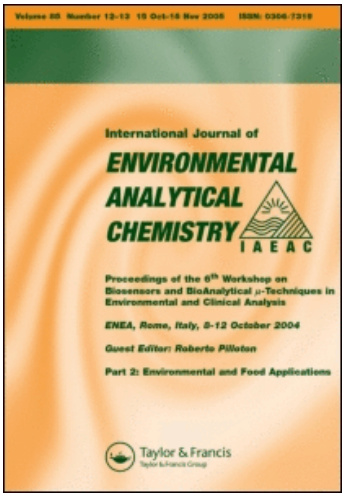

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Monitoring of Insecticides and Fungicides in Water and Sediment Samples in the Brazilian Environment

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MONITORING OF INSECTICIDES AND FUNGICIDES IN WATER AND SEDIMENT SAMPLES IN THE BRAZILIAN ENVIRONMENT

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Immunoassay techniques, either microtiter plate or magnetic particle-based ELISA and various gas chromatographic techniques (GC-ECD, GC-NPD and GC-MS) were applied to the determination of priority pesticides (chlorothalonil, metalaxyl, carbendazim, parathion-methyl and folpet) in water and sediment samples from Paty do Alferes at Rio de Janeiro State (Brazil). Water samples were directly analyzed in the field by immunoassay techniques and transported to Barcelona after being stored in SPE disks. Sediment samples were frozen and transported to Barcelona for further analysis. All results were confirmed by GC-MS under negative chemical ionization and using SIM and the protocol was extended to other pesticides like naled, fenitrothion and azinphos-ethyl that could not be measured with ELISA. The results showed that the majority of the water samples had pesticide levels below 5 ng/l with the exception of chlorothalonil and folpet that showed values up to 114 and 210 ng/l, respectively. The sediment samples showed values of 5–132 ng/g for folpet and one order of magnitude lower for chlorothalonil, being the two pesticides that exhibited the highest persistence in the Brazilian environment.

Keywords: Insecticides; ELISA; gas chromatography; pesticides; cross-reactivity

INTRODUCTION

The area of study, Paty do Alferes, Rio de Janeiro state (Brazil) comprises one of the provinces with cultivation of tomato, pepper and cauliflower. Greenhouses are also established in the region. Tomato is the highest cultivation in the area with a production per year of ca. 50–100.000 kg which is primarily sent to Rio de Janeiro central market for food supply. Pesticides are usually applied by manual

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spraying every week with a more intensive application from January to March with 10–20 tones per year of the following compounds: carbendazim, chlorothalonil, metalaxyl, parathion-methyl and folpet. Other pesticides used in minor amounts were fenitrothion, naled and azinphos-ethyl.

This research is part of the international and multidisciplinary research project financed by the European Union and entitled: *Development of Sustainable Farming Systems off Mountainous, Low Fertility Grazing Land in South America*. This research project links various institutions from European and South American countries to study the effects of unregulated farming systems.

Monitoring of organophosphorus pesticides and fungicides in water samples is usually carried out by solid phase extraction (SPE) procedures followed by GC and HPLC techniques [1, 2]. Pilot survey studies in the Mediterranean region jointly organized by the International Atomic Energy Agency indicated that low or undetectable levels of fungicides are found in estuarine waters of the various European rivers [3]. As regards to detectable organophosphorus pesticides, they were measured in estuarine and coastal waters just after application, with a half live of few hours. After one-two days the residue levels in water were undetectable [4–8].

During the last few years ELISA techniques were used by our group to determine residue levels of pesticides like atrazine, chlorpyrifos, carbaryl and fenitrothion in water [8–15]. Applications to residue levels in soil/sediment samples were also reported for chlorpyrifos [16]. The use of the ELISA kits to the determination of chlorothalonil in water and agricultural products was also reported [17, 18].

Scarce information is available on the new generation of pesticides (non organochlorinated) in tropical countries. The persistence of various organophosphorus pesticides in different Central America sediments was reported for parathion-methyl, malathion and fenitrothion with levels below 0.1 ng/g, except for chlorpyrifos that had levels up to 34 ng/g [19, 20]. Organophosphorus pesticides levels in surface water were below 1 μg/l. Chlorothalonil was detected in sediment and water samples at levels of low ppb (sediments) and until 11 μg/l in a water sample. The major part of the other pesticides corresponded to organochlorinated. The analysis of pesticides were always carried out by chromatographic techniques and very few information is available on the monitoring of water and sediment samples using immunoassay techniques in tropical countries.

