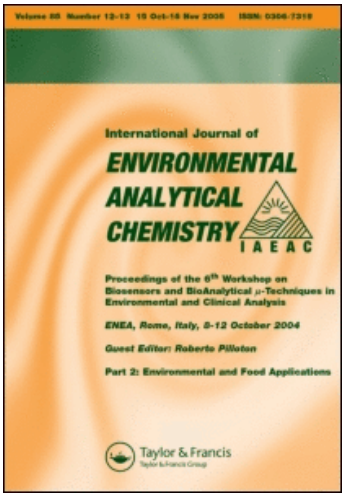

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Guzzella, L. , De Paolis, A. , Bartone, C. , Pozzoni, F. and Giuliano, G.(1996) 'Migration of Pesticide Residues from Agricultural Soil to Groundwater', International Journal of Environmental Analytical Chemistry, 65: 1, 261 — 275
To link to this Article: DOI: 10.1080/03067319608045560
URL: http://dx.doi.org/10.1080/03067319608045560

Intern. J. Environ. Anal. Chem., Vol. 65, pp. 261–275
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MIGRATION OF PESTICIDE RESIDUES FROM AGRICULTURAL SOIL TO GROUNDWATER

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(Received, 9 September 1995; in final form, 15 March 1996)

The pesticide content of agricultural soil and groundwater was investigated by monitoring three sampling sites located in the Northern Italy and cultivated with maize crop. The GC analyses of soil samples allowed the description of vertical distribution of pesticide residues in the soil profile. High concentrations of pesticide were determined in the superficial horizons while in the deeper layers the contamination reached values less than 0,2 μg/kg. The gas chromatographic results were positively correlated to the ELISA test ones, proving the possibility of utilizing immunoassay tests as a screening procedure.

KEY WORDS: Aquifer, herbicide, soil, gas chromatographic analysis, ELISA test.

INTRODUCTION

Most of the sampling and extraction methodologies that were developed for the analysis of pesticide residues are concerned with the liquid-phase material of the aquifers, i.e. water samples collected from the saturated zone. In past years the solid part of the aquifer material was scarcely considered from the analytical point of view in environmental monitoring studies of pesticide residues. However, the study of distribution of pesticide residues in the soil profile has a considerable importance in the investigation of environmental persistence of this contamination.

Excluding the most surficial layer, that is the so called agricultural soil (30–40 cm), usually heavily contaminated by the applied pesticides[1], the concentration of pesticide residues in the deeper solid-phase samples hardly reached high concentrations. Helling *et al.*[2] demonstrated that below a depth of 30 cm the concentrations of the applied compounds were less than 100 μg/kg in treated soil samples collected in a corn field. However, the continuous accumulation of pesticide residue in the unsaturated zone can constitute in the long term a serious problem for safeguarding the quality of the underground water resources[3]. Leaching phenomenon from surface to lower soil horizons can in fact allow migration of the chemicals to the saturated zone.

The present study was supported by the European Union (CT EV5V 93-0322) in order to develop practical techniques for the determination of low concentrations of pesticides and their transformation products (TPs) in the unsaturated aquifer zone. Ad hoc developed sampling and extraction techniques[4] were applied in the present investigation in order to evaluate the possible migration of pesticide residues from agricultural soil to aquifer.

The results obtained with traditional analytical methodologies were also compared with those obtained with more practical methods such as enzyme-linked immunosorbent assay (ELISA) techniques. The immunoassays were included in the project because they could be suitable for a practical pesticide determination. Recent studies demonstrated that ELISA tests look promising as a more rapid and cheaper alternative to GC or HPLC analysis[5]. The immunoassay tests have been extensively used in the past years for the quantification of pesticide residues in water samples but only recently were applied to soil samples[6,7].

In the present investigation the selection of pesticides to analyze was done on the basis of their use in the Northern part of Italy, the knowledge of contaminated aquifers, the availability of analytical techniques and immunoassay kits like the ELISA test. The most utilized pesticides in Italy in the last ten years were: atrazine and alachlor, while terbuthylazine and metolachlor are at the present the most sold compounds. Those four pesticides were selected in the present investigation together with some main transformation products (TPs). A small number of the main TPs was selected on the basis of the commercial availability of pure standards: atrazine-desethyl (DEA) and atrazine-deisopropyl (DIA) for atrazine, terbuthylazine-desethyl (DET) for terbuthylazine, 2,6-diethyl aniline (DAN) for alachlor and 2-ethyl-6-methylaniline (EMA) for metolachlor.

The selection of the sampling sites consisted in the satisfaction of several requirements or decision criteria. First, the history of the site had to be known as regards to the application of chemical products (type and rates). The application of concerned pesticides had to be continued for several years, so that the contamination of the deeper levels of the underground profile was reasonably occurred. Secondly, the underground profile in terms of permeability features had to be favourable to migration toward the groundwater body. Thirdly, the occurrence of a shallow water table was necessary in order to check the actual loading of the groundwater as a final result of contamination processes. Fourthly, the sites had to be representative of geo-pedological and agricultural conditions in the area. The intersection of the selected priority pesticides and the environmental conditions, taking into account operational constraints, resulted in the identification of the three sampling sites in Northern Italy.

EXPERIMENTAL

Sampling site location and sample collection

The sampling sites are located in the middle part of Lombardy plain (Figure 1). Two, i.e. Cervignano d'Adda (C) and Boffalora d'Adda (B) sites, are located 25 km S.E. of Milan, near the Adda river; the third, Manerbio (M) site, is located about 15 km South of Brescia, near the Mella river, a tributary of the Oglio river. They have practically the same cote and pertain to the fundamental level of Po plain. A substratum of sandy or gravel-sandy type occurs in the three sites. A water table is found at a depth variable from 5 to 10 meters in the different sites.

For each site farms and fields to perform sampling activities were chosen according to operational and agricultural practices. In all the selected fields the main crop was maize, sometimes alternated with barley or other cereals. Pesticide application (Primagran TZ and Lasso byproducts) was performed once in April-May. Flooding was applied in June in normal weather conditions.

