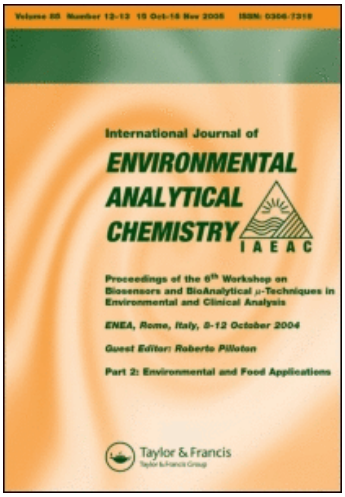

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A SIMPLE METHOD FOR SCREENING OF SOIL SAMPLES FOR ORGANOCHLORINE INSECTICIDE RESIDUES BY THIN-LAYER CHROMATOGRAPHY

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A simple, rapid and sensitive screening procedure requiring no costly equipment is described for the estimation of chlorinated hydrocarbon insecticide residues in soil samples by semi-quantitative TLC. Three published insecticide residue extraction procedures were evaluated for the recovery of spiked as well as field-acquired and naturally weathered residues from soil samples. Extraction of soils with methanol–water (2:1, v/v) and partitioning of extracts with *n*-hexane, followed by TLC on alumina *G* plates gave minimum interference of co-extractives and complete recovery of the residues. The average recoveries of dieldrin and *p,p'*-DDT as determined by GC for the validation of the method were 93 and 110%, respectively. Additional column chromatographic clean-up on alumina neutral was necessary for soil samples rich in organic matter. Insecticide residues could be estimated down to 0.02 $\mu g\,g^{-1}$; the procedure has been used for the determination of organochlorine residues in more than 100 soil samples.

KEY WORDS: Insecticide residues, screening method, soils, thin-layer chromatography, TLC.

INTRODUCTION

Due to an increasing awareness about the significance of the presence of small amounts of foreign chemical species in our surroundings, the contamination of the human environment with residues of persistent organochlorine compounds has received attention all over the world and stimulated research to develop and refine analytical techniques for the estimation of such residues.[1–4] Soils form an environmental reservoir which is continuously contaminated by usage of insecticides for agricultural purposes. As insecticide residues in soil can move into the atmosphere, water or living beings and give rise to several adverse effects, monitoring of this component of the terrestrial environment has been repeatedly stressed.[5–8]

Most of the insecticide residue estimation procedures currently employed are quite elaborate and require expensive instruments.[9–13] Coupled with the requirements of high purity reagents, adequately trained manpower and expert instrument maintenance, it is difficult to use such methods in remote areas or developing countries.[13] Moreover, high sensitivity provided by sophisticated methods of residue analysis is not required in all situations and the need for simple and inexpensive screening procedures has been emphasized by several workers.[13,14] The use of affordable and rapid screening methods can help not only in analyzing relatively large numbers of samples at laboratories with limited facilities and

manpower to indicate dimensions of the problem of environmental pollution in a region, but also aid in recognizing samples containing residues that are high enough to require further, more accurate quantitation.[13–16]

Because of its speed, simplicity and relatively low cost, thin-layer chromatography (TLC) has been suggested as an attractive alternative over other methods of quantitation of insecticide residues.[13,15–17] The present communication evaluates three methods of insecticide residue extraction and application of TLC as a rapid screening method for the determination of residues of insecticides in soil extracts without elaborate clean-up. The results obtained are validated by comparison of recovery values estimated by TLC with those determined by the analysis of sample extracts by gas chromatography (GC).

EXPERIMENTAL

Instruments

In addition to common laboratory apparatus, the following equipment was used in the analysis.

Gas chromatographic system Packard Model 7624 equipped with a tritium-source electron capture detector and a 165 cm × 2 mm i.d. glass column packed with 5% OV-210 on 80–100 mesh Gas Chrom Q. The operating conditions were: injector, 205 °C; column, 195 °C; detector, 210 °C; outlet, 220 °C; carrier gas (nitrogen) flow-rate, 100 ml/min.

Thin-layer chromatographic equipment 20 × 20 cm glass plates, slurry applicator, TLC drawing board (Perfit); developing tanks (Kontes) and 120-W UV lamp without filter (Toshniwal Instruments).

Reagents

Solvents Acetone, benzene, *n*-hexane and methanol were used as purchased or after distillation in all-glass apparatus.

Other chemicals Alumina G, alumina neutral, ammonium chloride, anhydrous sodium sulphate and silver nitrate.

The suitability of all the reagents for residue analysis work was ensured by running reagent blanks.

Insecticide reference standards Analytical-grade technical insecticides obtained from Applied Science Laboratories (PA, USA) were used as reference standards.