In view of the reported data in the literature as regards the determination of pesticide residues in tropical areas and the limited or scarce use of ELISA techniques for mesasuring residue levels of a new generation of pesticides in tropical countries, the purpose of this work was : (i) to supply a fast and effective monitoring scheme for the determination of a priority list of organophosphorus pesti-

cides and fungicides in the area of Paty do Alferes (Rio de Janeiro State, Brazil) in water and sediment samples. ELISA was used in the field with a portable instrument, for the first screening and afterwards, only the positive samples were analyzed and confirmed by GC techniques (ii) to compare the data obtained by ELISA with chromatographic techniques following the pretreatment of water and sediment samples and (iii) to show evidences, for the first time in Brazil, of the presence of organophosphorus pesticides and fungicides by a combination of powerful analytical techniques. It is also the intention that the present analytical scheme could be applied in the near future for the monitoring of pesticides in developing countries, where known problems in the analysis of pesticides exist.

EXPERIMENTAL SECTION

Chemicals

Methanol, acetone, dichloromethane and HPLC-grade water were purchased through Merck (Darmstadt, Germany). Ethyl acetate was obtained from Panreac Química S.A. (Montcada i Reixac, Spain). Azinphos-ethyl, Folpet, Parathion-methyl. Metalaxyl, Naled, Benomyl were acquired through Dr. Ehrenstorfer (Ausburg, Germany). Captan, Chlorothalonil, Fenitrothion, Carbendazim were from Promochem (Warsaw, Poland).

Sampling Strategy

Sampling of water and sediments in the area of Paty do Alferes, Rio de Janeiro State, Brazil, (see Figure 1) took place in 1995. The samples were collected in various lagoons, just below the fields where crops of tomatoes were grown. Water from the lagoons corresponded to drainage water from irrigation of the cultivated field. The total number of water and sediment samples collected was 35 and 14, respectively. Water samples were collected in jars below the water surface layer and at a water depth varying between 15 and 30 cm. without field spillage following previous protocols [21]. From few lagoons, sediment samples were also collected from the bottom sediments at 0.5 m depth approximately. From the same lagoons, various water samples were collected and pooled together.

Water samples were immediately kept in amber glass bottles and stored at 4 °C. After collecting the water samples, immunoassay techniques were applied for the determination of the target pesticides (chlorothalonil, metalaxyl and beno-

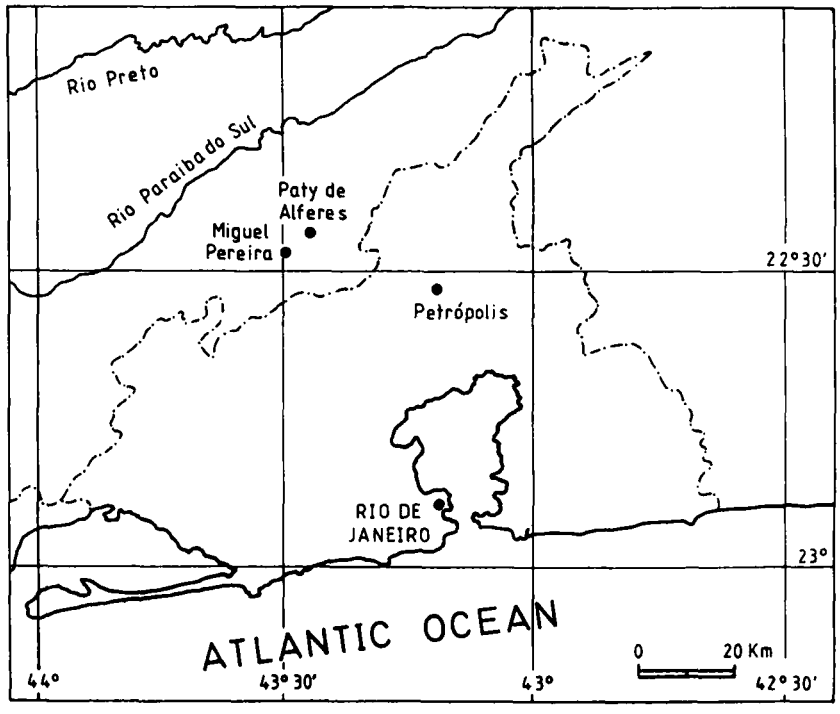

FIGURE 1 Map of the area of Rio de Janeiro State, Brazil where the monitoring took place

