CHAPTER-2

Adsorption-desorption studies of Tebuconazole in soils using HPLC

2.1 Introduction:

In general, the consumption of diazole and triazole fungicides has slowly increased in India as well as in the world (FAOSTAT, 2016); therefore, studies dealing with the environmental behavior of these fungicides are needed to better understand and evaluate the risks associated with their uses. The frequent application of tebuconazole could result in its accumulation in agricultural soils and consequently adversely affect on soil ecosystems (Komarek M. et al., 2010).

In general, adsorption is the main process that influences pesticide accumulation (retention) and movement in soil profile (Arias-Estevez M.et al., 2008). Retention refers to the ability of the soil to hold a pesticide in place and not allow it to be transported. Sorption is the primary process of how the soil retains a pesticide and is defined as the accumulation of a pesticide on the soil particle surfaces and is mainly affected by physico-chemical properties of the pesticide and soil properties (Gevao B. et al., 2000). The kinetics of sorption is inextricably linked to the forces involved in the sorptive interactions i.e. Vander Waals-London interactions, hydrophobic bonding, and charge transfer. In a complex matrix such as soil, a number of interactions are possible, and these have been discussed in detail by Pignatello, J.J. et al. (Pignatello, J.J. et al., 1996). Soil sorption is characterized by a distribution coefficient (K_d) that describes the partitioning of pesticide between solid and liquid phases. It is mathematically defined as the amount of pesticide in soil solution divided by the amount adsorbed to the particle of soil surface. For the most of the pesticides, organic matter is the most important soil property controlling the degree of adsorption. According to Kamrin M.A. et al., tebuconazole is considered to have low solubility in water (36 mg/L at 20 ^oC) (Kamrin M.A., et al., 1997) and it is less mobile in the soil environment (Tomlin CDS, 2009). Further, the relatively high log Kow

value indicates that tebuconazole would be sorbed to soils and sediments and less leaching would occur (Chamberlain K., et al., 1996). Tebuconazole degradation and its possible accumulation in soils are further influenced by the content of soil organic matter (Berenzen N., et al., 2005), which is in accordance with the relatively high value of the distribution coefficient Koc (describing pesticide distribution between soil organic carbon and the liquid phase). Pesticide sorption is also influenced by other soil components, especially clays (Singh N., 2005) and mineral-hydroxide content (Sukop M.et al., 1992). Nevertheless, tebuconazole has been detected in surface water streams situated near agricultural fields (Berenzen N., et al., 2005), probably as a consequence of runoff. Therefore, tebuconazole behavior in contrasting natural soil types and soil amended by dead plant leaves should be investigated and monitored. To the best of our knowledge, there is only limited information concerning tebuconazole sorption onto contrasting natural soil types.

The investigation of tebuconazole sorption was under taken in five types of soil (as OECD No. 106, 2000). To meet the requirement of test guideline, four different soils were taken from Gujarat and one soil was taken from Maharashtra and the tebuconazole content was monitored using HPLC technique. The HPLC method was validated before using as described below.

2.2 HPLC method validation

2.2.1 Preparation of solutions

2.2.1.1 Tebuconazole reference standard solution

A quantity of 25.10 mg tebuconazole reference standard (99.24% purity) was weighed into a volumetric flask of 25 ml capacity and dissolved in 5.0 ml acetonitrile and the volume was made upto the mark with acetonitrile [Concentration 1000 µg/ml, identified as stock solution (A)]. An aliquot of one ml solution from stock solutions

(A) was transferred into a volumetric flask of 10 ml capacity and the volume made upto the mark with acetonitrile. The concentration of tebuconazole working solution was 100µg/ml and it was identified as working solution (B).

2.2.1.2 Tebuconazole technical sample solution

Tebuconazole technical samples were supplied by UPL, Mumbai and it was used for HPLC method validation. A quantity of 10.36 mg tebuconazole technical sample was weighed into a volumetric flask of 10 ml capacity and 5.0 ml acetonitrile was added to dissolve the material, and the volume made upto the mark with acetonitrile. The concentration of the technical sample solution was 999.74 µg/ml and it was identified as stock solutions (a). An aliquot of one ml solution from technical sample stock solution (a) was transferred into a volumetric flask of 10 ml capacity and volume made upto the mark with acetonitrile. The concentration of tebuconazole in the technical sample working solution was 100µg/ml and it was identified as technical sample working solution b.

2.2.2 Validation of HPLC method

The instrument parameters for monitoring Tebuconazole during adsorption desorption studies in soil samples using Shimadzu, LC-2010 CHT are:

Column : C-18 [25 cm x 4.6 mm (i.d.) x 5 µm particle size]

Wave length : UV/220 nm

Flow rate : 1.0 ml/min.

Injection volume : 10 µL

Mobile phase : Acetonitrile: Water (80: 20, v/v)

The method adopted was validated for its suitability to monitor Tebuconazole during adsorption desorption studies in soil samples.

2.2.2.1. Specificity

The method specificity for tebuconazole was studied by injecting individual solutions of tebuconazole standard, tebuconazole technical sample, mobile phase, 0.01 M $CaCl_2$ solution(to simulate same ionic strength as soil solution), acetonitrile (solvent used for solution preparation) and control of each soil for any interference between components, with each other or with any of their impurities. The concentrations of reference standard solution, sample solution (4.0 µg/ml) were prepared by serial dilution of individual working solution (B and b). Since no interference was observed between components, with each other or with any of their impurities as well as the solvent, the method was considered to be specific.

2.2.2.2 Linear dynamic range

Reference standard solutions of five different concentrations viz. 1.0, 2.0, 4.0, 6.0 and 8.0 µg/ml (Table 2.1) were prepared by serial dilution of the working solution (B) in acetonitrile. The standard solutions were injected onto the HPLC in two replicates and the mean peak area was plotted against concentration (µg/ml). The intercept (a), slope (b) and correlation co-efficient (r) were calculated. The coefficient of correlation (r) was 0.999 (Figure 2.1).

Table 2.1: Linear dynamic range data of tebuconazole by HPLC

Concentration (µg/ml)	Replication	Area Count	Mean Area Count	% Variation
1.0	R1	235942	235601.50	0.29
	R2	235261		
2.0	R1	436956	436818.50	0.06
	R2	436681		
4.0	R1	894715	895024.50	0.07
	R2	895334		
6.0	R1	1359850	1360135.00	0.04
	R2	1360420		
8.0	R1	1778142	1777331.00	0.09
	R2	1776520		
Typical Calculation				
$\%\ \text{Variation} = \frac{\text{Max Peak Area} - \text{Min Peak Area}}{\text{Max Peak Area}} \times 100$			$= \frac{235942 - 235261}{235492} \times 100$	= 0.29%