The underground profiles were investigated from surface level to a depth variable

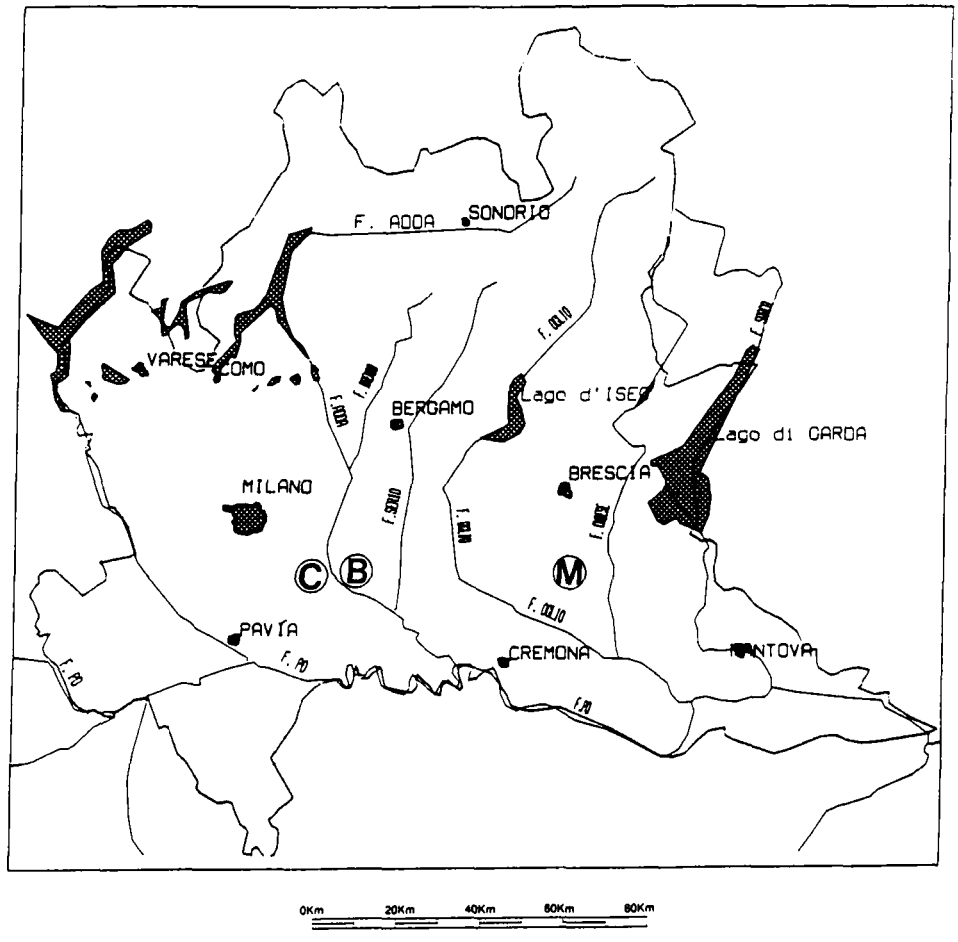

Figure 1 Location of sampling sites in Lombardy—Italy.

from 115 to 150 cm in the different sites. The sampling procedure started with the opening of a pit of one meter squared (Figure 2) by a reverse-arm machinery. For the geo-pedological analyses the samples were collected in the middle of the different soil pedological horizons while for the pesticide determinations the samples were obtained by the insertion of a battery of special metal boxes (4 cm of height) in the open profile wall. The upper 30 cm sample was sampled by a metal shovel in the middle part of the thickness. Sampling with shovel was also preferred to that with boxes in the presence of stone enriched profile.

One pit was excavated for each site at two times, in spring (April–May) and in autumn (November). Only in the B site two pits were excavated in spring for a preliminary check of the variability of the underground profile features before the pesticide application. In the November sampling campaign some average samples were collected: one mixing samples collected from the four walls of the pit and another mixing five different samples collected in the four corners and one in the middle of the field.

Groundwater (2l in glass, dark bottles) was sampled, in correspondence with the autumn campaign, collecting the water from the wells located in the selected farms.

