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## **Validation of a Spectrofluorimetric Method for the Determination of Thiram and Thiophanate Methyl Fungicides in Environmental Samples**

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The intensive use of thiram and thiophanate methyl fungicides in agriculture leaves residues on crops and contaminates groundwater and surface water bodies through leaching. For the purpose of monitoring pollution arising from their presence, a spectrofluorimetric method has been validated for screening these fungicides in environmental samples *viz.* water, soil and foodstuffs. The measurement of fluorescence intensity of cerium(III) species at 365 nm resulting from ceric ammonium nitrate oxidation of dimethyl dithiocarbamate and *o*-phenylene bis-thiourea (products of the reaction of thiram and thiophanate methyl, respectively, with potassium *tert*-butoxide) formed the basis of the method. As little as 0.18 and 0.22  $\mu\text{g ml}^{-1}$  of thiram and thiophanate methyl can be determined. A simple and rapid solid-phase extraction and purification procedure, prior to the spectrofluorimetric determination, show high recoveries of these fungicides from spiked water and grain samples in the range 86.0-98.0% and 88.5-97.5% with a maximum RSD of 2.60% indicating a good accuracy and precision of the method. The risk of water bodies' contamination by the two, groundwater ubiquity score (GUS), has also been evaluated using adsorption study on four soils; the values in the range -0.48 to 0.98 classify them as non-leacher fungicides.

**Keywords:** Thiram, Thiophanate methyl, Spectrofluorimetric determination, Environmental samples

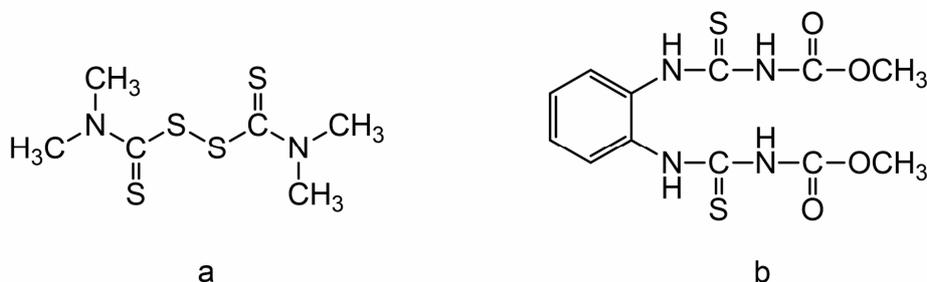
### **INTRODUCTION**

Thiram (tetramethylthiuramdisulfide) (Fig. 1a) and thiophanate methyl (1,2-bis (3-methoxy carbonyl-2-thioureido) benzene) (Fig. 1b) are wide-spectrum fungicides finding extensive use to control many fungal diseases of fruits, vegetables, field and plantation crops [1-2]. Their excessive use leaves residues on agricultural products and their ability to permeate the soil surface through leaching leads to the contamination of water bodies. The consumption of contaminated food and drinking water is of serious concern to human health. Hence, the judicious use of these fungicides is required to control their concentration in soil, water and agricultural samples for the purpose of monitoring pollution and health hazards. This necessitates the development and validation of simple and rapid methods for the analysis of the thiram and thiophanate methyl

fungicides.

The determination of these fungicides has been reported by gas chromatography [3-5], high performance liquid chromatography (HPLC) [2,6-8], liquid chromatography mass spectrometry (LCMS) [9-11], surface-enhanced Raman spectroscopy (SERS) [12-14], spectrophotometry [15-19], chemiluminescence [20-22] and capillary electrophoresis [23-24]. Despite the precision and accuracy of chromatographic and capillary electrophoresis methods, these are time-consuming, expensive, requiring high analytical skill thus limiting their wide applicability. Methods based on spectrophotometry and spectrofluorimetry find wide acceptance due to their simplicity and relatively low cost and allowing analysis in less time. Spectrofluorimetric analysis being highly sensitive has an edge over the spectrophotometric analysis, but very less work has been done in developing spectrofluorimetric methods in pesticide analysis [25-27]. This prompted us to apply the advantages

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**Fig. 1.** Chemical structure of a: thiram, b: thiophanate methyl.

of spectrofluorimetry in analysis of thiram and thiophanate methyl. The quantitative cleavage of disulphide linkage of thiram and amide linkage of thiophanate methyl with potassium *tert.*-butoxide to dimethyl dithiocarbamate and *o*-phenylene bis-thiourea, respectively, and their oxidation with ceric ammonium nitrate in acidic medium forming fluorescent cerium(III) with emission at 365 nm and linear relationship of fluorescence intensity with concentration of each fungicide are the bases of the proposed method. The method has been successfully validated for the determination of the above fungicides in commercial formulations, spiked water samples and agricultural products and also for their adsorption study on four soils to evaluate the leachability and subsequent risk of contamination of surface and ground water. Formulation analysis is essential to get reliable residue data.