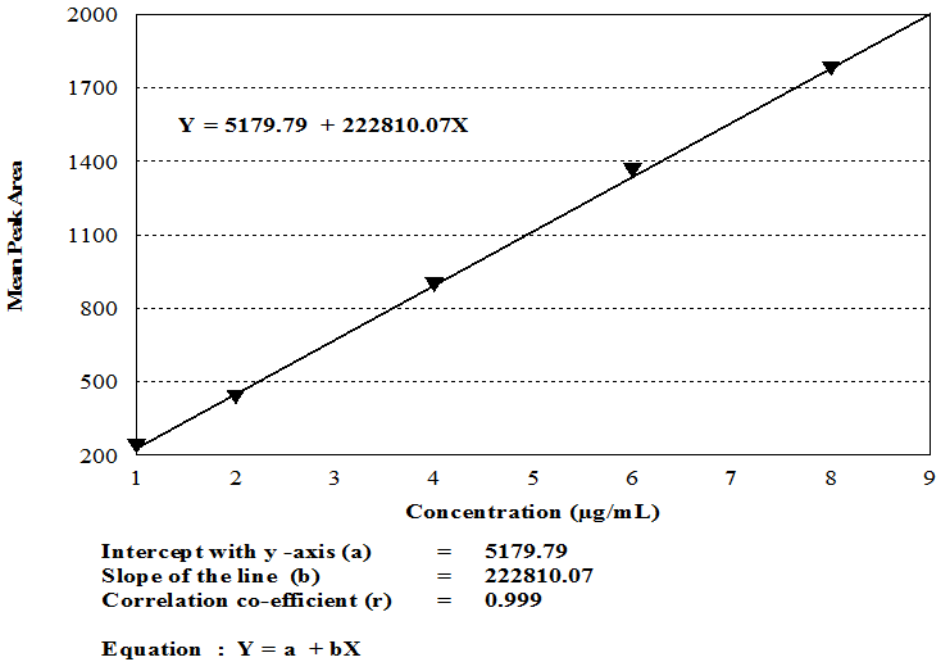

Figure 2.1: linear dynamic curve of Tebuconazole by HPLC

2.2.2.3 Limit of detection (LOD) and limit of quantitation (LOQ)

The three different concentrations of tebuconazole reference standard solutions viz. 0.1, 0.08 and 0.04 µg/ml were prepared and injected onto the HPLC in two replicates. The minimum concentration of tebuconazole which could be detected with signal to noise ratio (S/N) 3 ± 0.5:1 was considered as limit of detection (LOD). The

lowest detectable concentration (LOD) determined by the method was 0.04 µg/ml (Table 2.2). The minimum concentration of tebuconazole which could be quantified with signal to noise ratio (S/N) 5:1 to 10:1 was considered as LOQ. The LOQ determined by the method was 0.08 µg.ml^{-1} Table 2.2.

Table 2.2: Limit of detection (LOD) and limit of quantization (LOQ) of tebuconazole by HPLC

<table>
<tr><th>Solution Concentration (µg/ml)</th><th>Repli-cation</th><th>Area Count</th><th>Mean Peak Area (A)</th><th>Mean Noise</th><th>Signal to Noise Ratio (S/N)</th><th>LOD</th><th>LOQ</th></tr>
<tr><td rowspan="2">0.10</td><td>R1</td><td>24798</td><td rowspan="2">24654.00</td><td rowspan="6">3464.32</td><td rowspan="2">7.12</td><td rowspan="2">-</td><td rowspan="2">-</td></tr>
<tr><td>R2</td><td>24510</td></tr>
<tr><td rowspan="2">0.08</td><td>R1</td><td>19527</td><td rowspan="2">19439.50</td><td rowspan="2">5.61</td><td rowspan="2">-</td><td rowspan="2">LOQ</td></tr>
<tr><td>R2</td><td>19352</td></tr>
<tr><td rowspan="2">0.04</td><td>R1</td><td>9782</td><td rowspan="2">9819.50</td><td rowspan="2">2.83</td><td rowspan="2">LOD</td><td rowspan="2">-</td></tr>
<tr><td>R2</td><td>9857</td></tr>
<tr><td colspan="5">Average Noise Peak Area of Blank (B)</td><td colspan="3">3464.32</td></tr>
<tr><td colspan="8">Typical Calculation:</td></tr>
<tr><td colspan="2" rowspan="2">$Signal\ to\ Noise\ Ratio = \frac{A}{B}$</td><td colspan="3">Limit of Detection</td><td colspan="3">Limit of Quantitation</td></tr>
<tr><td colspan="3">$\frac{9189.50}{3464.32} = 2.83$</td><td colspan="3">$\frac{19436.50}{3464.32} = 5.61$</td></tr>
<tr><td colspan="2"></td><td colspan="3">0.04 µg/ml</td><td colspan="3">0.08 µg/ml</td></tr>
</table>

2.2.2.4 Precision (% RSD)

Ten separate sets of test sample solution (5.0 µg/ml) were prepared by fortifying 0.01 M $CaCl_2$ solution with 100 µg/ml working solution (b), separately. For this purpose, 0.5 ml working solution (b) was added to ten separate volumetric flasks of 10 ml capacity, containing 5.0 ml of 0.01 M $CaCl_2$ solution and the volume was made upto the mark with 0.01 M $CaCl_2$ solution,.

$$\text{Tebuconazole concentration, (µg/ml)} = \frac{Y - a}{b} \times D$$

Where,

Y = peak area of the sample

a = Intercept

b = Slope of the line

D = Dilution factor

$$\text{Precision (\% RSD)} = \frac{\text{Standard Deviation}}{\text{MeanComponent content}} \times 100$$

The precision (% RSD) of tebuconazole in 0.01 M $CaCl_2$ solution was 0.20 % (Table 2.3).

Table 2.3: Precision (% RSD) of method for tebuconazole

Solution	Fortification Level		Area	Recovered (µg/ml)	Mean Concentration (µg/ml)	Standard Deviation	% RSD
	Replic-ation	Concentration (µg/ml)					
0.01 M $CaCl_2$	R1	5.0	1121566	5.01	5.03	0.01	0.20
	R2		1120731	5.01			
	R3		1124059	5.02			
	R4		1127044	5.04			
	R5		1126720	5.03			
	R6		1122547	5.01			
	R7		1130649	5.05			
	R8		1127491	5.04			
	R9		1128466	5.04			
	R10		1125815	5.03			

Typical Calculation:

Intercept with y-axis (a) = 5179.79
Slope of the line (b) = 222810.07
Correlation of coefficient (r) = 0.999
Refer figure 2.1 from linear dynamic curve of tebuconazole standard

Dilution Factor (D) = 1.0

Tebuconazole A.I. Concentration (µg/ml)	Precision (% RSD)
$= \frac{Y - a}{b} \times D$ $= \frac{1121566 - 5179.79}{222810.07} \times 1.0$ = 5.01	$= \frac{\text{Standard Deviation}}{\text{Mean Concentration}} \times 100$ $= \frac{0.01}{5.03} \times 100$ = 0.20 %

2.2.2.5 Accuracy (% Recovery)

Three sets of 6 samples (2.0, 4.0 and 6.0 µg/ml in two replicates) were prepared for 0.01 M $CaCl_2$ solution by fortifying the 0.01 M $CaCl_2$ solution with 100 µg/ml working solution (b). For this purpose 0.2, 0.4 and 0.6 ml of 100 µg/ml working solution (b) was added in separate volumetric flasks of 10 ml capacity, containing 5.0

ml 0.01 M $CaCl_2$ solution and the volume was made upto the mark with 0.01 M $CaCl_2$ solution, respectively.

$$\text{Tebuconazole concentration, } (\mu g/ml) \quad = \frac{Y - a}{b} \times D$$

Where,

Y = peak area of the sample

a = Intercept

b = Slope of the line

D = Dilution factor

The Accuracy (% Recovery) was calculated using the following formula

$$(\%\ \text{Recovery}) \quad = \frac{\text{Quantity recovered}}{\text{Quantity fortified}} \times 100$$

The % Recovery of tebuconazole in 0.01 M $CaCl_2$ solution at 2.0, 4.0 and 6.0 µg/ml was 101.00, 100.13 and 100.17%, respectively and means recovery was 100.43% (Table 2.4).