myl/carbendazim) near the area of sampling, in a warehouse from the city of Paty do Alferes using a field portable spectrophotometer. The Ohmicron RPA-III is a microprocessor based, single-channel photometer for use with Ohmicron's RaPID Assays. The RaPID Assays kits can be used as a qualitative (yes/no) or a semi-quantitative procedure for the detection of the different pesticides in waters, and the protocol run at the same way as described in Immunoassay Procedure for Carbendazim, Chlorothalonil, Captan and Metolachlor kits.

In the next step, water and sediment samples were transported to Rio de Janeiro (about 200 km from Paty do Alferes). The sediment samples were frozen and stored at −20 °C in order to be analyzed in Spain, whereas the water samples were first filtered through fiber-glass filters (Millipore Corp. Bedford, MA) of 0.45 μm to eliminate the particulate matter, and preconcentrated using solid phase extraction disk, C_{18} Empore disks (J.T. Baker and Analytichem International, The Netherlands) following a previous protocol developed in our laboratory [1]. After preconcentrating, the Empore disks containing the various

pesticides were stored at 4 °C and transported to Spain after four days. Water samples are stable in Empore disks for a period that can vary from one to various months [22, 23].

Sample preparation.

Water samples were analyzed following the protocol described in a previous paper [1, 8]. After preconcentrating the samples, the pesticides analyzed by ELISA were: chlorothalonil, folpet, benomyl/carbendazim, metalaxyl and paration-methyl. Therefore, two pesticides were introduced this time (folpet and parathion-methyl).

Recoveries for all the analytes are above 70%, except for carbendazim, that exhibited a low recovery of 40% when it was analyzed in river water samples containing humic acids [1]. In a previous work, a method for the determination of organophosphorus pesticides in sediment samples was optimized [24]. However, this method needs to be improved since the recoveries were too low for some of them (naled, azinphos-ethyl, metalaxyl, chlorothalonil and folpet).

TABLE I Mean percentage recovery and coefficient of variation (CV) of different pesticides in sediment samples and surface waters (1) using Empore extraction disks. (Spiking level was at 1 mg/kg for the sediments and 2 µg/l for surface waters, n=5)

Compounds	*Sediments*		*River Water*	
	% Recovery	*% CV*	*% Recovery*	*% CV*
Naled	77	2	80	4
Metalaxyl	93	2	104	3
Fenitrothion	94	2	112	5
Azinphos-ethyl	102	3	115	6
Chlorothalonil	85	3	107	6
Methyl Parathion	82	4	90	6
Captan	91	4	85	7

The developed method consisted in freeze-drying of the sediment samples. Before extraction, the freeze dried sediment was sieved through a 120 µm sieve. Afterwards, 10 g of sediment were Soxhlet extracted for 12 h. with 100 ml of acetone:dichloromethane (3:1). The extract was concentrated in a rotary evaporator, transferred and carefully evaporated to dryness (nitrogen stream). Afterwards

dissolved in 0.5 ml of methanol by adding 100 ml of HPLC-grade water. The clean-up step was carried out using C_{18} Empore disks. The activation of the disk was performed using ethyl acetate, methanol and water (10 + 15 + 15 ml of each, respectively) with the vacuum on. Drying of the disk took place during 30 min. to remove the water that could cause the hydrolysis of many pesticides on the silica surface of the disks [22]. The elution took place with 4 × 10 ml of ethyl acetate. Afterwards, a final volume of 0.3 ml in ethyl acetate was collected.

To investigate the recoveries of the various pesticides (see Table I), clean sediment samples were spiked with a mixture of the target pesticides naled, metalaxyl fenitrothion, azinphos-ethyl, chlorothalonil, methyl parathion and captan with a final concentration in the sediments of 1 mg/kg.