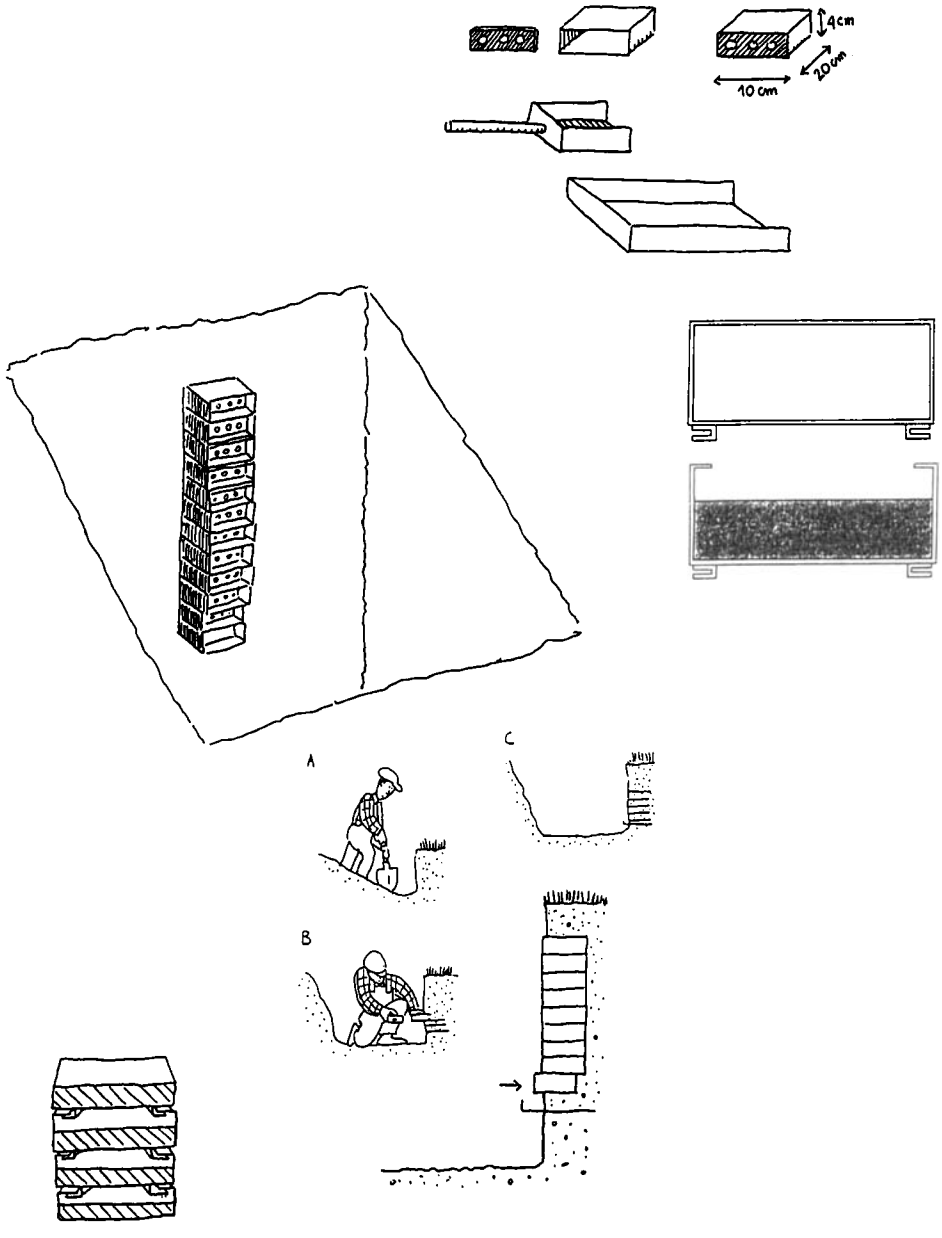

Figure 2 Sampling technique and necessary equipments.

Treatment of samples and pesticide extraction

After collection, all soil samples were frozen in sealed dark glass bottle and stored at –20°C. The geo-pedological characteristics of the different soil samples (pH in water,

percent of organic carbon, texture classification, exchangeable cationic capacity, exchangeable acidity, total carbonate content) were determined in the different horizons according to Italian Standard Methodology[8].

For the determination of pesticide content a large sample of soil (400–500 gr) was defrosted and dehydrated at air condition and laboratory temperature (20–22°C) for 96 hours. The determination of the water content of the air dried soil samples was done in laboratory treating 10 g of soil in a stove at 105°C for 24 h. The dry sample was sieved with 2 mm sieve. The skeleton (the amount of soil sample superior to 2 mm) was weighted and discarded.

The extraction procedure was the following. An amount of 50 gr of air-dried and sieved soil sample was treated with pesticide-free grade methanol (150 ml) in a Soxhlet extraction apparatus for 8 hours. The extract was then evaporated in Rotavapor in a water bath under light negative pressure condition and at 40–45°C. 1–2 ml of Milli Q water are added to the methanol-water obtained mixture in order to reach 3 ml of volume. The water mixture was passed through a 3 ml Extrelut filled column (Merk, Germany). The function of the diatomaee flour powder contained in the Extrelut column is that of removing the water content of the extract while the pesticide content is subsequently eluated with 15 ml of ethylacetate. The ethylacetate extract is then reduced to a volume of 0.25 ml and utilized for gas chromatographic analysis. A known amount of the ethylacetate extract (100 µl) was than diluted in 2 ml of MilliQ water, the ethylacetate was evaporated under a gentle stream of N_2 gas and utilized for ELISA test.

The analyses of water samples (1 l) were conducted by concentration on a column filled with a special graphitized carbon, Carbograph (Alltech), as described in Guzzella *et al.*[9].

GC analyses of soil samples

GC analyses of the herbicides and of the transformation products were performed on a Carlo Erba 5160 Mega Series gas chromatograph equipped with a nitrogen-phosphrous selective detector (NPD40) and with a fused capillary column (Sil 13—Chrompack, 50 m × 0.25 mm.

RESULTS

Geo-pedological analyses

Description of typical pedological profiles as well as textural and physico-chemical data for each horizons are reported in Table 1. The profiles were characterized on the basis of information provided by mean samples (5–6 for each profile) of each pedological horizons. All sampled profiles exhibit a well defined distribution of pedological fundamental horizons A/B/C.

In the B site, soils are scarcely evoluted, loamy-sandy textured with a sand content over 55%. From a taxonomic standpoint (USDA) they may be classified as typical Eritrochrepts, sandy skeleton, mixed mesic. The textural analysis show a decrease in loam with the depth. Below a deeper agricultural horizon, loose sand and gravel dominates. Carbonates occur mainly in the B deeper horizon. The organic carbon content is very high in the Ap horizon but rapidly decreases with depth.

In the C site, the pedological situation is complex showing soils rather evoluted. The

Table 1 Pedological characteristics of the soil samples

Site B (Date: 19 April 1994) Horizon	*AP*	*Bw1*	*Bw2*	*2CB*	*2Cl*
Depth of sample (cm)	0–36	36–50	60–61	61–66	66–100
pH in H_2O	6.7	7.7	7.8	7.7	8
Org. carb. (%)	2.77	0.52	0.37	0.39	0.06
Texture class	sandy loam	sandy loam	sandy loam	sandy loam	sand
CEC (meq/100 g)	17.6	9.4	5.9	4.7	0.2
Exchangeable acidity (meq/100 g)	0.4	0	0	0	0
Tot. carbonate content (%)	0	9.1	11.1	6.6	3.8
Site C (Date: 19 April 1994) Horizon	*Ap*	*Bt1*	*Bt2*	*Bt(g)*	*BC(t)*
Depth of sample (cm)	0–35	35–55	55–78	78–98	98–115
pH in H_2O	6.1	6.7	6.9	6.5	6.6
Org. carb. (%)	1.59	0.47	0.27	0.74	0.18
Texture class	loam	loam	sandy loam	loam	sandy clay loam
CEC (meq/100 g)	13.8	9	7.4	11.5	10.5
Exchangeable acidity (meq/100 g)	0.35	0.35	0.3	0.25	0.3
Tot. carbonate content (%)	0	0	0	0	0