Table 2.4: Accuracy (% Recovery) of method for tebuconazole

Solution	Fortification Level		Area	Recovered (µg/ml)	Recovery (%)	Mean Recovery (%)	Mean Accuracy (%)
	Replication	Concentration (µg/ml)					
0.01 M $CaCl_2$	R1	2.0	454564	2.02	101.00	101.00	100.43
	R2		454778	2.02	101.00		
	R1	4.0	896336	4.00	100.00	100.13	
	R2		898533	4.01	100.25		
	R1	6.0	1346571	6.02	100.33	100.17	
	R2		1342596	6.00	100.00		
Typical Calculation							
Intercept with y-axis (a) = 5179.79 Slope of the line (b) = 222810.07 Correlation of coefficient (r) = 0.999 Refer figure 2.1 from linear dynamic curve of tebuconazole standard					Dilution Factor (D) = 1.0		
Tebuconazole A.I. Concentration (µg/ml)					% Recovery		

$= \frac{Y - a}{b} \times D$	$= \frac{\text{Quantity Recovered}}{\text{Quantity Fortified}} \times 100$
$= \frac{454564 - 5179.79}{222810.07} \times 1.0$	$= \frac{2.02}{2.00} \times 100$
$= 2.02$	$= 101.00\ \%$

The validated method was used for further studies.

2.3 Adsorption – Desorption studies

The adsorption-desorption studies were conducted as described in OECD test guideline (OECD No. 106, 2000). A batch or slurry mixing experiment is usually done to measure the distribution coefficient wherein a mass of soil m_s (g) is mixed with a volume V (ml) of 0.01M $CaCl_2$ to simulate same ionic strength as soil solution and minimize cation exchange. A mass m_p g of pesticide is added to the slurry, such that an initial concentration Ci= m_p/V of pesticide was in the liquid phase. The slurry is then mixed gently in order to disturb the soil structure as little as possible for a period typically from 2 to 72h, with 24h being something of a standard. The contents in liquid phase were then analyzed by HPLC for the pesticide concentration remaining in liquid phase at equilibrium'. The distribution coefficient K_d is then calculated assuming that all pesticide removed from the solution is sorbed on the soil surface. Pesticide sorption isotherms in soils are usually well described by the Freundlich model (Coquet Y., 2003b). Sorption strength in different soils is often estimated using the K_{oc} value, which is the ratio of K_d and % OC, the amount of organic carbon (%) in the soil *Koc = Kd. 100/* % OC (Wauchope R.D. *et al.*, 2002).

2.3.1 Collection of soils

Gujarat state lies on the west coast of India between 20º 6` - 24º 42` N latitude and 68º 10` -74º 28` E longitude (Figure 2.2). This study was performed to determine

the physico-chemical properties of soils taken from different part of Gujarat and one soil taken from Maharashtra State. The four top soils (alluvial, black, deep-black clayey and sand/ Sandy Loam) from depth of 0-15 cm were collected from fields growing paddy and peanut, mango orchid, and also from a farm house in the tropical agro-climatic zone of south Gujarat medium and heavy rainfall zone (Geographical details of Gujarat-India; 2015), viz., Paria, Vansada, Bardoli, and Nanivahiyal locations and one soil (loamy) was collected from crop land of Phondaghat Maharastra using a hand auger. Care was taken that there was no history of application of pesticides in collected soils for last six months. Collected soil samples were passed through 2.0 mm sieve and stored at 4 ºC until use. The Nanivahiyal soil was amended with dead plant leaves and characterized for different physico-chemical parameters.

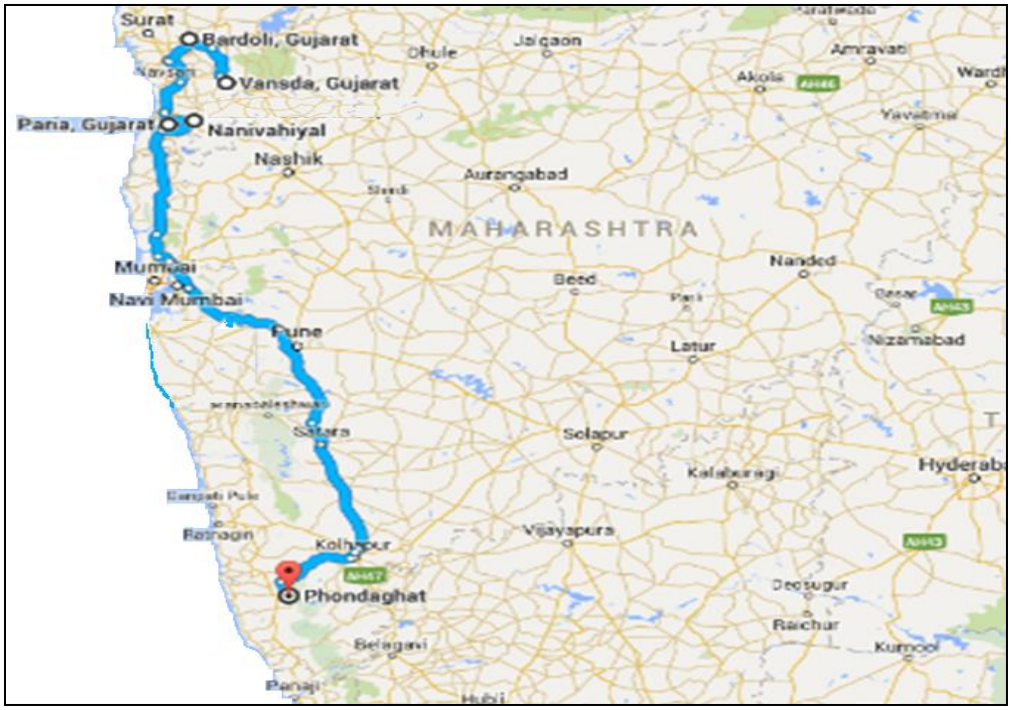

Figure 2.2: Locations of soil collection in Gujarat and Maharashtra

2.3.2 Characterization of soils

The soil samples were characterized for different physical-chemical properties as described below.