Apparatus for Immunoassay Determinations

For EnviroGard Parathion Plate Kit, immunoassay experiments were carried out in 96-well microplates washer SLT 96PW (SLT, Salzburg, Austria) and the absorbances were read at 450 nm in a microtiter-plate ELISA reader Multiskan Plus (Labsystems, Helsinki, Finland). Data acquisition and calculations were performed using the commercial software package Genesis (Labsystems). A four-parameter logistic equation was used for the calibration curves. The spectrophotometric measurements for the Chlorothalonil, Captan, Metolachlor and Carbendazim RaPID ELISA kits were determined using the RPA-I RaPID Photometric Analyzer and a field portable spectrophotometer RPA-III Analyzer (Ohmicron. Newtown, PA, USA).

Immunoassay Procedure for Carbendazim, Chlorothalonil, Captan and Metolachlor kits

The samples were assayed according to the RaPID Assay package insert. A total of 200 µl of the sample to be analyzed is added to a disposable test tube. along with 250 µl of pesticide (carbendazim or chlorothalonil, captan or metolachor) hapten-horseradish peroxidase (HRP) enzyme conjugate, and 500 µl of rabbit anti-pesticide magnetic particles (anti-carbendazim or anti-chlorothalonil or anti-metolachlor or anti-captan) attached covalently. Both pesticides of the sample and the enzyme labeled pesticide competed for antibody sites on the magnetic particles. Tubes were vortexed and incubated for 20 min, in the case of Carbendazim, and 30 min. in the case of Chlorothalonil. Captan and Metolachlor at room temperature. The reaction mixture was magnetically separated using a specially designed magnetic rack. After separation, the magnetic particles were

washed twice with 1.0 ml of distilled water to remove unbound conjugate and eliminate any potential interfering substances. Pesticide and enzyme labeled pesticide remained bound to the magnetic particles in concentrations proportional to their original concentration. The presence of labeled pesticide was detected by adding a total of 500 µl of a 1:1 mixture of a solution containing substrate and chromogen (hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB). The tubes were vortexed to resuspend particles and incubated for another 20 min at room temperature to allow color development. The color reaction was stopped by the addition of 500 µl of sulfuric acid solution (0.5%). The final concentration of pesticide for each sample was determined using the RPA-I RaPID or RPA-III Photometric Analyzer by determining the absorbance at a wavelength of 450 nm.

Immunoassay Procedure for Parathion kit

The plates coated with antibody were filled with 100 µl/well of standard/sample and 100 µl/well of Parathion-enzyme conjugate. The wells were covered to prevent evaporation and incubated at room temperature for 60 min. The plates were washed five times with distilled water with the washer SLT 96PW and then, 100 µl/well of substrate/chromogen were added. After incubation for 30 min. at room temperature, the reaction was stopped with a stopping solution (100 µl/well) and mixed thoroughly. This turned the solution yellow and the optical density at 450 nm. was determined with a microtiter-plate ELISA reader Multiskan Plus.

Gas Chromatographic Analysis

GC-NPD and GC-ECD

Gas chromatography was performed with a Mega Series 5300 gas chromatograph (Carlo Erba, Milan Italy) equipped with a nitrogen phosphorus detector (GC-NPD) and another chromatograph Hewlett Packard 5890A with an electron capture detector (GC-ECD).

Two different columns were used. For GC-NPD, a DB-1701 and for GC-ECD, a DB-5 fused silica capillary column from J & W Scientific, Folsom, CA (USA) of 30 m × 0.25 mm × 0.25 µm. For GC-NPD, hydrogen was employed as carrier gas at 50 cm s^{-1} and helium as make up gas at 30 ml/min. For GC-ECD, helium was used as carrier gas at 2.8 ml/min and nitrogen was used as the make up gas at 70 ml/min. The temperatures of the injector and detector of were maintained at 280 and 300 °C, respectively. The column temperature was programmed from 70 to 280 °C at 4 °C/min, being the initial and final times of 1 and 10 min, respectively.

GC-MS-CI-NEG

Fisons MD800 (Quadrupole Analyzer Fisons Instruments, V.G. Manchester, U.K.) in splitless -mode (keeping the split valve closed for 40 s.). The column used was a 15 m × 0.15 mm i.d. fused-silica capillary column coated with chemically bonded cyanopropylphenyl DB-225 (J & W Scientific, Folsom, CA, US). Helium was used as the carrier gas (30 cm s^{-1}). The temperature of the injector was maintained at 280 °C. The gradient was programmed from 70 to 280 °C. The ion source and the transfer line were maintained at 150 and 280 °C, respectively. Methane was used as the reagent gas (Abell6, Barcelona, 99.995% of purity).