Site M (Date: 1 May 1994) Horizon	*Ap1*	*Ap2*	*Bw*	*BC*	*C1*	*C2*
Depth of sample (cm)	0–25	25–32	32–50	50–84	84–102	102–135
pH in H_2O	7.2	7.7	7.6	7.6	7.5	8.1
Org. carb. (%)	1.77	1.03	0.31	0.14	0.15	0.04
Texture class	sandy loam	loam	clay loam	sandy clay loam	sandy loam	sand
CEC (meq/100 g)	13.9	12.3	11.3	10.5	6.1	1.5
Exchangeable acidity (meq/100 g)	0	0	0	0	0	0
Tot. carbonate content (%)	2	0.2	0	0	0	17.7

profile is characterized by some deep (80–100 cm) reddish argillitic-eluviation horizons. Above these some transitions horizon occur. At a greater depth fine loam and clay prevail along with sand fraction as a subordinate. Carbonates are absent throughout the profile.

In the M site, the superficial horizon is reworked with a high content of clay. Below one meter the soil is mainly sandy with low clay content. The organic carbon content is high only in the Ap upper horizon. The soil is well evoluted and typical of middle sandy plain.

Recovery experiment

The literature review of different published papers[10,11] conditioned the choice of the best extraction techniques. Particularly, the methanol solvent and the Soxhlet apparatus were chosen as the best extraction techniques for the analysis of the pesticide in soil samples. The same extraction was besides supported by the standard German method for the analysis of triazine herbicides and desalkyl metabolites in soil samples published in 1979[12].

In the present study the recovery experiment was conducted by mixing one ml of the standard pesticide solution with 25 ml of pure methanol and 50 grams of air dried soil

sample in order to obtain the following standard concentrations: atrazine, terbuthylazine, DEA and DET = 10 μg/kg; alachlor, metolachlor, DAN and EMA = 20 μg/kg. The liquid suspension was stirred for two hours and let it dried for a night at room temperature. The soil samples were then treated as described previously with the Soxhlet apparatus. The results of the recovery experiments are reported in Table 2 as the mean of three different replicate extractions .

An experiment for evaluating the repeatibility of the adopted procedure was also undertaken. A superficial Manerbio soil sample (5–22 cm, June 1995) was extracted three times and the variation coefficient values are reported in Table 2.

Gas chromatographic results

The Boffalora results before and after the pesticide treatment are reported in Figure 3. The higher content of pesticide was determined in the most superficial layer (5–30 cm). It is evident that in this layer the concentration of terbuthylazine, alachlor and metolachlor are higher one month after the treatment of the agricultural soil than after six months due to actual decay and degradation. The terbuthylazine contamination is more superficial and below 64 cm of depth it cannot be quantified anymore. The DET is present only in the June sample, just after the pesticide application. Metolachlor seems to move quite quickly from the superficial to the deeper layers while the quantification of EMA, the main transformation product of metolachlor, was impossible for the presence of a matrix interference. Alachlor also seems to follow the same process of leaching from the upper layers to the deeper ones. The main metabolite of alachlor, DAN, is present in high concentration especially in November samples. The contamination for atrazine in the soil profile seems to be quantitatively limited but constant (about 0.4 μg/kg) while DEA is present only in the upper layers. The atrazine and DEA content of the soil samples is probably a memory effect of the past use of this pesticide. None contamination of DIA was determined in all the samples.

Very similar results were obtained for the Cervignano and the Manerbio soil profiles with a maximum concentration in the top soil and a decreasing trend in the below horizons. However in the Cervignano samples (Figure 4) both DET and EMA were

Table 2 Results of recovery and reproducibility experiments

	Soil samples of Manerbio (Bs)		
	Recovery (%)	*Reproducibility*	
Depth of sample (cm)	74–78	5–22	
Solvent	Methanol	Methanol	
Sampling date	01/05/94	16/06/95	
		(1)	(2)
ATRA	100	0.77 ± 0.11	11
DEA	99		
TER	87	7.00 ± 0.46	10
DET	87	2.50 ± 0.43	22
ALA	83		
DAN	71		
METO	98	6.73 ± 1.18	24
EMA	80	1.39 ± 0.28	25