Residue Analysis

Preparation of the sample Soil samples were thoroughly mixed and stones and plant materials were removed. Two 50 g portions were drawn, one of which was used for the estimation of insecticide residues while the other was employed for determination of the moisture content.

Optimization of the extraction procedure The following three methods, based on previously published procedures, were compared for their extraction efficiency for spiked as well as field-acquired and naturally weathered insecticide residues from soil samples.

Method 1 A 50 g soil sample, premoistened for 15 min with 70 ml of 0.2 M ammonium chloride in a 250 ml Erlenmeyer flask, was swirled for about 1 min with 100 ml of *n*-hexane–acetone (1:1, v/v) and allowed to stand for 24 h. Next, the extract was filtered through a Whatman No. 1 filter paper into a 500 ml separatory funnel and washed with 100 and 50 ml portions of distilled water. The *n*-hexane layer was dried over anhydrous sodium sulphate and concentrated to about 1 ml[18] (by rotary vacuum evaporation to about 10 ml and next, by evaporation under a gentle stream of nitrogen).

Method 2 A 50 g soil sample, premoistened for 15 min with 70 ml of 0.2 M ammonium chloride in a 250 ml Erlenmeyer flask, was swirled for 1 min with 100 ml of benzene–acetone (1:1, v/v) and allowed to stand for 24 h. Next, the extract was filtered through a Whatman No. 1 filter paper into a 500 ml separatory funnel and washed with 100 and 50 ml portions of distilled water. The benzene layer was dried over anhydrous sodium sulphate and concentrated (cf. above) to 1 ml.[19]

Method 3 A 50 g soil sample was taken in a 250 ml Erlenmeyer flask and 100 ml of methanol–water (2:1, v/v) was added. The flask was swirled for 1 min and allowed to stand for 24 h. Next, the extract was filtered through a Whatman No. 1 filter paper into a 500 ml separatory funnel and partitioned with 100 and 50 ml portions of *n*-hexane. The combined *n*-hexane layers were dried over anhydrous sodium sulphate and concentrated (cf. above) to 1 ml.[19,20]

TLC screening of soil samples The soil samples were screened for organochlorine insecticide residues by following a TLC procedure based on the technique described by Abbott *et al.*[21] TLC plates of 0.25 mm thickness were prepared from a slurry of alumina G in 0.2% (w/v) aqueous silver nitrate. The coated plates were air-dried and then activated at 110 °C for 45 min. On plates cooled to room temperature, aliquots of soil extracts equivalent to a 10 g soil sample were co-chromatographed with standards of organochlorine compounds. The plates were developed to about 10 cm with *n*-hexane, removed from the developing tank, air-dried and irradiated with UV light for 20 min when insecticides appeared as black

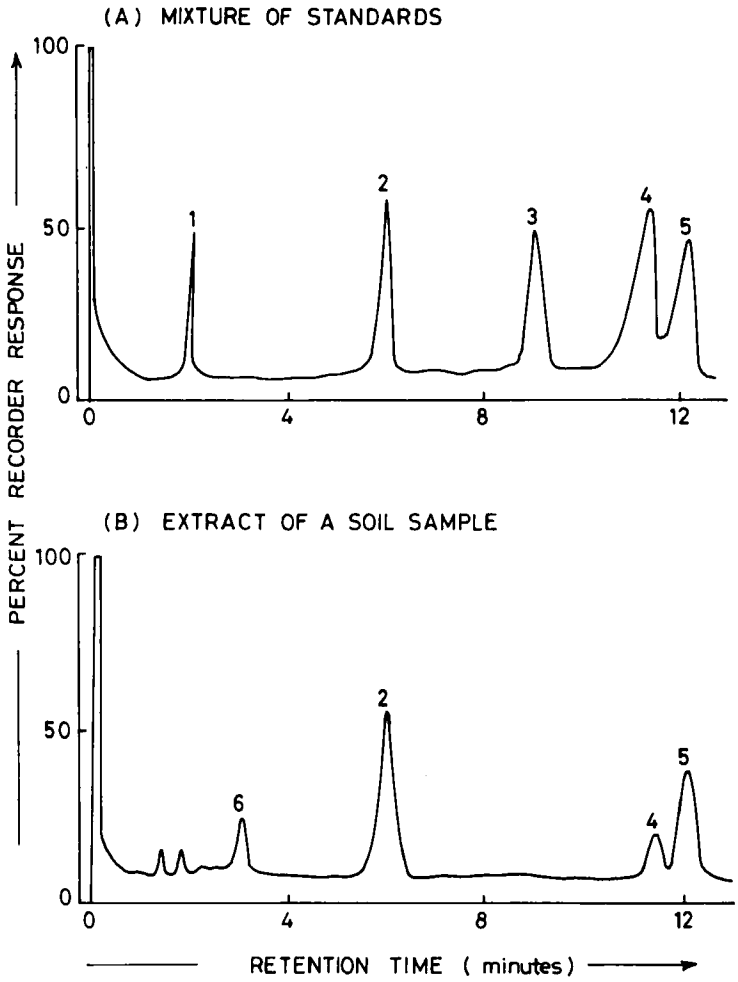

Figure 1 GC-ECD of (A) mixture of standards and (B) extract of a soil sample. 1, Lindane; 2, *p,p'*-DDE; 3, Dieldrin; 4, *p,p'*-DDD; 5, *p,p'*-DDT; 6, Unidentified peak.