The retention time in the various used GC columns and the GC-MS with SIM ions used for the confirmation are indicated in Table II.

TABLE II Retention time (Rt) in minutes, and typical fragment ions of the target pesticides analyzed by GC-MS with NICI. *Main ions of Folpet analyzed by Electro Impact with a DB 5 column

Compounds	*Rt (DB 225 column)*	*m/z (relative abundance)*
Fenitrothion	30.48	168 (100) / 141 (20) / 277 (30)
Naled	22.48	241 (68) / 275 (100)
Methyl Parathion	28.07	141 (85) / 154 (100) / 263 (82)
Metalaxyl	28.75	192 (100) / 216 (10) / 278
Chlorothalonil	29.07	266 (100) / 230 (52)
Captan	32.80	150 (100) / 182 (5)
Folpet	32.89	146 (100)
Folpet*	26.91	104 (100) / 260 (20) / 297 (10)

GC-MS-EI

Fisons MD800 (Quadrupole Analyzer Fisons Instruments, V.G. Manchester, U.K.) in splitless-mode (keeping the split valve closed for 40 s.). The column was a DB-5 fused silica capillary column from J & W Scientific, Folsom, CA (U.S.) of 30m × 0.25mm × 0,25 μm. Helium (SEO, 99.9990% of purity) was used as the carrier gas (30 cm/s). The temperature of the injector was maintained at 280 °C hot needle injection The gradient was programmed from to 70 to 280 °C. The ion source and the transfer line were maintained at 200 and 280 °C, respectively.

RESULTS AND DISCUSSION

General remarks

An important aspect to be considered before starting with immunoassay determinations is to know the value of cross-reactivity of the compounds to be analyzed. For all the compounds measured by ELISA, an extensive cross-reactivity study has been carried out in a previous work [14]. Table III indicates the sensibility values (IC_{50}) and the cross-reactivities of the target analytes determined by ELISA techniques. Two cross-reacting analytes, like metalaxyl, that is analyzed using the Metolachlor ELISA kit, and folpet, that is analyzed using the Captan ELISA kit were included in this table. Another important point that it should be considered is that these kits are able to respond to the presence of other cross-reactants (structurally similar analytes to the analyte of interest) giving a 100% of response if the cross-reactant is the only analyte present in the sample.

TABLE III Values of the IC_{50}, 50% inhibition concentration (doses of the analyte necesssary to displace 50% of the bound labeled tracer) and the percentage of cross-reactivity from two commercial suppliers. The RaPID-magnetic particle-based ELISA assay and EnviroGard Parathion plate kit. * IC_{50} in mg/l

Compounds	*IC_{50} (μg/l)*	*%CR*
Carbendazim	1.60	100
Benomyl	5.61	28.52
Chlorothalonil	1.12	100
Metalaxyl	5.60	15.18
Metolachlor	0.85	100
Captan*	0.42	100
Parathion-methyl	0.30	100
Folpet*	>10	4.2

This behavior was observed for fenitrothion in a previous paper [8], and now it is being applied for the determination of folpet and metalaxyl in water samples. since it was shown that captan and metolachlor were not present in the water and sediment samples.

Confirmation by GC techniques always remains necessary after ELISA determinations in real environmental samples. GC-ECD and GC-NPD are common methods. Confirmation by GC-negative chemical ionization-MS is an effective tool for organophosphorus pesticides and other analytes with electron withdrawing groups in the molecule [25].

TABLE IV Water sample from Paty do Alferes (Rio de Janeiro State, Brazil) analyzed by Chlorothalonil, Metolachlor and Benomyl/Carbendazim RaPID ELISA kits before (with and without a filtration directly in the field) and after solid phase extraction using C_{18} Empore disks. Values expressed in µg/l. n.d. non detectable values

Sample	***Date***	***Chlorothalonil***			***Metalaxyl***			***Benomyl/Carbendazim***		
		Non Filtered	*Filtered*	*After Extraction*	*Non Filtered*	*Filtered*	*After Extraction*	*Non Filtered*	*Filtered*	*After Extraction*
DBF/MBCNI	31/10/95	0.1–1	0.08	n.d.	0.1–1	0.26	n.d.	0.1–1	n.d.	n.d.