(1) Mean ± standard error (μg/kg)
(2) Relative standard deviation

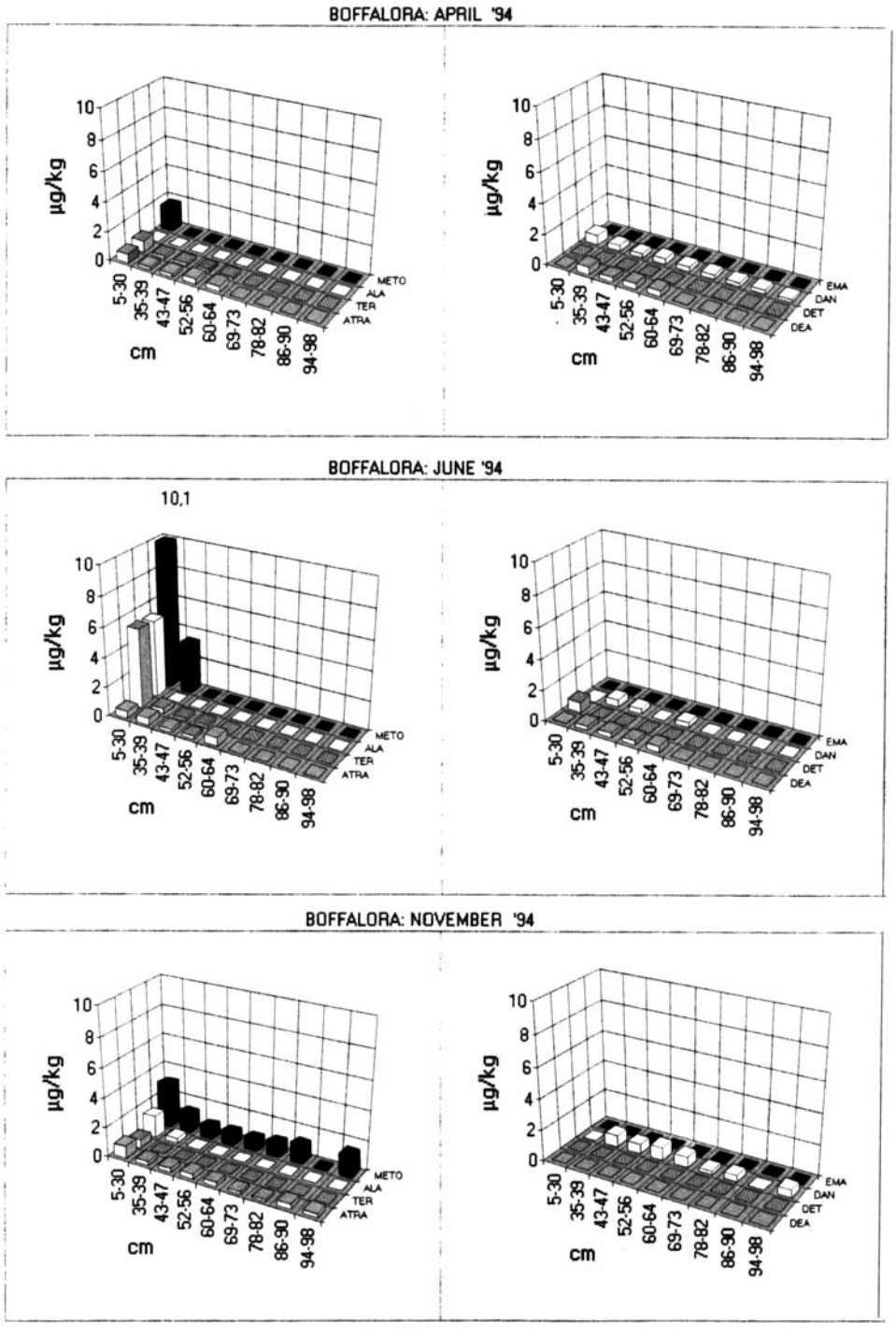

Figure 3 Concentration of herbicides and their TPs in Boffalora soil samples (April, June and November 1994).

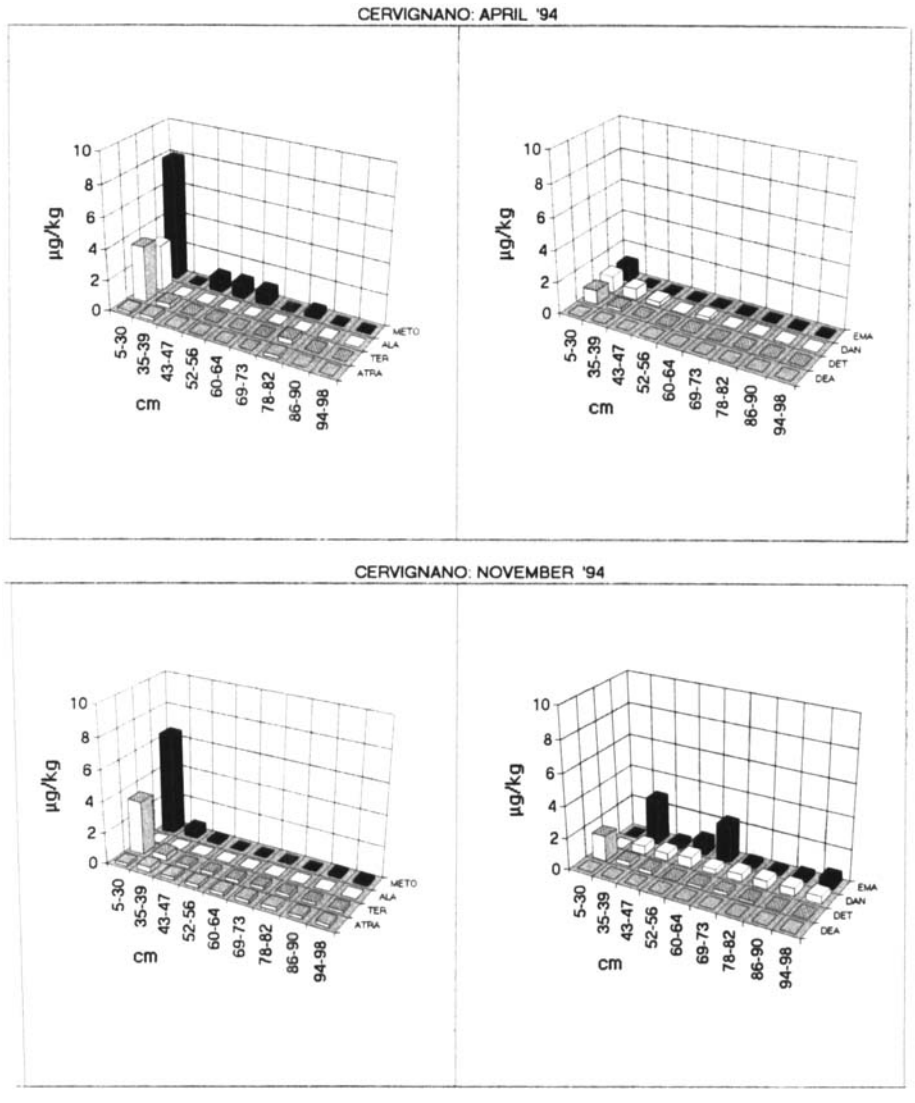

Figure 4 Concentration of herbicides and their TPs in Cervignano soil samples (April and November 1994).

determined even in 90–98 cm soil samples. Alachlor pratically disappeared in the November samples and only its metabolite, DAN, can be detected. Atrazine profile is very similar to that of Boffalora while DEA and DIA were absent. The atrazine was quantified only in the November samples because the detection limit of the instrument was improved during this period from 0.2 to 0.1 µg/kg.