2.3.2.1 Soil pH

- **Preparation of 0.01M $CaCl_2$ solution**

A quantity of 1.47 g calcium chloride dihydrate was weighed into a volumetric flask of 1.0 liter capacity, dissolved in 400 ml distilled water and the volume was made upto the mark with distilled water.

- **Procedure**

Ten-gram air-dried soil was suspended in 25 ml 0.01M $CaCl_2$ solution and 25 ml distilled water, separately kept overnight for equilibration. After equilibration period, the soil suspension was disturbed once and the pH was measured using a calibrated pH meter (Jackson, M. L., 1967).

2.3.2.2 Soil organic carbon (SOC) contents

- **Preparation of reagents**

Potassium dichromate solution (1N): A quantity 24.52 g $K_2Cr_2O_7$ was dissolved in 500 ml distilled water.

Ferrous ammonium sulphate solution (0.5N) : A quantity of 98.00 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in 200 ml distilled water containing 3.8 ml of concentrated H_2SO_4 into a 500 ml volumetric flask and the volume was made upto the mark with distilled water.

Concentrated H_2SO_4 with 1.25% Ag_2SO_4: A quantity of 1.25g Ag_2SO_4 was dissolved in 100 ml conc. H_2SO_4.

Orthophosphoric acid: 88 - 93%

Diphenylamine indicator: A quantity of 0.5 g diphenylamine was dissolved in 20 ml distilled water and 100 ml concentrated H_2SO_4 was added.

- **Procedure** (Walkley, A. et al., 1934)

One gram soil was weighed and transferred into a 500 ml conical flask in two replicates. Ten ml 1N $K_2Cr_2O_7$ solution was added with thorough mixing followed by 20 ml concentrated H_2SO_4. The flask was swirled 2-3 times and allowed to stand for 30 minutes on an asbestos sheet for the reaction to complete. The mixture was diluted with 200 ml distilled water followed by addition of 10 ml orthophosphoric acid. The mixture was titrated with 0.5N ferrous ammonium sulphate solution using 1.0 ml diphenylamine indicator until the color changed from violet through blue to bright green. Blank titration (without soil) was also carried out in a similar manner.

- **Calculation**

Volume of 1N $K_2Cr_2O_7$ used for oxidation of C = 0.5 x (B-S) ml

[1 ml of 1N $K_2Cr_2O_7$ (=1 meq) = 3 (=12/4) mg of organic C = 0.003 g of organic C]

Assuming that an average of 77% recovery of organic C was found by this method; the correction factor would be 100/77 = 1.3

% Organic C in soil = 0.5 x (B - S) x N x 0.003 x (100/W) x 1.3

Where,

W = Weight (g) of soil taken

B = Volume (ml) of 0.5N Fe $(NH_4)_2(SO_4)_2$ solution used for blank titration

S = Volume (ml) of 0.5N Fe $(NH_4)_2(SO_4)_2$ solution used for sample titration

N = Normality of $K_2Cr_2O_7$

2.3.2.3 Total nitrogen content

- **Preparation of reagents**

0.1N H_2SO_4**:** A volume of 1.35 ml conc. H_2SO_4 was mixed with 400 ml distilled water into 500 ml volumetric flask and the volume made upto mark with distilled water.

40 % NaOH : A quantity of 400 g sodium hydroxide was dissolved in 500 ml distilled water in 1000 ml volumetric flask and volume was made upto the mark with distilled water.

NaOH (0.1N) : A quantity of 4.0 g sodium hydroxide was dissolved in 800 ml distilled water in 1000 ml volumetric flask and volume was made upto the mark with distilled water.

- **Procedure** (Michael, G. J., 1972).

A quantity of 5.0 g soil sample was transferred in to 500 ml Kjeldahl flask in two replicates followed by addition of 30 ml conc. H_2SO_4 and 2 g salicylic acid. The contents were shaken thoroughly and allowed to stand for 30 minutes with frequent shaking. Five gram sodium thiosulphate was added followed by heating the solution for 5.0 minutes and subsequent cooling. Then 20 ml water, 10 g K_2SO_4 and 1.0 g digestion accelerator (20 g $CuSO_4.5H_2O$, 3g HgO and 1.0 g selenium powder) was added and digested until light green color appeared. Blank was also processed following the same procedure without soil sample. Digestion was carried out on the digestion assembly with low flame for the first 10-30 minutes, until frothing completely subsided and then gradually, more strongly until the sample was completely charred. The content was cooled and diluted to 100 ml with distilled water and transferred into a 1000 ml distillation flask.

A few glass beads were added to prevent bumping and a piece of Zn to prevent liberation of nascent hydrogen in a fine stream. The Kjeldahl flask was attached to the delivery tube and a condenser. The end of condenser was connected to a tube dipped into 50 ml 0.1N H_2SO_4 solution, contained in a conical flask, with few drops of methyl red indicator. A volume of 125 ml 40% NaOH solution was added to the distillation flask along the sides and the content were heated to liberate the ammonia (NH_3), which was absorbed in 0.1N H_2SO_4 solution. The absorbed ammonia (NH_3) was titrated with 0.1N NaOH solution until color changed from pink to yellow.

- **Calculation**

a.	Weight of the soil sample	=	5.0 g
b.	Normality of H_2SO_4	=	0.1N
c.	Volume of H_2SO_4	=	V (50 ml)
d.	Normality of NaOH	=	0.1N
e.	Volume of NaOH	=	V_1 ml
f.	meq. of H_2SO_4 taken	=	0.1 x V
g.	meq. of NaOH used in the titration	=	0.1 x V_1
h.	meq. of H_2SO_4 used in sample titration	=	(0.1 x V) – (0.1 x V_1)
	Indicated 0.1 (V - V_1)	=	S
i.	meq. of H_2SO_4 used in blank titration	=	B
j.	corrected meq. of H_2SO_4 used in absorption	=	(S - B)
k.	1 ml of 0.1N H_2SO_4 (= 0.1 meq. H_2SO_4)	=	0.0014 g of N
	(Since 1 meq. of H_2SO_4	=	14 mg of N)
l.	(S – B) meq. of 0.1N H_2SO_4	=	(S - B) x 0.0014 g of N
Thus, W g sample contains		=	(S - B) x 0.0014 g of N
Hence, 100 g sample contains		=	(S - B) x 0.0014 x 100 / W

Therefore, total % N in soil = (S - B) x 0.0014 x 100 / W

2.3.2.4 Total sand, silt and clay content analysis (Particle size analysis),

(Black, C.A. 1965 and Baruah, T. C. *et al.*, 1997**).**

- **Preparation of reagents**

Hydrogen peroxide (30%)

HCl (2N) : A quantity of 87.40 ml concentrated HCl was mixed with 400 ml distilled water into a 500 ml volumetric flask and volume was made upto the mark with distilled water.

$AgNO_3$ (0.1N) : A quantity of 1.70 g silver nitrate was dissolved in 80.0 ml distilled water into a 100 ml volumetric flask and volume was made upto the mark with distilled water.

Phenolphthalein indicator.