Water samples

Direct Measurements in Water by ELISA

Chlorothalonil, metalaxyl and benomyl/carbendazim were measured directly in the field using a field portable spectrophotometer. The measurements were carried out with and without a previous filtration. Only one of 35 measured water samples was positive (see Table IV) using this type of test. Sample DBF/MBCNI was positive for chlorothalonil and metalaxyl, although this positive result only indicated the presence of the pesticides at levels between 0.1–1 μg/l, so a semi-quantitative response was obtained. Benomyl/carbendazim kit presented negative results for all samples with the exception of the DBF/MBCNI. This sample point was positive without a filtration, but after this step, a non detectable value was obtained, thus indicating the matrix effect in the determination without filtration.

Table IV also presents the results before and after a filtration step and after a solid phase extraction using C_{18} Empore disks to show that a matrix effect is affecting the final value of this sample SPE disks act as a clean up step, so pesticides contained in water samples can be measured more precisely. The log K_{oc} of these compounds varies between 2.1 (captan) – 3.9 (chlorothalonil), and consequently, exhibits lower values than typical compounds that absorb into the particulate matter like PAHs or PCBs with log K_{oc} of 5–6 [26]. Big differences between filtration and no filtration of the tropical waters from Paty do Alferes can be noticed in the Table IV. In addition, the data reported in the following tables indicates that very low values of pesticides were determined in waters. The half-life of folpet and chlorothalonil varied from 4.3 to 10 days, respectively. Due to the short half-life, the difference between ELISA and GC data are most likely to be attributed to degradation. Generally, the ELISA values overestimated the chromatographic data, although in some cases good agreement was observed.

After the first measurements were carried out in the field, the samples were submitted to the preconcentration step using solid phase extraction disks and the obtained results were analyzed by ELISA and GC-ECD. This time, five kits were employed, Parathion, Metolachlor (to detect metalaxyl), Benomyl/Carbendazim, Captan (to detect Folpet) and Chlorothalonil. First, all samples were negative for parathion. metalaxyl and benomyl/carbendazim when they were analyzed by ELISA and gas chromatography. Table V presents the positive results for the determination of chlorothalonil showing a 37% of false positives with the analysis by ELISA. The same behavior could be observed in Table VI with a 17% of false positives in the determination of folpet. However, it is remarkable that not false negatives were obtained.

TABLE V Water samples from Paty do Alferes (Rio de Janeiro State, Brazil) analyzed by the RaPID ELISA for Chlorothalonil after SPE and GC-ECD for the determination of Chlorothalonil. Values expressed in ng/l. n.d. non detectable

Samples	*Date*	*ELISA*	*GC-ECD*
JO/MBC NI 2	25/10/95	26	n.d.
JO/MBC NI 3	25/10/95	10	n.d.
LC/MBC ND	25/10/95	9	n.d.
OB/MBC ND	25/10/95	9	n.d.
JO/MBC NI 1	31/10/95	114	14.3
JS/MBC NI	31/10/95	12	n.d.
JD/MBC ND	31/10/95	19	n.d.
SA/MBC ND	31/10/95	28	n.d.
CO/MBC ND	31/10/95	28	n.d.
JF/MBC ND	31/10/95	25	n.d.
JF/MBC NI	31/10/95	82.5	7.4
DI/MBC ND	31/10/95	33	n.d.
DI/MBC NI	31/10/95	24	n.d.
IS/MBC ND	31/10/95	29	n.d.
BE/MBC ND	31/10/95	10	n.d.

TABLE VI Water samples from Paty do Alferes (Rio de Janeiro State, Brazil) analyzed by the RaPID ELISA for Captan (to determine folpet) and GC-ECD for the determination of Folpet.Values expressed in ng/l

Samples	*Date*	*ELISA*	*GC-ECD*
JO/MBC NI 1	25/10/95	27	76
JO/MBC NI 2	25/10/95	210	156.65
LC/MBC ND	25/10/95	39	76.25
OB/MBC NI	25/10/95	23	48.61
JS/MBC NI	25/10/95	35	76.83
JO/MBC NI 3	31/10/95	10	29.09
JS/MBC NI	31/10/95	22	n.d.
JO MBC NI 1	31/10/95	17	n.d.
NI/MBC ND	31/10/95	9	n.d.
OB/MBC NI	31/10/95	29	32.31
JD/MBC ND	31/10/95	174	175.70
JF/MBC NI	31/10/95	18	n.d.
DIMBC ND	31/10/95	7	n.d.
IS/MBC ND	31/10/95	19	n.d.
BE/MBC ND	31/10/95	42	48.61