## EXPERIMENTAL

### Instruments

Perkin Elmer LS 55 Fluorescence Spectrometer (Perkin Elmer, United Kingdom) with 1 cm matched quartz cells was used for fluorescence intensity measurements. All spectral measurements were processed by FL-WinLab software. Incubator Shaker PT-422 (Popular Traders, Ambala Cantt.) was used for equilibration of fungicides in soil solutions during adsorption study. Sigma 3-30KS centrifuge was used for the centrifugation of the soil samples.

### Materials and Reagents

The analytical standards of thiram (Fluka, Switzerland) and thiophanate methyl (Sigma-Aldrich Munich, Germany)

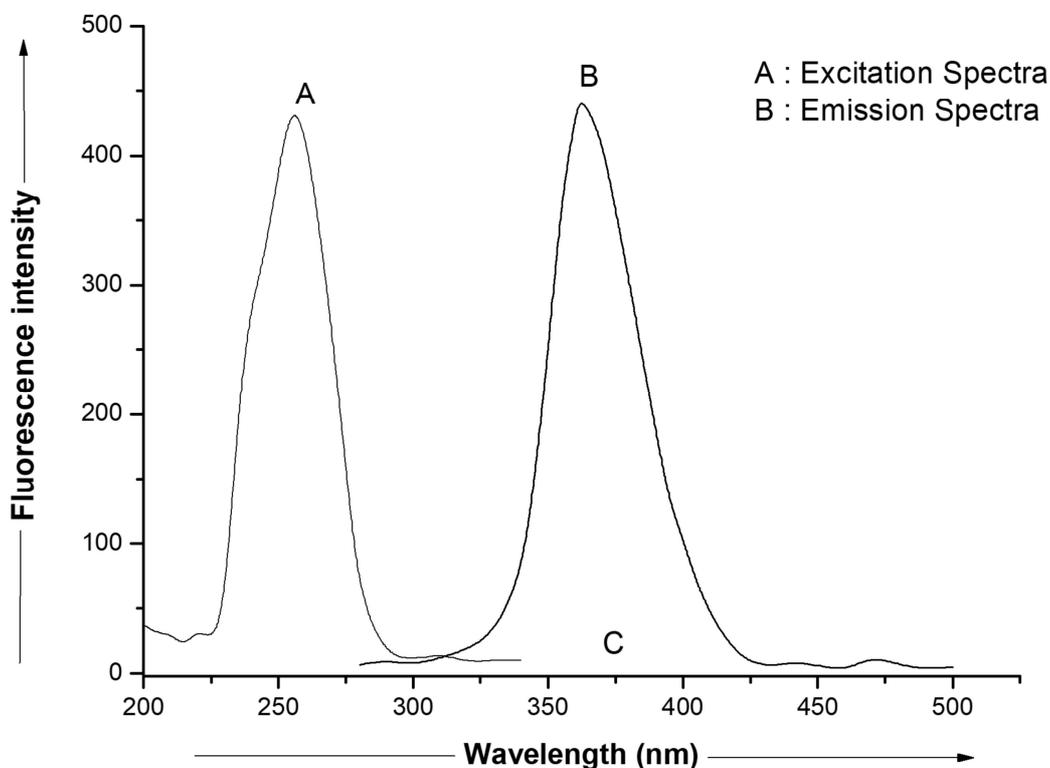
fungicides were used. The purity of each compound was checked by a known method [28]. Acetonitrile (HPLC grade, Merck) was used. Potassium *tert.*-butoxide (Lobachemie), 0.02 M, was prepared by dissolving 0.056 g of pure compound in 25 ml *tert.*-butanol. Perchloric acid (Merck, AR), ~1.0 M, was prepared in distilled water. Ceric ammonium nitrate (Merck, AR), 0.01 M, in sulfuric acid was prepared by dissolving a little more than the calculated amount of the oxidant in 1.0 M sulfuric acid.

### Preparation of Standard Solutions

Accurately weighed 5 mg of each of thiram and thiophanate methyl was dissolved in 10 ml of acetonitrile. The 5 ml of this solution was further diluted to 100 ml to obtain a working standard solution of 25  $\mu\text{g ml}^{-1}$ .

### Procedures

**Preparation of calibration graph for each pure compound.** Aliquots of 0.1-2.0 ml of thiram and 0.1-2.5 ml of thiophanate methyl of the standard solution in acetonitrile were taken separately in 10 ml measuring flasks and volume made to 2 ml with acetonitrile. Each solution was mixed with 0.25 ml of 0.02 M solutions of potassium *tert.*-butoxide and heated in microwave for 60 s. Each solution was then mixed with 0.5 ml of 0.002 M ceric ammonium nitrate solutions and kept at room temperature (~25 °C) for 5 min and finally made to 10 ml with 1.0 M perchloric acid solution. The emission intensity measured at 365 nm with an excitation at 255 nm in each case. A typical excitation-emission spectrum due to cerium(III) observed with both fungicides is shown in Fig. 2. The calibration curve was prepared in each case by plotting fluorescence intensity



**Fig. 2.** Fluorescence spectra of Ce(III) produced during the reaction.

values against the concentration of the fungicide and is shown in Fig. 3.