- **Procedure**

Treatment with hydrogen peroxide

Twenty gram of air dried soil was weighed and transferred into a 500 ml beaker followed by addition of 15 ml H_2O_2 in two replicates. The beaker was swirled and allowed to stand for 10 minutes to complete the reaction. The beaker was then placed on a water bath to digest the sample with intermittent stirring until the reaction completely subsided. As the frothing persisted, the procedure was repeated. The beaker was then cooled and the walls were rinsed with distilled water.

Treatment with hydrochloric acid and filtration

To remove $CaCO_3$ present in the soil, 25 ml 2N HCl was added to the same beaker and contents were stirred. The content was diluted to 250 ml with distilled water and allowed to react for one hour with intermittent shaking. The contents were

then filtered using Whatman filter paper No- 50. The soil was washed with distilled water until the filtrate was free from chloride ($AgNO_3$ solution test).

Dispersion and separation of coarse sand

The soil sample was transferred from the filter paper to a 500 ml polypropylene bottle with a jet of distilled water and the volume was made upto 500 ml with distilled water. Few drops of phenolphthalein indicator were added to the mixture followed by addition of 0.1NaOH until the whole suspension turned pink. The content of the bottle was then stirred for dispersion. After dispersion, content was transferred to a 70 mesh sieve and the coarse sand was separated out. The contents on the sieve were then washed with a jet of distilled water until no more clay and silt remained over the sieve. The coarse sand was dried at 105 ºC in oven to a constant weight and the weight was recorded.

Determination of silt + clay

After separation of coarse sand, the suspension was transferred into a 1000 ml cylinder and volume was made upto 1000 ml with distilled water. The content was mixed thoroughly and kept in constant temperature chamber (25 ºC) to ensure minimum variation of temperature between the two samples. Ten ml samples withdrawn at different time intervals were transferred to pre-weighed dishes and dried at 105 ºC to a constant weight and the weight was recorded.

Determination of fine sand

To separate the fine sand, the bulk of the suspension was decanted and sediment was transferred to a 500 ml beaker with a jet of distilled water. The sediment was washed with distilled water and turbid solution was decanted until the liquid above the sediment no longer stand turbid. The sediment was dried at 105 ºC to a constant

weight and the weight was recorded. The moisture content of the test soil was determined simultaneously.

- **Observations and calculation**

a. Weight of the air dried soil taken = X g

b. Moisture content of the soil = M % (on dry weight basis)

Therefore, oven dry wt. of the soil (g) = $(100 \times X) / (100 + M) = W$

c. Weight of the dish (g) = W_1

d. Weight of the dish with coarse sand (g) = W_2

Percent coarse sand, P = $(W_2 - W_1) / W \times 100$

e. Temperature of the suspension = T ^oC

f. Sediment commencement time = t_0

g. Time of the sampling (silt + clay) = $t_0 + t_{sic}$

h. Time of the sampling (clay) = $t_0 + t_c$

i. Weight of the dish (g) = W_3

j. Volume of the suspension taken for analysis = 10 ml

k. Oven dry weight of dish and silt + clay (g) = W_4

l. Weight of silt + clay (g) = $(W_4 - W_3)$

$$\text{Per cent silt + clay, } P_{SIC} = \frac{W_4 - W_3}{W} \times \frac{1000}{10} \times 100$$

m. Weight of the dish (g) = W_5

n. Volume of the suspension taken for analysis = 10 ml

o. Oven dry weight of dish + clay (g) = W_6

p. Weight of clay (g) = $(W_6 - W_5)$

$$\text{Per cent clay, } P_C = \frac{W_6 - W_5}{W} \times \frac{1000}{10} \times 100$$

Per cent silt, P_{SI} = P_{SIC} - P_C

q. Weight of the dish (g) = W_7

r. Oven dry Weight of dish + fine sand (g) = W_8

Per cent of the fine sand, $P_{FSb} = \frac{W_8 - W_7}{W} \times 100$

2.3.2.5 Water holding capacity

- **Procedure** (Froster, J. C., 1965)

A quantity of 50 g air-dried soil was weighed and transferred into a funnel with a Whatman filter paper No:1 fitted inside a funnel and clamped on a stand. The water was added into the funnel to moist the soil upto saturation, which was judged by excess water dripping from the funnel. The wet filter paper with wet soil was transferred into a porcelain crucible when the water from the funnel stopped dripping. Wet and dry filter paper along with porcelain crucible with and without wet soil was weighed. The samples were placed in the oven for drying at a temperature of 105 °C until constant weight was observed. The samples were taken out from the oven and weighed for dry soil weight with filter paper and crucible. Two replicate analyses were performed.

- **Calculation**

The water holding capacity of soil (mass basis) as the percentage of dry soil was calculated using the following formula:

Water holding capacity (%) of the soil = [(A – B) / (B)] x 100

Where,

Weight (g) of wet soil (A) =

(Weight of wet soil with filter paper plus crucible) – (Weight of wet filter paper with crucible)

Weight (g) of dry soil (B) =

(Weight of dry soil with filter paper plus crucible) – (Weight of dry filter paper with crucible)

2.3.2.6 Determination of cation exchange capacity (CEC)

- **Preparation of reagents**

1. Sodium acetate solution:

A quantity 136 g of $NaC_2H_3O_2.3H_2O$ was dissolved in 900 ml distilled water in 1000 ml volumetric flask, pH of solution was adjusted to 8.2 and volume was made upto mark with distilled water.

2. Ammonium acetate solution :

A volume 114 ml of 99.5% glacial acetic acid in 1000 ml distilled water, followed by addition of 138 ml of NH_4OH, diluted with distilled water, and the pH was adjusted to 7 and finally the volume was made upto 2000 ml.

- **Procedure**

A quantity of 5 g soil was taken in 50 ml centrifuge tube in two replicates; 33ml sodium acetate solution (136 g $NaC_2H_3O_2.3H_2O$ in 1000 ml volumetric flask, pH of solution adjusted with acetic acid and NaOH to 8.2) was added and shaken for 5 minutes. The content was centrifuged at 8,000 rpm for 10 minutes. After centrifugation, the supernatant was decanted completely. This procedure was repeated three times to remove any excess free sodium acetate. Sample was suspended in 33 ml of 85% ethanol, shaken for 5 minutes, the content was centrifuged and the supernatant was discarded. This procedure was repeated three times. The absorbed Na^+ was exchanged with 3 x 33 ml ammonium acetate (prepared by mixing 114 ml 99.5% glacial acetic acid in 1000 ml water followed by addition of 138 ml NH_4OH, diluted

to 1980 ml with distilled water, adjusted the pH to 7 and finally making the volume up to 2000 ml) washing.

The volume of the wash solution was made up to 100 ml. The samples were diluted to necessary dilutions with ammonium acetate and analyzed by atomic absorption spectrometer (AAS, make Perkin Elmer model AAnalyst 100) for Na^+ concentration.

