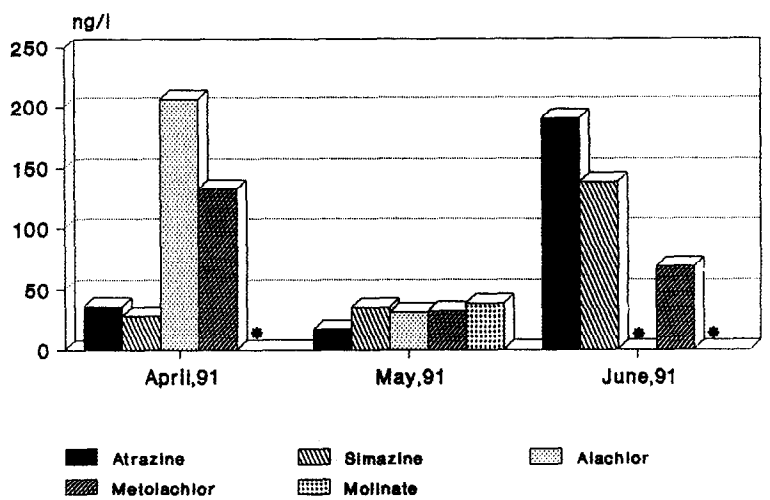

Fig. 4. Environmental levels of herbicides at a station located on the Ebro River (Tarragona, Spain) during April–June 1991. Concentration levels are indicated in ng/l (ng/l). Asterisks denote herbicides not detected.

UV and GC–MS confirmation, respectively, has been applied to the determination of trace levels of herbicides in relatively clean water samples. The reported screening method is applicable for monitoring most of the herbicides considered, with a few exceptions, in water samples under the restrictive measures (0.1 μg/l) imposed by the European Community of herbicide residues in water for human consumption.

The possibilities of peak identification in LC–DAD when working at very low detection limits were discussed. Such positive identification depends on the concentration of the analyte and on its UV spectrum. When absorption maxima in the spectra are at higher wavelengths (above 210 nm), the L.O.D. is lower (10 ng/l) than those for herbicides with absorption maxima in the region of 190–210 nm (100 ng/l).

The use of the two chromatographic methods offers a great advantage in the screeninig of trace levels of a variety of herbicides in water samples. GC–NPD allowed determinations at very low limits of detection and permitted the separation of alachlor and metolachlor, which was impossible by LC–DAD, whereas LC–DAD permitted the direct determination of bentazone without any derivatization.

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