In the Manerbio samples (Figure 5) terbuthylazine and metolachlor reach the highest concentrations, 12 and 7 µg/kg respectively, in the superficial horizon while in the deeper samples the contamination decreases to level of 0.2–0.3 µg/kg. EMA, the metabolite of metolachlor, was detected also in the bottom layers. The presence of alachlor and DAN can be attributed to the past use of this compound as for atrazine. This is confirmed even

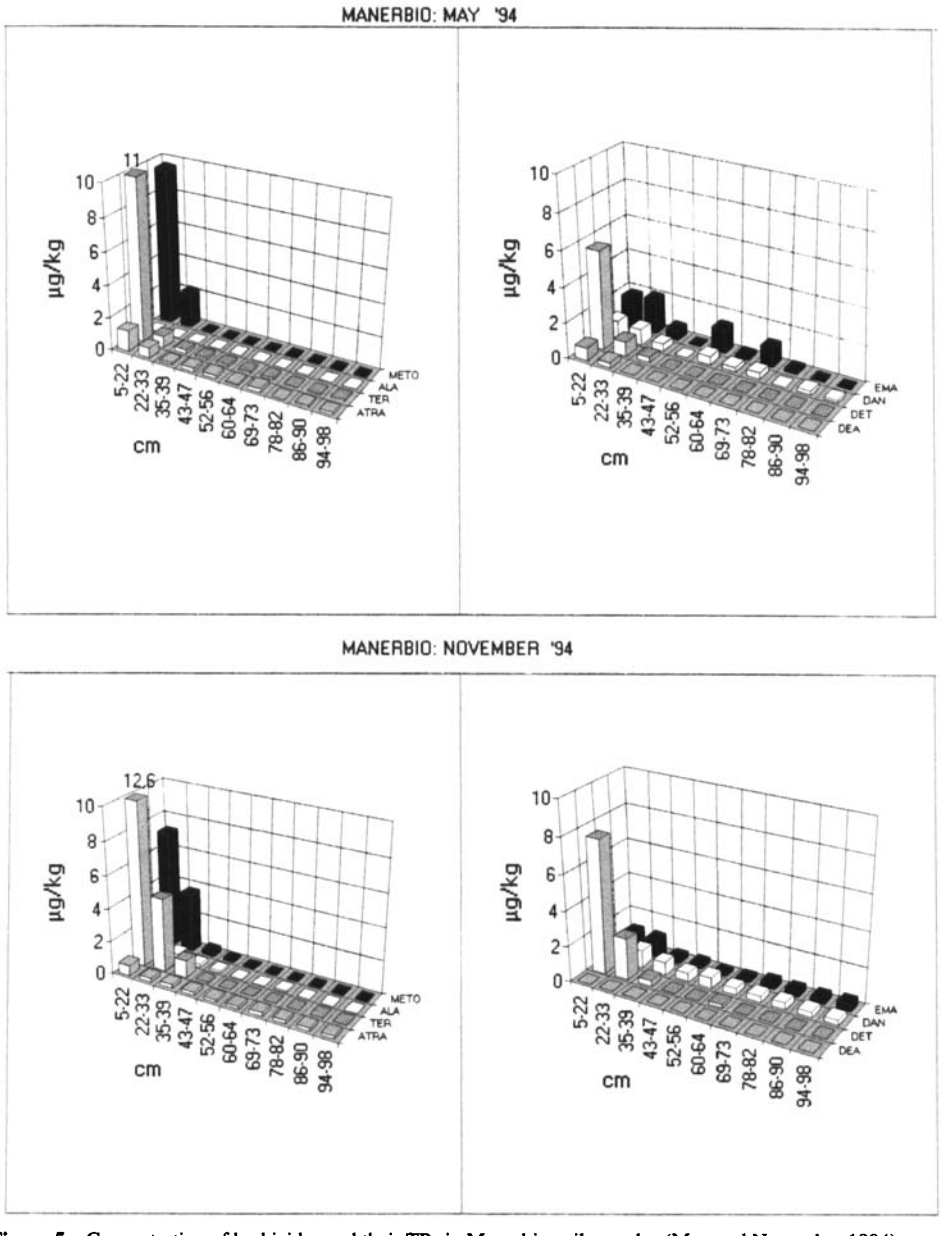

Figure 5 Concentration of herbicides and their TPs in Manerbio soil samples (May and November 1994).

by the fact that DAN contamination prevails to that of the original pesticide, alachlor. The concentrations of atrazine are higher in the superficial layers and decrease to 0.2 µg/kg in the deeper horizons as in Boffalora soil samples. The observed difference of atrazine content in May and November samples can be attributed probably to a variability in the sampling site.

The pesticide concentration in the groundwater samples are reported in Table 3. All

Table 3 Concentration of pesticides and their TPs in groundwater (μg/l).

Well (depth)	*Sampling date*	*Atrazine*	*DEA*	*Terbuthylazine*	*DET*	*Alachlor*	*DAN*	*Metolachlor*	*EMA*
B (14 m)	14/11/94	0.052	0.052	0.033	0.118	0	0	0.072	0
B (16 m)	14/11/04	0.038	0.032	0.026	0.076	0	0	0	0
M (120 m)	22/11/94	0	0	0	0.02	0	0	0	0
C (20 m)	17/11/94	0	0	0	0	0	0	0	0
M (20 m)	16/06/95	0.006	0	0	0	0	0	0	0
M (120 m)	16/06/95	0	0	0	0	0	0	0	0

B = Boffalora, M = Manerbio, C = Cervignano

the analyzed pesticide and their transformation products (TPs) are present at level inferior to 0.1 μg/l with the only exception of DET in Boffalora groundwater samples collected from the 14 m well. Atrazine, DEA, terbuthylazine, DET and metolachlor were detected mainly in Boffalora samples. All the pesticides were absent in Cervignano water sample.