- **Calculation**

Weight of the soil	=	W g
Volume of ammonium acetate extract prepared	=	100 ml
Dilution factor	=	F
Concentration of sodium obtained from AAS	=	C ppm (mg/L)
1000 ml of extract contains	=	C x F ppm
Therefore, 100 ml of extract contains	=	$\frac{C \times F \times 100}{1000}\ mg\ of\ Na$
W g of soil contents	=	$\frac{C \times F \times 100}{1000}\ mg\ of\ Na$
Therefore, 100 g of soil contains	=	$\frac{C \times F \times 100}{1000 \times W} \times 100\ mg\ of\ Na$
	=	Y mg of Na
As 23 mg of Na/100 g of soil	=	1 meq. of Na per 100 g of soil
Therefore, Y mg of Na/100 g of soil	=	Y/23 meq. of Na/100 g of soil
Hence, CEC of the soil (meq./100 g of soil)	=	Y/23

The details of soils characterization data was given below in Table 2.5.

Table 2.5: Characterization of soil samples

Soil Sampling Site (Location)	Soil Properties									Soil Classification as Per OECD N° 106
	Moisture Content on dry basis (%)	Total Nitrogen (%)	Organic Carbon (%)	pH ($0.01MCaCl_2$)	pH Distilled Water	Particle Size				
						Total sand (%)	Silt (%)	Clay (%)	Water Holding Capacity	
Bardoli, Gujarat	8.90	0.09212	1.3	4.80	4.88	22	10.00	68.0	73.29	Clay (Deep Black Clayey)
Vansda, Gujarat	1.73	0.0456	0.7	7.27	7.30	40.0	17.00	43.0	66.68	Clay loam (Black)
Nanivahiyal amended soil	4.14	0.1302	10.90	6.97	7.02	72.21	17.19	10.6	104.26	Sand/Sandy Loam
Paria,	7.76	0.1068	2.5	5.86	5.91	35	44	21	67.43	Silt Loam (Alluvial)
Phondaghat	5.13	0.1140	3	4.83	4.87	35.8	37.7	26.5	-	Loamy

2.3.3 Adsorption-desorption test procedure

2.3.3.1Reagents and standards

A tebuconazole reference standard (purity 99.24%) was supplied by United Phosphorus Ltd (UPL), Mumbai, India, calcium chloride (AR grade, Merck India) and acetonitrile (HPLC grade) were purchased from Rankem. Milli-Q distilled water was used. A quantity of 1.47 g calcium chloride dihydrate was weighed into a 1000 ml capacity of volumetric flask and contents were dissolved in 400 ml distilled water followed by sonication and the volume was made up to the mark with distilled water to prepare 0.01 M $CaCl_2$ solution. A quantity of 10.36 and 12.95 mg tebuconazole (99.24% purity) were weighed separately into 1000 and 500 ml capacity volumetric flasks containing 1 ml acetonitrile and made up to mark with 0.01 M $CaCl_2$ solution to get 10 and 25.00 µg/ml of tebuconazole solutions respectively. The solutions were identified as test substance solutions and labeled as Stock Solutions I and II respectively.

2.3.3.2Equilibration of Bardoli and Nanivahiyal soil with 0.01 M $CaCl_2$ solution

The soil samples in each tube (poly propylene, 50 ml capacity) were equilibrated with 0.01M $CaCl_2$ solution wherein different soil- solution ratios (1:1, 1:5 and 1:25) were maintained. About 1 gram (dry wt.) of Nanivahiyal amended and Bardoli soil was transferred into 14 numbers of tubes (in duplicate) separately containing 25 ml 0.01M $CaCl_2$ (1:25 soil- solution ratio) and equilibrated for 7 different time intervals at 250 rpm for 24 h on end over shaker.

A blank was prepared by taking 12.5 ml of tebuconazole solution of concentration 10 mg/ml (prepared in 0.01 M $CaCl_2$) and 12.5 ml of 0.01 M $CaCl_2$ solution were taken separately into two tubes (polypropylene) without adding soils to monitor, the adsorption of tebuconazole on surfaces of test vessels.

Two background Controls were also run for each soil, using 0.01M $CaCl_2$ (without tebuconazole). All blank and control samples were shaken in orbital shaker, filtered and analyzed for tebuconazole content by HPLC using validated method.

Further, after equilibration with 0.01 M $CaCl_2$, soil-slurry content in tubes was centrifuged at ≈ 4000 rpm for 20 minutes and the supernatant was removed (half of the total volume of the aqueous phase). An equal volume of 10 µg/ml test substance solution (Tebuconazole stock solution I) was added for 1: 1, 1: 5 and 1: 25 soil - solution ratio into Bardoli soil and for 1: 5 and 1: 25 soil – solution ratio into Nanivahiyal-amended soil. In case of Nanivahiyal-amended soil, for 1:1 soil – solution ratio, 3 ml supernatant was removed and 3 ml of 25 µg/ml test substance solution (Stock Solution II) was added.

2.3.3.3Sampling and processing of samples drawn at different time intervals

At the particular time interval (2, 4, 8, 16, 32, 48 and 72 h), all the pre-labeled sample tubes were drawn from shaker and centrifuged at ≈ 4000 rpm for 20 minutes. The supernatant was filtered through Millipore disposable AP25 filters and the concentration of tebuconazole was determined in the supernatant using validated HPLC method. The decreases in the concentration of fungicide in supernatant after different time interval shaken were taken as the amount of fungicide sorbed by the soil.

2.3.3.4Adsorption kinetics

The adsorption kinetics of the other three soils, namely Paria, Vansda and Phondaghat were studied at 1:25 soil-solution ratio as described in preliminary test. Therefore, one gram (dry mass) of Paria, Vansda and Phondaghat soils were transferred into the centrifuge tube (polypropylene, 50 ml capacity) for 7 time intervals in duplicate and 25 ml 0.01 M $CaCl_2$ was added into each tube. All the tubes

were shaken overnight in orbital shaker at 250 rpm. After equilibration with 0.01 M $CaCl_2$, the tubes were centrifuged at ≅ 4000 rpm for 20 min and a volume of 12.5 ml supernatant was discarded and same volume of test substance (from 10 µg/ml tebuconazole stock solution I) was added into the each tube. The content was mixed thoroughly and shaken in an orbital shaker at 250 rpm for different time intervals (2, 4, 8, 16, 32, 48 and 72 h). After shaking at different time intervals (2, 4, 8, 16, 32, 48 and 72 h), the samples were centrifuged at ~ 4000 rpm for 20 minutes and 20 ml supernatant (aqueous phase) was processed and analyzed for tebuconazole contents using validated HPLC method. The pH of the soil suspension (test substance solution in 0.01 M $CaCl_2$) was measured. The pH of the aqueous phase was 4.81, 4.41, 4.91, 5.90, 7.34 and 5.28 for Bardoli, Nanivahiyal-amended, Phondaghat, Paria, Vansda and blank soils, respectively.