#### **Assay of Thiram and Thiophanate Methyl in Commercial Formulations**

The commercial formulations of thiram, Thirid-75 WP containing 75% active ingredient, and thiophanate methyl, Topsin-M WP containing 70% active ingredient, were used. A single large sample of each formulation, equivalent to 50 mg of active ingredient, was dissolved in acetonitrile, filtered and volume made to 100 ml with the same solvent. 5 ml of this solution was further diluted to 100 ml with acetonitrile to obtain a solution with a working concentration of  $25 \mu\text{g ml}^{-1}$ . Suitable aliquots of the above solution were taken and processed for the analysis in the same manner as described above for pure compounds.

#### **Assay of Thiram and Thiophanate Methyl in Spiked Water and in Agricultural Samples**

Aliquots of standard solutions of each fungicide were

added to 25 ml tap water samples and 5 g of grains (wheat and rice). The samples were extracted with 2 instalments of 5 ml chloroform and the extracts were purified through the silica column extractor at a flow rate of  $0.7 \text{ ml min}^{-1}$  [29]. The eluate was collected and dried with nitrogen gas dryer and the remainder was dissolved in 2 ml acetonitrile and analysed in the same manner as discussed for the pure compounds.

#### **Procedure for Soil Adsorption Study: Evaluation of Leaching Potential**

Adsorption studies were conducted on four Indian soils by the batch equilibrium method. Bulk samples of soils were air dried and passed through 40 mesh sieves to remove stones and large particles. The soil characteristics viz. pH, organic carbon and cation exchange capacity were determined by a reported method [30] and are summarized in Table 1.

Adsorption isotherms of each fungicide on four soil types of different soil characteristics were obtained at

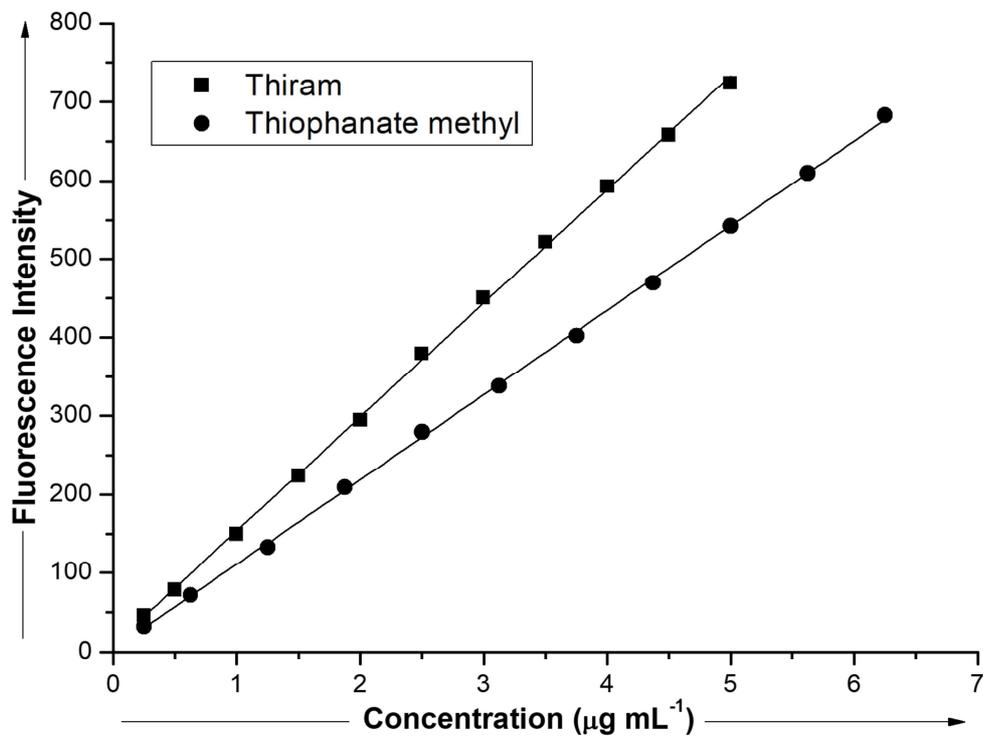


Fig. 3. Relationship between fluorescence intensity and concentration of fungicides.

Table 1. Characteristics of the Different Indian Soils Used in the Adsorption Study of Fungicides

Soil Sample	pH	Clay (%)	Organic carbon (%)	Cation exchange capacity (meq/100 g)
I	7.44	32.6	0.6	13.1
II	7.38	9.3	0.7	12.2
III	7.41	18.2	1.0	12.9
IV	7.48	10.2	0.8	12.8

25 ± 1 °C. Soil samples (2 g) were equilibrated with 2 ml acetonitrile solution of the fungicide in the concentration range from 4.8-43.3 µg ml<sup>-1</sup> and 6.8-61.6 µg ml<sup>-1</sup> of thiram and thiophanate methyl respectively and 8 ml distilled water in 50 ml conical flask. The contents of each soil sample were agitated by stirring in the incubator shaker over night for equilibrium to be reached between fungicide adsorbed

by soil and in solution. After 24 h, each sample was centrifuged and the supernatant was extracted with 10 ml of chloroform. The extracts were purified through the silica column extractor at a flow rate of 0.7 ml min<sup>-1</sup>. The eluate was collected and dried with nitrogen gas dryer and the remainder was dissolved in 10 ml acetonitrile. The equilibrium concentrations (C<sub>e</sub>) were determined in each

case by taking 1 ml of above acetonitrile solution for analysis and analysed in the same manner as discussed for pure compounds. The study has been carried out in triplicate to minimize the error. The adsorption parameters were evaluated using Freundlich and Langmuir adsorption models (Table 2).