ELISA test results

The Millipore and Ohmicron kits for atrazine, metolachlor and alachlor were used in the present study for the ELISA tests because at the present specific kits for terbuthylazine analysis are not commercially available. All the results of ELISA tests for Boffalora,

Table 4 Comparison between pesticide concentration with GC and ELISA test (μg/kg) in Boffalora d'Adda samples (November 1994).

Depth cm	*Atrazine*			*Alachlor*			*Metolachlor*		
	GC	*Millipore*	*Ohmicron*	*GC*	*Millipore*	*Ohmicron*	*GC*	*Millipore*	*Ohmicron*
5–30	0.86	0.97	1.32	1.43	0.90	1.05	3.10	n.d.	1.24
4L (5–30)	0.82	1.28	1.01	1.49	1.11	1.18	3.31	n.d.	1.55
Mix (5–30)	1.36	1.68	1.34	2.26	3.24	1.72	3.27	n.d.	2.00
37–55	0.92	0.80	1.21	0.32	0.16	0.11	0.26	n.d.	0.42
4L (40–55)	0.12	0.27	0.30	0.22	0.20	0.17	0.96	n.d.	0.33
Mix (40–55)	0.16	0.19	0.23	0.15	0.15	0.10	0.70	n.d.	0.30
55–70	0.69	0.84	0.20	0	0.04	0.06	0.67	n.d.	0.17
5–30	0.86	0.91	1.32	1.43	0.90	1.05	3.10	n.d.	1.24
35–39	0.28	0.32	0.32	0.37	0.08	0.07	1.34	n.d.	1.72
43–47	0.24	0.39	0.42	0.15	0.07	0.09	0.73	n.d.	0.34
52–56	0.40	0.47	0.37	0.15	0.09	0.10	0.73	n.d.	0.29
60–64	0.30	0.34	0.33	0.20	0.05	0.07	0.71	n.d.	0.21
69–73	0.11	0.19	0.17	0.19	0.15	0.20	0.89	n.d.	0.34
78–82	0.09	0.17	0.06	0.17	0.08	0.10	1.20	n.d.	0.20
86–90	0.35	0.17	0.04	0	0.03	0.04	0	n.d.	0.15
94–98	0.24	0.10	0.07	0	0.12	0.10	1.43	n.d.	0.28
Groundwater (14 m)	0.05	0.01	0.06	0	0	0	0.07	n.d.	n.d.
Groundwater (16 m)	0.04	0.01	0.02	0	0	0	0	n.d.	n.d.

n.d. = not determined

Table 5 Comparison between pesticide concentration with GC and ELISA test (μg/kg) in Cervignano d'Adda samples (November 1994).

	Atrazine			*Alachlor*			*Metolachlor*		
Depth (cm)	*GC*	*Millipore*	*Ohmicron*	*GC*	*Millipore*	*Ohmicron*	*GC*	*Millipore*	*Ohmicron*
5–30	0.10	0.35	0.40	0	0.69	0.98	6.53	n.d.	2.54
4L (5–30)	0.10	0.30	0.30	0.80	0.80	0.97	7.56	n.d.	2.53
Mix (5–30)	0.10	0.42	0.34	0.89	2.10	2.19	5.44	n.d.	2.51
37–55	0	0.06	0.11	0	0.10	0.29	1.23	n.d.	0.44
4L (40–55)	0.13	0.08	0.20	0	0.04	0.15	0.45	n.d.	0.39
Mix (40–55)	0	0.06	0.13	0	0.13	0.22	1.87	n.d.	0.34
55–80	0.15	0.05	0.06	0	0.02	0.10	0	n.d.	0.27
5–30	0.10	0.35	0.40	0	0.69	0.98	6.53	n.d.	2.54
35–39	0.41	0.33	0.18	0	0.15	0.27	0.61	n.d.	0.60
43–47	0.16	0.06	0.12	0	0.06	0.14	0.16	n.d.	0.36
52–56	0.18	0.07	0.07	0	0.02	0.07	0	n.d.	0.27
60–64	0.17	0.07	0.07	0	0.02	0.09	0	n.d.	0.21
69–73	0.21	0.06	0.10	0	0.01	0.06	0	n.d.	0.27
78–82	0.23	0.04	0.05	0	0.01	0.05	0	n.d.	0.26
86–90	0.15	0.03	0.04	0	0	0	0.12	n.d.	0.19
94–98	0.15	0.04	0.06	0	0.01	0.05	0.14	n.d.	0.17
Groundwater (20 m)	0	0	0	0	0	0	0	n.d.	n.d.

n.d. = not determined

Table 6 Comparison between pesticide concentration with GC and ELISA test (μg/kg) in Manerbio samples (November 1994).

	Atrazine			*Metolachlor*		
Depth (cm)	*GC*	*Millipore*	*Ohmicron*	*GC*	*Millipore*	*Ohmicron*
0–22	0.65	0.92	1.10	7.19	n.d.	3.52
22–33	0.23	1.16	0.96	6.13	n.d.	2.67
4L (5–30)	0.45	1.34	0.82	6.48	n.d.	4.60
Mix (5–30)	0.52	1.12	0.70	8.32	n.d.	3.73
37–55	0.19	0.07	0.06	0	n.d.	0.30
4L (40–55)	0.18	0.07	0.08	0	n.d.	0.35
Mix (40–55)	0.27	0.08	0.10	0.31	n.d.	0.49
55–73	0.22	0.06	0.09	0	n.d.	0.58
35–39	0.26	0.18	0.30	0.39	n.d.	0.36
43–47	0.20	0.11	0.08	0	n.d.	0.18
52–56	0.18	0.11	0.10	0	n.d.	0.21
60–64	0.18	0.06	0.09	0	n.d.	0.17
69–73	0.25	0.10	0.11	0	n.d.	0.21
78–82	0.20	0.09	0.09	0	n.d.	0.17
86–90	0.20	0.13	0.10	0	n.d.	0.23
94–98	0.17	0.05	0.06	0	n.d.	0.32
Groundwater (120 m)	0	0	0	0	n.d.	n.d.

n.d. = not determined

Table 7 Results of the statistical correlation between GC and ELISA results.