2.3.3.5 Desorption kinetics

Desorption was studied in the same soils as used for adsorption. After adsorption, the supernatant was decanted and it was replaced with 20 ml of fresh 0.01 M $CaCl_2$ solution. Again soil-water suspension was shaken on end over shaker for time intervals of 2, 4, 8, 16, 32, 48 and 72 h. The samples were centrifuged at ≅ 4000 rpm for 20 min and supernatant was analyzed for tebuconazole contents using validated HPLC method for desorption kinetics. The amount of tebuconazole desorbed was calculated by subtracting the amount of tebuconazole in the entrapped solution after adsorption experiment from the solution concentration measured after the desorption experiment.

2.3.3.6Adsorption and desorption isotherm

After equilibration of the soils with 0.01 M $CaCl_2$ solution, the content of each pre-labeled tubes were centrifuged at ≈ 4000 rpm and a volume of 20 ml supernatant

was discarded. Thereafter, 17.5, 15.0, 10.0, 5.0, 0.0 ml of 0.01 M $CaCl_2$ solution and 2.5, 5.0, 10.0, 15.0 and 20.0 ml test substance solution (10 µg/ml, Stock Solution I) was added for maintaining 1.0, 2.0, 4.0, 6.0 and 8.0 µg/ml concentrations level respectively. The content was mixed thoroughly and shaken in an orbital shaker at 250 rpm for 48 hour for adsorption equilibrium. After adsorption equilibrium was reached, the tubes were centrifuged at~4000 rpm for 20 minutes; the supernatant (20 ml) was drawn from each tube, processed as describe in preliminary test and analyzed for tebuconazole contents using validated HPLC method.

The adsorption isotherm of tebuconazole in all five soils (Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda) was studied at1:25 soil-solution ratio at 1.0, 2.0, 4.0, 6.0 and 8.0 µg/ml concentrations level in duplicate.

Adsorption and desorption data were fit into the linearized Freundlich Equation,

$$LogC_{ads} = logKf_{ads} + {}^{1}/_{n_{ads}}\, Log\ C_{aq}$$

Where C_{ads} is the quantity of test substance adsorbed per gram of soil, K_{ads} and $1/n_{ads}$ are empirical Freundlich constants and C_{aq} is the equilibrium concentration in aqueous phase (μg/ml). Kf$_{ads}$ represents the degree of adsorption, and $1/n_{ads}$ takes into account the nonlinearity in the adsorption or desorption isotherm.

2.3.3.7Calculations

In all the calculations, the weight of 1 cm^3 (ml) aqueous phase was considered as 1 gram.

- **Adsorption**

The distribution co-efficient (Kd) was calculated at equilibrium using the following formula:

$$k_d = \frac{C_{soil}}{C_{water/aqueous\ phase}}\ where, C = contents\ of\ test\ substance$$

The distribution coefficient (*K*d) was calculated from the linear fit of the adsorption data. The *K*oc values were calculated by normalizing adsorption constant *K*oc with the OC content of the soil as follows:

$$k_{oc} = k_d \times \frac{100}{\%\ OC}\ cm^3/g\ \ where, \%OC = \%\ Organic\ carbon\ in\ soil$$

Simple correlation was worked out between $Kf_{ads}(1/n_{ads})$ as the adsorption parameter and the characteristics of the soils using statistical graphic system.

- **Desorption**

The mass of the test substance (Tebuconazole) desorbed from soils at time interval "ti' was calculated as

$$m_{aq}^{des}(ti) = m_{m}^{des}(ti)\left[\frac{V_o}{V_r^i}\right] - m_{aq}^{A}$$

Where,

$\mathbf{m_m^{des}(ti)}$ = Mass of the test substance analytically measured from the aliquot taken at time t_i in desorption study (µg)

$\mathbf{V_r^i}$ = Volume of the aliquot taken from the tube (i) for measurement of test substance in desorption kinetics experiment

And the mass of the test substance (Tebuconazole) test substance or substance adsorbed in soil from the solution at equilibrium was calculated as

$$\mathbf{m_{aq}^{A} = m_{aq}^{ads}(eq)\left(\frac{V_O - V_R}{V_O}\right)\ \mu g}$$

$\mathbf{m_{aq}^{ads}(eq)}$ = Mass of the test substance in the solution at adsorption equilibrium(µg)

Where,

V_R =Volume of the supernatant removed from the tube after the attainment of adsorption equilibrium and replaced by the same volume (ml) of 0.01M $CaCl_2$.

V_O = Initial volume (ml) of the aqueous phase in contact with the soil during the adsorption test.

The apparent desorption co-efficient (K_{des}) was calculated as the ratio between the content of the test substance remaining in the soil phase and the mass contents of the desorbed test substance in the aqueous solution.

K_{des} = [{T.S. adsorbed on soil at adsorption equilibrium (µg) – T.S. desorbed at desorption equilibrium (µg)} / T.S. desorbed at desorption equilibrium (µg)] x [Total volume of aqueous phase (ml)/ Weight of soil taken (g)] or as

$$k_{des} = \frac{\text{T.S.adsorbed on soil at Ads Eq} - \text{µg T.S.desorbed at Des Eq}}{\text{µg T.S.desorbed at Des Eq}} \times \frac{Total\ Volume\ (25) ml}{Soil\ weight\ (1) g}$$

The desorption isotherm data was fitted into the linearised form of the Freundlich desorption equation, in the similar way as described for the adsorption isotherm, and ($1/n_{des}$) - slope and (K_{des}) - intercept was calculated.

2.3.3.8 Adsorption-desorption test

2.3.3.8.1 Selection of soil-solution ratio and determination of adsorption kinetics

The Bardoli and Nanivahiyal-amended soil was used during preliminary study for determination of the adsorption kinetics at 1:1, 1:5 and 1:25 soil-solution ratios. The adsorption of tebuconazole was studied at 5.0 µg/ml concentration. The adsorption equilibrium time for both the soils was 48 h at 1: 25 soil-solution ratio. The adsorption in Bardoli soil after 48 h at 1:1, 1:5 and 1:25 soil-solution ratios were 96.08, 75.50 and 45.58%, respectively. The adsorption in Nanivahiyal-amended soil after 48 h at 1:1, 1:5 and 1:25 soil- solution ratio was 97.80, 89.76 and 65.57%, respectively. The adsorption in Bardoli soil with 1:1 and 1: 5 soils - solution ratio was 87.55 and 62.75% and in Nanivahiyal-amended soil adsorption was 96.39 and 82.03%

at 2 h, respectively. Similarly, the experiment was done with 1:1, 1:5 and 1:25 soil-solution ratio for 4, 8, 16, 32 and 72h shaking (Figure 2.3 - 2.4). Therefore, to achieve better adsorption pattern, soil-solution ratio (1: 25) was selected for the main study. Since, the percentage adsorption of tebuconazole was more than 20% in Bardoli and above 50% in Nanivahiyal-amended soil. The percentage adsorption in Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda at 48 h was more than 20%.

The adsorption for at 1:25 soil-solution ratio in Phondaghat, Paria and Vansda soils was, 43.15, 34.98 and 21.58%, respectively. In all the soils, adsorption after 48 h was > 20%, and reached plateau at 72 h. The adsorption kinetics of tebuconazole in Phondaghat, Paria and Vansda soils at 1:25 soil-solution ratio is given in Figure 2.5.