spots on a white background. On the basis of comparison of R_f values, size and intensity of the spots for sample extracts with those of standards, a semi-quantitative estimation of the amount of the detected residues was made.

The TLC analyses were carried out in duplicate.

GC analysis Suitable aliquots of soil extracts were injected into the GC so as to obtain satisfactory peak heights (Fig. 1). The compounds were identified and quantitated by comparing the retention times and peak heights in the sample chromatograms with those of the insecticide reference standards run under identical operating conditions.

Column chromatographic clean-up Further clean-up of the soil extracts was generally not required. However, certain samples containing high organic matter

produced interferences during TLC or GC, and a column chromatographic clean-up was standardized to remove the co-extractives in such cases. Alumina neutral was activated at 110 °C for 1 h, equilibrated for 24 h with 5% water in a stoppered flask and a 20 g portion was packed in a glass column (40 cm × 2 cm i.d.) between 1-cm layers of anhydrous sodium sulphate. The column was prewashed with 50 ml of *n*-hexane, and a soil sample extract concentrated to about 5 ml was added to it. The column was then eluted with 100 ml of *n*-hexane and 50 ml of *n*-hexane–acetone (4:1, v/v). The fractions thus obtained were then subjected to TLC or GC analysis as described earlier.

Recovery studies In order to judge the efficiency of the analytical procedures, recovery experiments were performed by spiking soil samples with *p,p'*-DDT and dieldrin at the level of 0.05 $\mu g\, g^{-1}$ each. The samples were extracted and cleaned-up by the methods described above. Unfortified samples were also processed in a similar manner to find out levels of background contamination of the soils used for recovery studies. For calculating recovery values, residue levels observed for unfortified samples were subtracted from the levels obtained for fortified samples.

Confirmatory procedure In addition to 10% of the total number of samples analysed, all sample extracts found to have an unusual pattern of insecticide residues or exceptionally high residue levels when estimated by TLC were subjected to GC determination to confirm the findings.

Expression of Results

50 g undried soil sample was taken in a pre-weighed petri plate and kept in an oven at 110 °C. The sample was removed from the oven after 24 h, allowed to cool to room temperature and the per cent weight loss after oven drying was taken as the moisture content of the soil. The insecticide residue levels in a soil sample determined by TLC or GC were subjected to a correction based on the moisture content of the sample and were expressed on a dry weight basis.

RESULTS AND DISCUSSION

In this study, benzene–acetone (1:1, v/v) was found to be the least efficient extraction solvent system as it gave low recoveries of incurred as well as spiked residues in comparison with the other two solvent mixtures (Tables 1 and 2). Good and identical recoveries were obtained with *n*-hexane–acetone (1:1, v/v) and methanol–water (2:1, v/v). However, as extraction of soil samples with *n*-hexane–acetone (1:1, v/v) gave higher amounts of co-extractives which resulted in a dark background during TLC studies, only methanol–water (2:1, v/v) was used for further studies. The estimation of amounts of insecticide residues by visual comparison of the R_f values, size and intensity of the spots from sample extracts

Table 1 Comparison of recovery of incurred chlorinated hydrocarbon residues by three extraction procedures

Sample no.	*Extraction solvent*	*Residue level* ($\mu g\,g^{-1}$)		
		p-p'-DDE	*p,p'-DDD*	*p,p'-DDT*
1	*n*-Hexane–acetone (1:1, v/v)	0.05	0.05	0.05
	Benzene–acetone (1:1, v/v)	0.05	0.04	0.04
	Methanol–water (2:1, v/v)	0.05	0.05	0.05
2	*n*-Hexane–acetone (1:1, v/v)	ND	ND	0.02
	Benzene–acetone (1:1, v/v)	ND	ND	ND
	Methanol–water (2:1, v/v)	ND	ND	0.02

ND = Not detectable (less than 0.02 $\mu g\,g^{-1}$).