In Freundlich's adsorption equation (Table 2, Eq. (1)),  $X$  is the amount of fungicide adsorbed ( $\text{mg Kg}^{-1}$ ) on the adsorbent and  $C_e$  is the equilibrium solution concentration ( $\text{mg l}^{-1}$ ). The values of adsorption coefficients  $K_f$  and  $n_f$  were calculated from the intercept and the slope of the straight line of the linearized form of the Freundlich isotherm (Table 2, Eq. (2)). In the case of Langmuir isotherm (Table 2, Eq. (3)), constant  $k$  is related to the free energy of adsorption and  $b$  reflects the equilibrium constant for the adsorption process and is an indication of the affinity of the adsorbent for fungicides. The values of  $k$  and  $b$  were calculated from the intercept and the slope of the straight line of the linearized form of the Langmuir isotherm. The various parameters of these models for each fungicide adsorption on to soil samples along with the values of the coefficient of determination ( $r^2$ ) were evaluated and are given in Table 3.

## RESULTS AND DISCUSSION

### Optimization of Reaction Parameters

The proposed method is based on the measurement of fluorescence intensity of cerium(III) from oxidation of each fungicide. It is pertinent to mention here that the target fungicides as such undergo oxidation with ceric ammonium nitrate but reaction is slow; thiram being the oxidised product of dimethyl dithiocarbamate and thiophanate methyl is an ester of *bis*-phenylene thiourea and consequently reproducible results are not obtained. Through reaction with potassium *tert*-butoxide, thiram is reduced to a dithiocarbamate [31] and thiophanate methyl is cleaved into simple phenylene thiourea [32] thus not only speeds up the oxidation reaction but also increases sensitivity of the method. The excess of potassium *tert*-butoxide does not interfere with the reaction.

The effects of various experimental parameters on the stability and sensitivity of the fluorescence intensity, *vis-a-vis* development of the proposed method, have been studied

before applying it to the real analysis of these fungicides; *i.e.*, in commercial formulation, water and agricultural samples and adsorption study on soils. The time required in a microwave oven for the cleavage of disulphide and amide linkage with a view to obtain maximum fluorescence intensity was optimised as 60 s of heating. The effects of the concentration of potassium *tert*-butoxide and ceric ammonium nitrate on the fluorescence intensity were studied by varying concentration from 0.01-0.04 M of potassium *tert*-butoxide and from 0.001-0.01 M of ceric ammonium nitrate. It was observed that 0.25 ml of 0.02 M solution of potassium *tert*-butoxide was sufficient for the cleavage of disulphide (thiram) and amide (thiophanate methyl) linkage and the addition of 0.5 ml of 0.002 M ceric ammonium nitrate solution oxidised these cleaved products completely and consequently produced maximum fluorescence intensity. Among various acids *viz.* perchloric acid, hydrochloric acid, sulfuric acid and nitric acid were tested as the medium of above oxidation; perchloric acid with 1 M gave maximum fluorescence intensity. The fluorescence intensity has been found to be stable for at least 120 min with each fungicide.

### Validation of Proposed Method

The proposed spectrofluorimetric method was validated with the evaluation of various analytical parameters *viz.* linearity range, limit of detection, limit of quantification, accuracy and precision. Under the optimized experimental conditions, the relationship between concentration of each fungicide and the corresponding fluorescence intensity has been found to be linear in the range of 0.25-5.00 and 0.25-6.50  $\mu\text{g ml}^{-1}$  of thiram and thiophanate methyl solution, respectively. The linearity of calibration graphs was proved by the high values of correlation coefficients ( $r^2 = 0.999$ ). The various calibration characteristics and statistical data were calculated by the regression equation " $Y = mX + C$ " (where  $Y$  = fluorescence intensity,  $m$  = slope,  $C$  = intercept and  $X$  = concentration in  $\mu\text{g ml}^{-1}$ ) and are summarised in Table 4. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined using the equations:  $\text{LOD} = 3.3 \sigma/s$  and  $\text{LOQ} = 10 \sigma/s$  [33]; where  $\sigma$  is the standard deviation of the intercept of regression line and  $s$  is the slope of calibration curve and were found to be  $0.06 \mu\text{g ml}^{-1}$  and  $0.18 \mu\text{g ml}^{-1}$