Sediment Samples

One important point that should be indicated is that the determination of pesticides in sediments offers many more difficulties than water samples [16, 28], and higher differences in the determinations are expected. One of the main advantages of the immunoassay techniques is the possibility to measure the samples directly in the field and to avoid the clean-up steps. To know if the matrix effects could affect the ELISA results, two very polluted sediments were chosen, DBF/MBCNI and DB/MBCNI. Table VII shows that the results for folpet without a clean-up step are significantly high. 2.2 and 2.9 times, respectively. In the case of chlorothalonil. they are somewhat lower differences, between 2.3 and about 1 time high without the clean-up step. These results emphasize that even though the matrix effect is very evident, immunoassay techniques are able to detect the analyte of interest in sediment samples.

TABLE VII Sediment samples from Paty do Alferes (Rio de Janeiro State, Brazil) analyzed by the RaPID ELISA for Chlorothalonil and Captan for the determination of chlorothalonil and folpet, respectively, with and without a clean-up step. Values expressed in ng/g. n.d: non detectable (below detection limit, 0.65 ng/g)

Sediment samples	*Date*	*Folpet*		*Chlorothalonil*	
		Without Clean-up	*With Clean-up*	*Without Clean-up*	*With Clean-up*
DBF/MBCNI	31/10/95	290	132.43	2.50	1.08
DB/MBCNI	31/10/95	230	79.23	0.86	n.d.

Comparison between ELISA and GC Techniques

All the sediment samples presented non detectable values when they were analyzed using the parathion and the benomyl/carbendazim kits, and the same results were obtained using GC-ECD and GC-NPD. The results of the water samples already analyzed showed that the concentration of chlorothalonil and folpet indicated that these two analytes were the two most relevant pesticides from the area of the study.

Figure 2 presents the chlorothalonil values obtained using ELISA. GC-NPD and GC-ECD. The higher differences between the two techniques and the fact that ELISA values are lower than GC could be explained by the degradation of chlorothalonil caused by the different analysis time between GC and ELISA analysis (few months later for ELISA). To study the observed deviations, a chlo-

rothalonil degradation study was performed. A standard of 900 mg/l of chlorothalonil was prepared in ethyl acetate and stored at −20 °C for three months. Each month the standard was analyzed by GC-ECD and GC-NPD observing a degradation of 20.6% in the third month of study. So, taking into account these results. It can be concluded that a degradation effect is observed in these samples. In fact, degradation problems in a project about pollution monitoring and research in the Mediterranean seat (MED POL) were detected with this fungicide [29]. On the other hand, a false positive in the ELISA technique was observed for samples JO/MBCNI2 and OB/MBCNI.

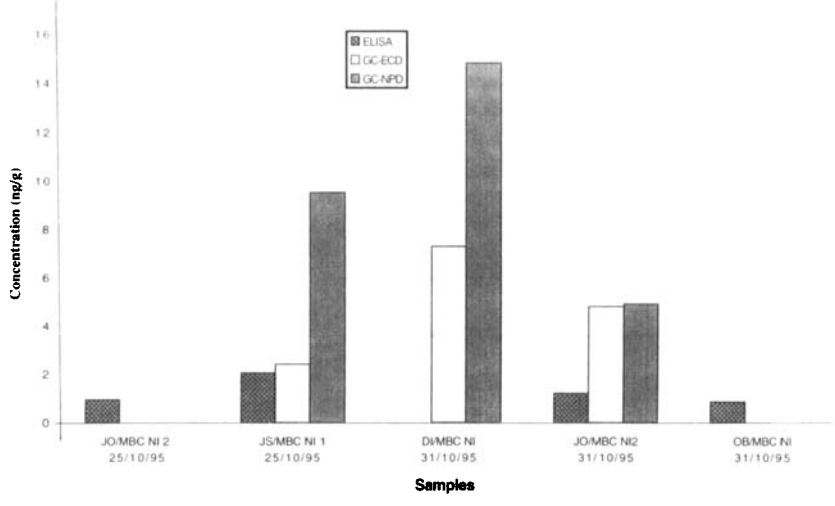

FIGURE 2 Sediment samples analyzed by the RaPID ELISA for chlorothalonil. GC-ECD and GC-NPD from Paty do Alferes (Rio de Janeiro State, Brazil) for the determination of chlorothalonil. Values expressed in ng/g