Table 2 Recovery of insecticide residues from soil samples fortified with *p,p'*-DDT and dieldrin at a level of 0.05 $\mu g\,g^{-1}$ each and extracted with different solvent mixtures

Sample no.	*Extraction solvent*		*Residue level* ($\mu g\,g^{-1}$)	
			p,p'-DDT	*Dieldrin*
TLC determination				
1	*n*-Hexane–acetone (1:1, v/v)	Unfortified	0.02	ND
		Fortified	0.07	0.05
	Benzene–acetone (1:1, v/v)	Unfortified	0.02	ND
		Fortified	0.07	0.04
	Methanol–water (2:1, v/v)	Unfortified	0.02	ND
		Fortified	0.07	0.05
2	*n*-Hexane–acetone (1:1, v/v)	Unfortified	0.05	ND
		Fortified	0.10	0.05
	Benzene–acetone (1:1, v/v)	Unfortified	0.04	ND
		Fortified	0.09	0.05
	Methanol–water (2:1, v/v)	Unfortified	0.05	ND
		Fortified	0.10	0.05
GC determination				
1	Methanol–water (2:1, v/v)	Unfortified	ND	ND
		Fortified	0.055	0.049
2	Methanol–water (2:1, v/v)	Unfortified	ND	ND
		Fortified	0.055	0.044

ND = Not detectable (less than 0.02 $\mu g\,g^{-1}$ for TLC and 0.01 $\mu g\,g^{-1}$ for GC).

with the spots of standards as followed in this study has been considered to be a convenient approach for the semi-quantitative determination of residue levels by other workers also.[13,15] Sherma[15] noted that errors of 15–50% are typical with such estimations, but this degree of accuracy is sufficiently adequate for a rapid and low-cost screening procedure to find out samples that contain residue amounts high enough for further, more accurate quantitation.

Table 3 R_f values, sensitivities and detection limits of some organochlorine compounds

Compound	*R_f value (solvent system n-hexane)*	*Sensitivity* (μg)	*Limit of detection* ($\mu g\,g^{-1}$)
p,p'-DDE	0.95±0.02	0.2	0.02
Heptachlor	0.89±0.03	0.1	0.01
p,p'-DDT	0.85±0.03	0.2	0.02
α-HCH	0.61±0.05	1.0	0.10
γ-HCH (lindane)	0.51±0.04	1.2	0.12
p,p'-DDD	0.42±0.05	0.2	0.02
Heptachlor epoxide	0.23±0.01	0.4	0.04
Dieldrin	0.19±0.01	0.2	0.02
Dicofol	0.06±0.01	0.1	0.01

As the detection limits of various organochlorine residues by this technique ranged from 0.1 to 1 μg, and soil extracts equivalent to 10 g samples could be spotted without affecting the resolution or darkening of the TLC background, the limits of determination for the various compounds ranged from 0.01 to 0.1 $\mu g\,g^{-1}$ (Table 3).

The basic requirement for the use of simplified insecticide residue analysis procedure is that the results acquired are comparable to those obtained by GC.[13] So the TLC screening methodology was validated by measuring and confirming residues in selected samples by GC. The studies by fortifying soil samples with *p,p'*-DDT and dieldrin revealed that the average recoveries for these compounds were 110 and 93%, respectively (Table 2).

Several workers have observed that as extraction of insecticide residues tends to be poor from very dry soils, wetting of the soil samples improves the recovery.[22–25] Woolson[25] and Horwitz[26] have recommended premoistening of air-dry soils with aqueous ammonium chloride before extraction to get maximum recovery of insecticide residues and this procedure was followed while extracting soil samples with *n*-hexane–acetone (1:1, v/v) or benzene–acetone (1:1, v/v). However, water in aqueous methanol was found to adequately desorb insecticide residues from soil samples and this resulted in recoveries equivalent to or greater than with the above-mentioned solvent systems. So methanol–water (2:1, v/v) was considered suitable in the regular screening of soil samples without any extra step for the pre-moistening of the soil samples.

Mostly the determination of insecticide residues in soil extracts was carried out without column chromatographic clean-up. This was possible because the organic content of soils is usually low in comparison with plant samples. Such analysis without clean-up was considered advantageous because clean-up procedures are usually time consuming and may result in the loss of extracted residues.[26,27] However, additional column chromatographic clean-up using alumina neutral as adsorbent was occasionally found necessary when interferences resulting in low sensitivity and altered R_f values were observed during TLC. This was particularly true when the organic content of soil samples was high. A similar effect of

coextractives on the determination of insecticide residues in muck soils and the need for additional clean-up of extracts from high-organic matter soil samples has also been reported by other workers.[18,25,28]

In conclusion, the proposed TLC technique is a simple, rapid and sensitive procedure for the screening of soil samples for the presence of organochlorine insecticide residues and has been satisfactorily used for the determination of organochlorine residues in more than 100 soil samples.

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