**Table 2.** The Adsorption Isotherm Models Used in the Present Study

Isotherm	Formula and definition
Freundlich	$X = K_f \times C_e^{n_f} \quad (1)$ $K_f = \text{Freundlich isotherm coefficient (l Kg}^{-1}\text{)}$ $N_f = \text{degree of freedom}$
Linearized form of Freundlich isotherm	$\log X = \log K_f + n_f \log C_e \quad (2)$
Langmuir	$\frac{C_e}{X} = \frac{1}{kb} + \frac{C_e}{b} \quad (3)$ $k = \text{Langmuir constant (l mg}^{-1}\text{)}$ $b = \text{maximum adsorption capacity (}\mu\text{g g}^{-1}\text{)}$

**Table 3.** Adsorption Constants and Coefficient of Determination ( $r^2$ ) of Thiram and Thiophanate Methyl on Four Indian Soils Based on the Freundlich and Langmuir Equations

Thiram	Soil	Freundlich			Langmuir		
		$K_f$	$n_f$	$r^2$	k	b	$r^2$
Thiram	I	5.71	0.79	0.91	0.25	18.89	0.64
	II	5.90	0.84	0.99	0.15	33.36	0.77
	III	10.58	0.73	0.93	0.44	20.07	0.91
	IV	8.51	0.75	0.92	0.24	18.89	0.76
Thiophanate methyl	I	4.41	0.96	0.96	0.27	15.08	0.45
	II	4.99	0.98	0.92	0.29	16.36	0.79
	III	12.57	0.77	0.96	0.20	46.34	0.62
	IV	7.63	0.87	0.93	0.13	52.54	0.53

for thiram and  $0.07 \mu\text{g ml}^{-1}$  and  $0.22 \mu\text{g ml}^{-1}$  for thiophanate methyl, respectively. The accuracy and precision of the proposed method were evaluated by performing five replicate analyses of pure fungicide solutions at five different concentration levels over the ranges  $0.5\text{-}4.5 \mu\text{g ml}^{-1}$  and  $0.5\text{-}6.0 \mu\text{g ml}^{-1}$  for thiram and thiophanate methyl, respectively (Table 5). The low values of the

relative standard deviation (1.80%) for both fungicides show good accuracy and precision of the method. The effect of several inorganic ions including  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{OAc}^-$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{Cl}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$  (commonly present in water and soil samples) on the fluorescence intensity was investigated for possible interference on the determination of thiram and thiophanate

**Table 4.** Calibration Characteristics and Statistical Data of Regression Equation

Characteristics	Thiram	Thiophanate methyl
$\lambda_{\text{excitation}}$ (nm)	255	255
$\lambda_{\text{emission}}$ (nm)	365	365
Linearity range ( $\mu\text{g ml}^{-1}$ )	0.25-5.00	0.25-6.25
Slope	135.55	107.89
Intercept	0.7104	3.1249
Correlation coefficient ( $r^2$ )	0.9996	0.9995
Limit of detection (LOD) ( $\mu\text{g ml}^{-1}$ )	0.06	0.07
Limit of quantification (LOQ) ( $\mu\text{g ml}^{-1}$ )	0.18	0.22

LOD and LOQ are calculated by known method [33].

**Table 5.** Assay Results of the Determination of Thiram and Thiophanate Methyl in Pure Compounds

Fungicides	Amount taken ( $\mu\text{g ml}^{-1}$ )	Amount found <sup>a</sup> $\pm$ SD ( $\mu\text{g ml}^{-1}$ )	RSD (%)
Thiram	0.5	0.49 $\pm$ 0.009	1.80
	1.5	1.48 $\pm$ 0.019	1.27
	2.5	2.49 $\pm$ 0.021	0.84
	3.5	3.52 $\pm$ 0.032	0.91
	4.5	4.47 $\pm$ 0.039	0.87
Thiophanate methyl	0.5	0.51 $\pm$ 0.009	1.80
	1.5	1.49 $\pm$ 0.021	1.40
	3.0	2.96 $\pm$ 0.037	1.23
	4.5	4.43 $\pm$ 0.064	1.42
	6.0	5.95 $\pm$ 0.047	0.78

<sup>a</sup>Values are expressed as mean  $\pm$  SD (n = 5).

methyl from water and soil samples to assess the validity of the proposed method and the method was found to be free from interferences due to these ions.

#### Assay of Thiram and Thiophanate Methyl in Commercial Formulations

The method has successfully been applied for the

**Table 6.** Assay Results of Thiram and Thiophanate Methyl from Commercial Formulations

Fungicides	Formulation	Maker's specification <sup>b</sup>	Amount taken ( $\mu\text{g ml}^{-1}$ )	Amount found <sup>a</sup> $\pm$ SD ( $\mu\text{g ml}^{-1}$ )	Recovery (%)	RSD (%)
Thiram	Thirid-75	75% Wettable powder	0.5	0.49 $\pm$ 0.010	98.00	2.00
			1.5	1.47 $\pm$ 0.033	98.67	2.20
			2.5	2.44 $\pm$ 0.053	97.60	2.12
			3.5	3.48 $\pm$ 0.044	99.43	1.26
			4.5	4.46 $\pm$ 0.041	99.11	0.89
Thiophanate methyl	Topsin-M	70% Wettable powder	0.5	0.49 $\pm$ 0.012	98.00	2.40
			1.5	1.48 $\pm$ 0.031	98.67	2.07
			3.0	2.94 $\pm$ 0.059	98.00	1.97
			4.5	4.49 $\pm$ 0.043	99.78	0.96
			6.0	5.96 $\pm$ 0.050	99.33	0.83

<sup>a</sup>Values are expressed as mean  $\pm$  SD (n = 5). <sup>b</sup>Maker's specification established separately by independent methods [16-17].