Figure 3 shows the results obtained for folpet using ELISA and GC-ECD for sediment samples. Differences were noticed, with higher values for ELISA in comparison with GC-ECD. This is not surprising since the application of ELISA to the determination of pesticides in sediments is more difficult than in water samples. and generally overestimation is noticed. Tropical sediments contained, in addition, high amounts of clay and organic matter components that can influence the ELISA determinations. Therefore, the observed differences in the ELISA determinations in samples with and without a clean-up step, were important due to the influence of clay material. The most relevant fact is the presence of folpet, that was confirmed by GC-MS using EI and NCI (see Table II). Although the K_{oc} of folpet is much lower than that of chlorothalonil [26], it has

been reported that folpet is strongly adsorbed into the soil and that will explain its higher stability in the analyzed sediment samples. Therefore, adsorption can be enhanced by the presence of great amounts of clay materials in the analyzed sediments.

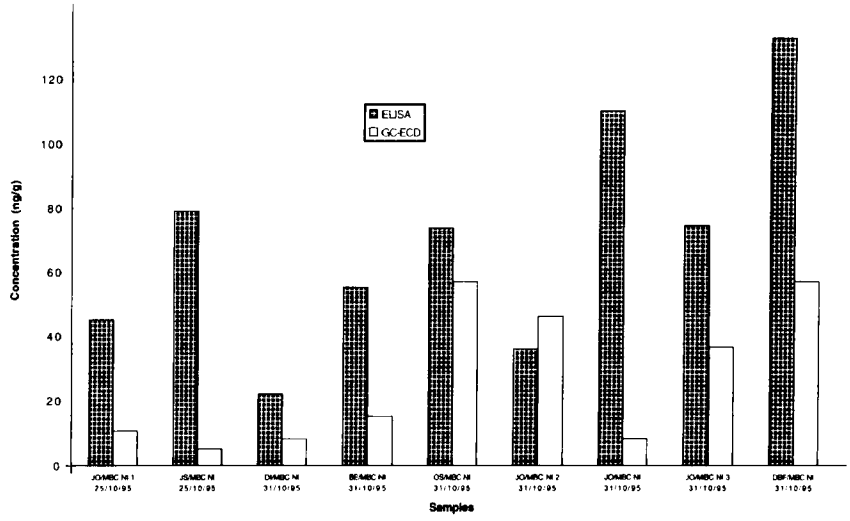

FIGURE 3 Sediment samples from Paty do Alferes (Rio de Janeiro State, Brazil) analyzed by the RaPID ELISA for Captan and GC-ECD for the determination of folpet. Values expressed in ng/g

No organophosphorus pesticides were detected in water and sediment samples. It can be explained by the fact that the parathion group can easily undergo photochemical degradation and volatilization under hot climatic conditions [8] and this is the reason that non detectable levels were observed in water and sediment samples. From the two detected pesticides, chlorothalonil has already been reported in samples from Central America countries, but folpet is reported in Brazil for the first time. The approach reported in this study, with direct pesticide measurements of water samples using ELISA, can be of easy implementation in developing countries and permits to screen in a relatively short time the presence of pesticides in water matrices. Attention should be paid to particulate matter present in tropical waters that influences ELISA determinations. For sediment samples, a previous extraction of the material is always needed, being the main limitation of this analysis.

As a general conclusion, the obtained results could indicate that probably the bottom sediments of the lagoons accumulate fungicides used during a long

period of time and that the folpet residues correspond to the various applications during the last few years. Fungicides are applied various times per week due to the high temperatures and humidity of the studied area. It is also important to notice that no organophosphorus pesticides were detected in the sediments and low levels, up to few ng/l in water samples, although an intensive application of compounds like parathion takes place in this area. The fast degradation of these organophosphorous pesticides under hot and humid conditions has been previously shown for fenitrothion in water [8] and sediments [5] and for other organophosphorus pesticides [6, 7]. The environmental problems of this specific area of Paty do Alferes are the fungicides, although the amounts used of organophosphorus pesticides are in the same order of fungicides (around 10–20 tones per year of each analyte).

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