Sampling site	*Compound*	*Comparison between*	*Correlation coefficient (r)*	*Linear regression line*
Boffalora d'Adda				
	Atrazine	GC – Millipore	0.94	y = 0.77x + 0.048
		GC – Ohmicron	0.88	y = 0.66x + 0.130
		Ohmicron – Millipore	0.85	y = 0.92x + 0.024
	Alachlor	GC – Millipore	0.96	y = 1.03x + 0.090
		GC – Ohmicron	0.88	y = 1.15x + 0.080
		Ohmicron – Millipore	0.8	y = –0.66x + 0.097
	Metolachlor	GC – Millipore	n.d.	
		GC – Ohmicron	0.82	y = 1.46x + 0.420
		Ohmicron – Millipore	n.d.	
Cervignano d'Adda				
	Atrazine	GC – Millipore	n.s.s.	
		GC – Ohmicron	n.s.s.	
		Ohmicron – Millipore	0.8	y = 1.16x + 0.042
	Alachlor	GC – Millipore	n.d.	
		GC – Ohmicron	n.d.	
		Ohmicron – Millipore	0.98	y = 1.05x + 0.110
	Metolachlor	GC – Millipore	n.d.	
		GC – Ohmicron	0.97	y = 2.76x – 0.490
		Ohmicron – Millipore	n.d.	
Manerbio				
	Atrazine	GC – Millipore	0.75	y = 0.22x + 0.190
		GC – Ohmicron	0.81	y = 0.31x + 0.180
		Ohmicron – Millipore	0.94	y = –0.72x + 0.047
	Metolachlor	GC – Millipore	n.d.	
		GC – Ohmicron	0.97	y = 1.98x – 0.440
		Ohmicron – Millipore	n.d.	

n.d. = not determined
n.s.s. = not statistically significant

Cervignano and Manerbio samples are reported in Table 4, 5 and 6 respectively. Millipore test for metolachlor was not performed as described in Material and Method section.

The ELISA results seem to be in a good agreement with those obtained with GC-NPD. The coefficient of correlation (Table 7) indicates in fact that in Boffalora samples the correlation is statistically significant both comparing GC and ELISA results and those of Millipore and Ohmicron ELISA tests.

For Cervignano samples it was impossible to determine GC-ELISA correlation because atrazine concentrations are very low and do not vary along the soil profile. In this condition the gas chromatographic error (10%) in the determination of atrazine residue becomes relevant in respect to the difference existing between the different samples. The alachlor level in the soil samples is also so low that the quantification with

GC-NPD instrument was possible in only two samples. Therefore it was impossible to verify any correlation with ELISA results. Ohmicron and Millipore results for alachlor were in a good agreement (r = 0.98) and also that between GC and Ohmicron (r = 0.97) for metolachlor.

ELISA determination of alachlor in Manerbio samples was not performed because this compound was not used in the agricultural practice during the investigation. The best correlation was observed comparing Ohmicron and Millipore results for atrazine and those of GC and Ohmicron for metolachlor.

The possibility of matrix interference, particularly in Cervignano and Manerbio soil samples, will be investigated with spiking experiment as soon as possible.

DISCUSSION AND CONCLUSION

A tentative correlation between the results of the gas chromatographic and pedological analyses was undertaken but a favorable correspondence was observed only between the organic carbon content of the soil samples and the determined levels of pesticide residues. The superficial layers are in fact characterized by high organic carbon content and high concentrations of the analyzed pesticides while in the deepest horizons the organic carbon content drastically reduces as far as the pesticide contamination.

Besides there is a limited correlation between the textural characteristics and pesticide content in the sense that pesticide contamination is very low in the soil samples where gravel content prevail. None of the other pedological characteristics of the soil samples seems to be correlated to pesticide content.

Besides as fár as regard the soil profile of atrazine, terbuthylazine, alachlor and metolachlor, the environmental contamination seems to be limited to the more superficial levels (0–30 cm of depth) while the vertical distribution of the main transformation products showed the possibility of leaching phenomenon into the deeper horizons. Particularly DAN and EMA metabolites reach easily 1 meter of depth even if the observed concentration are very low, about 0.2–0.4 μg/kg. The possibility of these compounds to reach the underground water table depends greatly on the physico-chemical characteristics of the considered soils. Only in the case of Boffalora, in fact, a limited amount of the analyzed pesticides and their TPs was detected. This phenomenon may be explained by the fact that Boffalora soil profile is mainly sandy loam in respect to the other ones. Sandy material has a poor capacity of keeping bound the organic compounds. The real availability of these compounds to migrate to the aquifer zone will be evaluated in laboratory by saturation experiments that are now in progress.

The ELISA test results demonstrated the positive comparison between Millipore and Ohmicron kits (r value superior to 0.8) for all the analyzed pesticide. As far as concerned ELISA and GC-NPD comparison, Boffalora soil samples do not evidentiate any phenomenon of matrix interference while those of Cervignano and Manerbio should have to be submitted to spiking experiments in order to verify some possible interferences.

Besides the sensitivity of the two considered ELISA kits is higher than that of GC analysis. The extracts utilized for ELISA tests in fact (see Material and Method section) are usually diluted 1 : 20 – 1 : 50 according to % Bo values. For the ELISA determination, therefore, it is not necessary to conduct the concentration of the solvent extract to a so excessive extent (150 to 0.25 ml) such as for GC analysis. The speed and

ease of ELISA analysis in respect to GC methodology make immunoassay technique an attractive screening method for pesticide determination in soil samples. If properly standardized, ELISA test can constitute an alternative and suitable method for analyzing low concentrations of pesticide in soil.

The research program supported by the Europen Union is now still in progress and the present manuscript may be considered as a preliminary report of the analyzed results.

Acknowledgement

The present investigation was conducted with the financial support of the European Union (CT EV5V 93-0322) and it was also included within the activities of the "Strategic Project" recently promoted by the National Research Council of Italy on the *Critical aspects of availability of water destined to human consumption.*

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