**Table 7.** Assay Results of Thiram and Thiophanate Methyl from Spiked Water Samples

Fungicides	Amount taken ( $\mu\text{g ml}^{-1}$ )	Amount found <sup>a</sup> $\pm$ SD ( $\mu\text{g ml}^{-1}$ )	Recovery (%)	RSD (%)
Thiram	0.5	0.47 $\pm$ 0.013	94.00	2.60
	1.0	0.93 $\pm$ 0.022	93.00	2.20
	2.0	1.89 $\pm$ 0.028	94.50	1.40
	3.0	2.94 $\pm$ 0.034	98.00	1.13
	4.0	3.81 $\pm$ 0.046	95.23	1.15
Thiophanate methyl	1.0	0.97 $\pm$ 0.023	97.00	2.30
	2.0	1.89 $\pm$ 0.041	94.50	2.05
	3.0	2.89 $\pm$ 0.037	96.33	1.23
	4.0	3.90 $\pm$ 0.055	97.50	1.37
	5.0	4.84 $\pm$ 0.064	96.80	1.28

<sup>a</sup>Values are expressed as mean  $\pm$  SD (n = 5).

determination of listed fungicides in their commercial formulations. The formulation analysis is essential not only to ensure the quality of the marketed samples of the fungicide but also to get reliable adsorption data. The recoveries of the fungicides were in the range 97.60-99.43% and 98.00-99.78% of the nominal content with relative standard deviations (RSD) in the range 0.89-2.20% and 0.83-2.40% for thiram and thiophanate methyl, respectively (Table 6). The maker's specification has also been established by the independent methods [16-17].

### Validation of Method for Residue Analysis and Soil Adsorption Study

A cost-effective solid phase extraction (SPE) (Silica based, 60-120 mesh, 1.2 cm i.d. homogeneous glass column) with 10 ml chloroform elution at room temperature has been used for further purification of the extract of each fungicide from water, agricultural and soil samples. Glass column of 15 cm length and 1.2 cm diameter was filled with silica slurry (prepared by dissolving 8 g silica in 15 ml of chloroform) slowly so that silica spreads homogeneously in the column [29]. The experimental residue and adsorption data were subjected to the analysis of procedural blanks and spiked water, agricultural and soil samples with each set of samples analysed. Thiram or thiophanate methyl was not detected in the procedural blanks.

### Assay of Thiram and Thiophanate Methyl in Spiked Water and Agricultural Samples

The method when applied to the assay of each fungicide from water and agricultural samples gave high recoveries in the range 86.00-98.00% and 88.50-97.50% for thiram and thiophanate methyl, respectively, with the maximum RSD value of 2.60% indicating good accuracy and precision of the method (Table 7 and 8).

### Evaluation of Leaching Potentials Based on Soil Adsorption Study

Following application, these fungicides enter soil and then to the aquatic environment through leaching which results from unadsorbed fraction of the fungicide. Two isotherms *viz.* Freundlich and Langmuir were used to study the adsorption; the coefficient value of determination was higher ( $r^2 > 0.91$ ) for Freundlich isotherm than that for

Langmuir isotherm ( $r^2 > 0.45$ ), indicating that data fitted better with Freundlich isotherm model and consequently can explain satisfactorily the results of adsorption in soils. This observation is in consistent with the several studies on adsorption of pesticides in soils showing Freundlich isotherm as the most reliable and well-known relationship applicable to the adsorption on heterogeneous surfaces with interaction between adsorbed molecules [29]. The adsorption isotherms for thiram and thiophanate methyl on four soils were drawn between the amount of fungicide adsorbed  $X$  ( $\text{mg Kg}^{-1}$ ) on soils and equilibrium concentration  $C_e$  ( $\text{mg l}^{-1}$ ) and are shown in Figs. 4 and 5. Other parameters for the adsorption process *viz.* distribution coefficient or soil-adsorption coefficient ( $K_d$ ), soil organic carbon partition coefficient ( $K_{oc}$ ), Gibb's free energy ( $\Delta G^\circ$ ) and Groundwater Ubiquity Score (GUS) were calculated as described earlier [29]. The GUS score is used to study the leaching behaviour of pesticides and these can be classified as leacher ( $\text{GUS} > 2.8$ ), transition ( $2.8 > \text{GUS} > 1.8$ ) and non-leacher ( $\text{GUS} < 1.8$ ). All Freundlich's adsorption parameters for the adsorption of thiram and thiophanate methyl are given in Table 9.

The strength of adsorption of fungicide to soils is related to two basic parameters: soil adsorption coefficient ( $K_d$ ) and soil organic carbon partition coefficient ( $K_{oc}$ ) of the fungicide. The values of  $K_d$  represent the extent of adsorption. The  $K_d$  coefficient is soil-specific and varies with soil texture and its organic matter content, but  $K_{oc}$  is less soil specific [34] and is calculated by normalizing adsorption coefficient ( $K_d$ ) with the organic carbon (OC) content of the soil. The  $K_{oc}$  values upto 300 correspond to weak adsorption, 300-1000 moderate and above 1000 strong adsorption [35-36]. The observed  $K_{oc}$  values (554.14-691.78) suggested moderate adsorption of fungicides on all soil types. The  $\log K_{oc}$  values of 2.74-2.77 and 2.82-2.84 for thiram and thiophanate methyl, respectively, are also in consistent with the literature values of 2.83 and 3.26 of these fungicides on similar soil types [37] which further supports the Freundlich's adsorption model.

The observed trend of adsorption of these fungicides in terms of the cation exchange capacity (CEC) and pH was the same as reported by other workers [37-38]. The values of Gibb's free energy ( $\Delta G^\circ$ ) for the adsorption of fungicides were also observed negative in all the cases suggesting the

**Table 8.** Assay Results of Thiram and Thiophanate Methyl in Agricultural Samples (Wheat and Rice)

	Amount taken ( $\mu\text{g ml}^{-1}$ )	Wheat			Rice		
		Amount found <sup>a</sup> $\pm$ SD	Recovery	RSD	Amount found <sup>a</sup> $\pm$ SD	Recovery	RSD
		( $\mu\text{g ml}^{-1}$ )	(%)	(%)	( $\mu\text{g ml}^{-1}$ )	(%)	(%)
Thiram	0.5	0.46 $\pm$ 0.012	92.00	2.40	0.43 $\pm$ 0.013	86.00	2.60
	1.0	0.91 $\pm$ 0.021	91.00	2.10	0.89 $\pm$ 0.022	89.00	2.20
	2.0	1.77 $\pm$ 0.029	88.50	1.45	1.79 $\pm$ 0.028	89.50	1.40
	3.0	2.78 $\pm$ 0.037	92.67	1.23	2.77 $\pm$ 0.034	92.33	1.13
	4.0	3.67 $\pm$ 0.044	91.75	1.10	3.63 $\pm$ 0.046	90.75	1.15
Thiophanate methyl	1.0	0.94 $\pm$ 0.026	94.00	2.60	0.91 $\pm$ 0.022	91.00	2.20
	2.0	1.78 $\pm$ 0.039	89.00	1.95	1.79 $\pm$ 0.039	89.50	1.95
	3.0	2.74 $\pm$ 0.045	91.33	1.50	2.68 $\pm$ 0.033	89.33	1.10
	4.0	3.73 $\pm$ 0.051	93.25	1.28	3.54 $\pm$ 0.037	88.50	0.92
	5.0	4.65 $\pm$ 0.053	93.00	1.06	4.58 $\pm$ 0.052	91.60	1.04

<sup>a</sup>Values are expressed as mean  $\pm$  SD (n = 5).

**Table 9.** Adsorption Parameters for the Adsorption of Thiram and Thiophanate Methyl on Four Indian Soils

	Soil	$K_d$	$K_{oc}$	$\log K_{oc}$	GUS	$\Delta G^\circ$
Thiram	I	3.38	563.88	2.75	-0.50	-2.696
	II	3.95	563.71	2.75	-0.50	-3.344
	III	5.54	554.14	2.74	-0.50	-4.171
	IV	4.75	593.96	2.77	-0.48	-3.797
Thiophanate methyl	I	3.97	661.63	2.82	0.94	-3.359
	II	4.72	677.51	2.83	0.97	-3.792
	III	6.92	691.78	2.84	0.98	-4.712
	IV	5.44	680.12	2.83	0.94	-4.127

energetically favourable adsorption process. The GUS values for thiram and thiophanate methyl were observed in the range -0.48 to 0.98 and thus classify thiram and

thiophanate methyl as non-leacher fungicides. These values are also comparable to literature values on similar soil types [39].

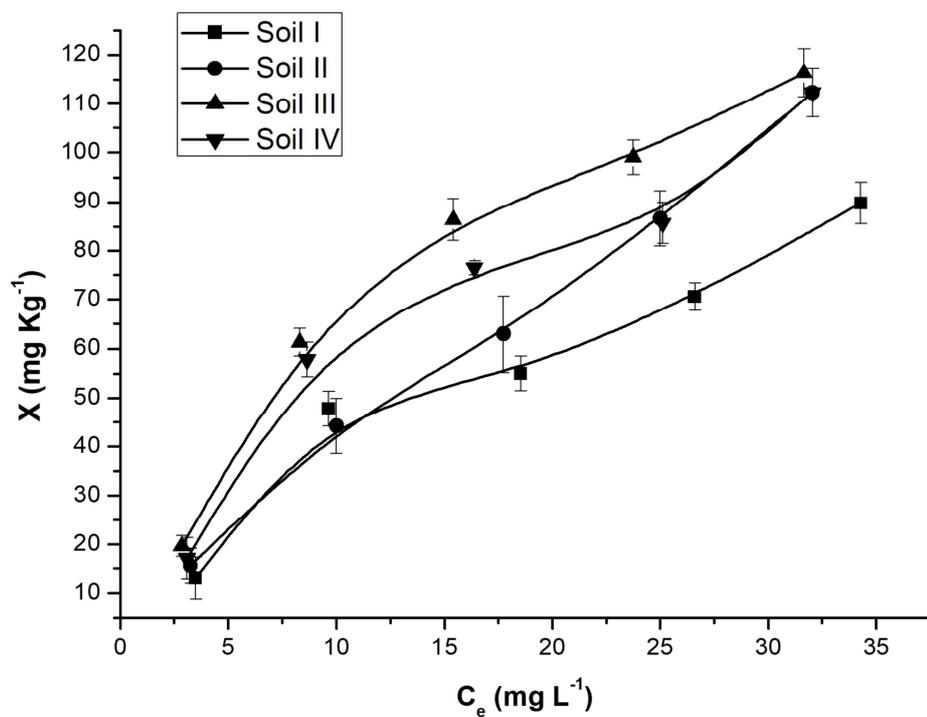


Fig. 4. Freundlich adsorption isotherms for thiram on four soils at 25 °C.

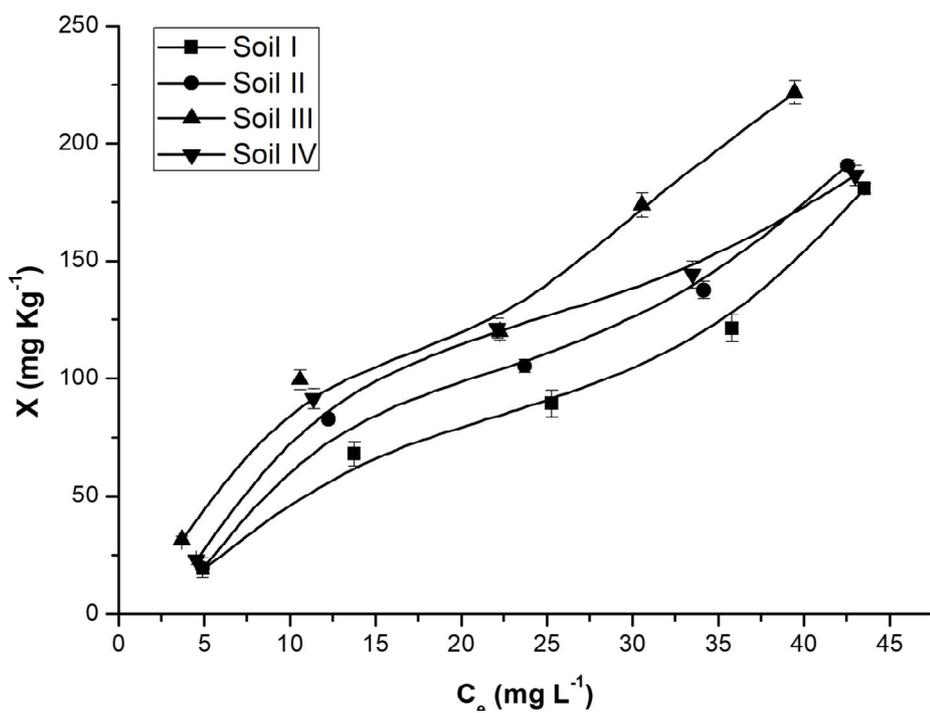


Fig. 5. Freundlich adsorption isotherms for thiophanate methyl on four soils at 25 °C.

## CONCLUSIONS

The applied fungicide ultimately enters soil and then to the aquatic environment through leaching, resulting in surface and ground water contamination, or it may desorb in relation to changes in the soil conditions (humidity), resulting in phytotoxicity of the subsequent crops. Thus, screening of these fungicides in environmental samples *viz.* water, soil and foodstuffs has been carried out by a new, rapid and sensitive spectrofluorimetric method based on the linear relationship of fluorescence intensity of cerium(III) at 365 nm and concentration of fungicide. The method is advantageous, so that analysis can be done at low concentration (LOQ, 0.18-0.22  $\mu\text{g ml}^{-1}$ ) without any interference from inorganic ions commonly present in water and soil samples coupled with the simplicity and rapidity of the procedure. The screening of the target fungicides in agricultural products (grains) and water, for the purpose of predicting health hazards, indicating high recoveries shows good accuracy and precision of the method. The residue data are an essential guide in setting regulatory limits for the assessment of the leaching risk and subsequent contamination of ground and surface water. Their adsorption on four soils has also been studied. The leaching potential in terms of GUS index of thiram and thiophanate methyl in the range -0.48 to 0.98 classifies both as non-leacher fungicides, thereby, these do not pose potential risk to aquatic environment if used judiciously.

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