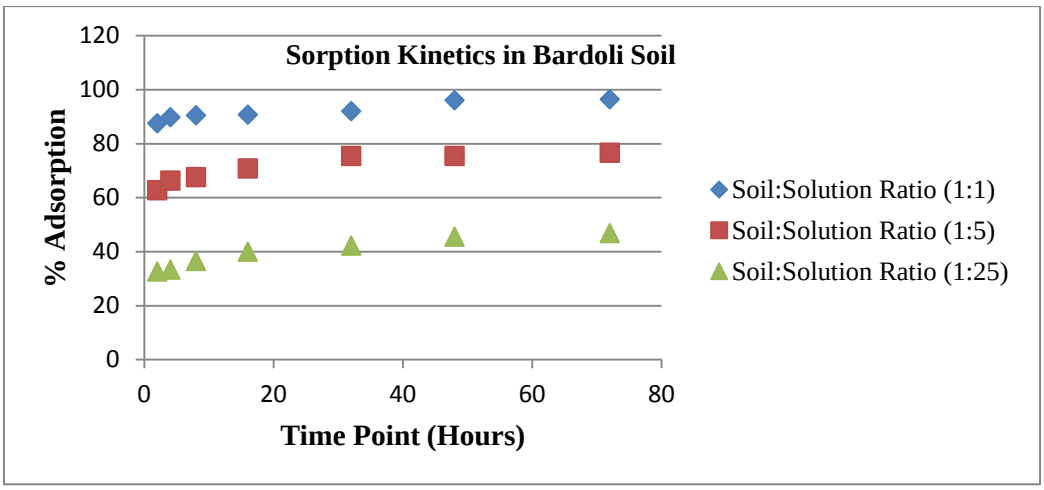

Figure 2.3: Graph of adsorption kinetics of tebuconazole in Bardoli soils at different soil - solution ratio

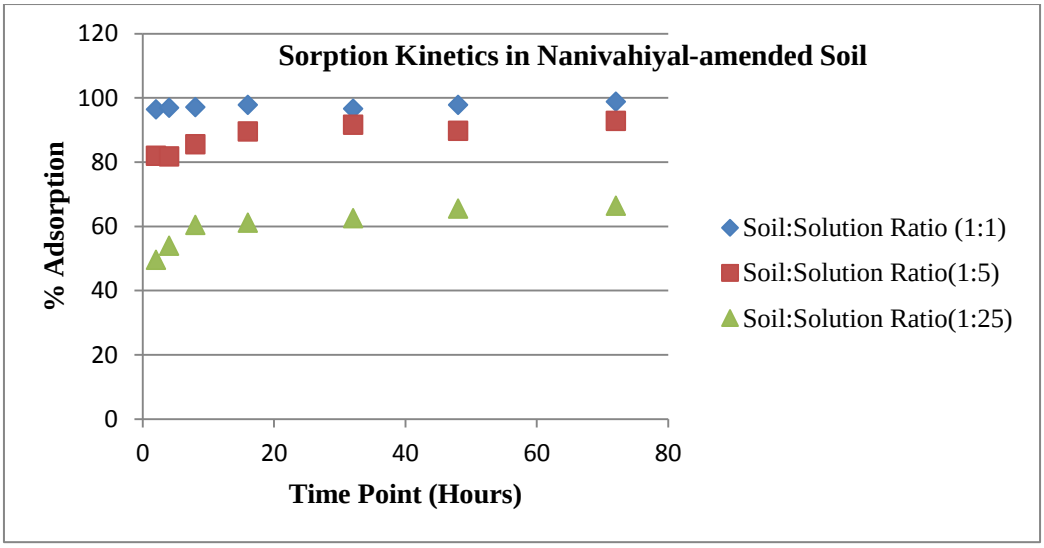

Figure 2.4: Graph of adsorption kinetics of tebuconazole in Nanivahiyal-amended soils at different soil - solution ratio

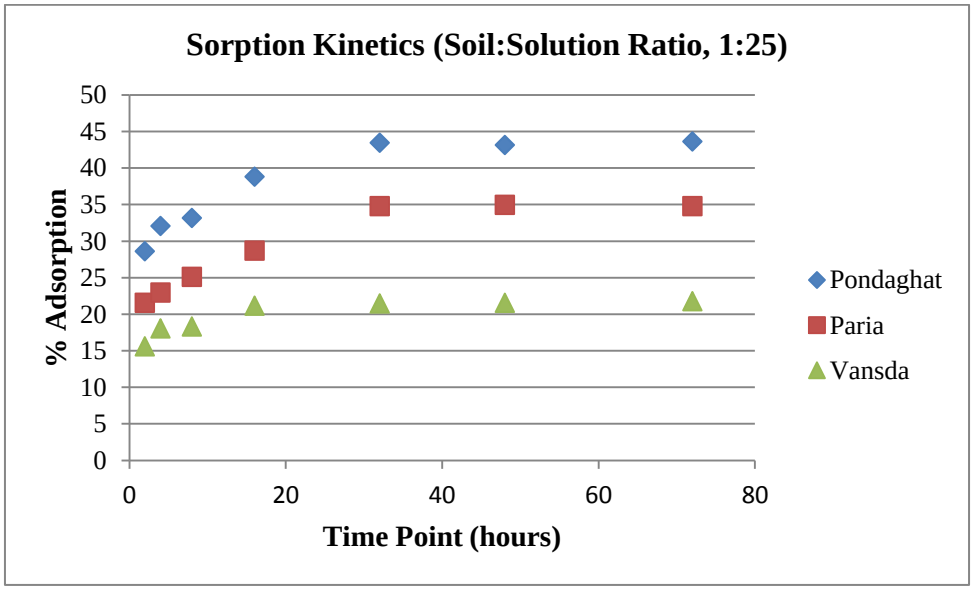

Figure 2.5: Graph of adsorption kinetics of tebuconazole in different soils at soil - solution ratio of 1:25

Typical Calculation:

<table>
<tr><td colspan="2">Intercept with y-axis (a) = 5179.79;
Slope of the line (b) = 222810.07
Correlation of coefficient (r) = 0.999
Refer Figure 2.1</td><td>Dilution Factor (D) = 1.0</td></tr>
<tr><td rowspan="2">A</td><td colspan="2">For Bardoli (after 2h shaking, Sample-1, Replication(R)-1</td></tr>
<tr><td>$$\text{Recovered (µg/ml)} = \frac{\text{Peak Area* - a}}{\text{b}} \times D$$
$$= \frac{141248 - 5179.79}{222810.07} \times 1.0$$
$$= 0.61$$
*= Peak Area taken from tebuconazole fortified soil samples analyzed by HPLC</td><td>$$\text{Recovery (\%)} = \frac{\text{Quantity Recovered}}{\text{Quantity Fortified}} \times 100$$
$$= \frac{0.61}{4.98} \times 100$$
$$= 12.25$$</td></tr>
<tr><td></td><td colspan="2">$$\text{Adsorption (\%)} = \frac{\text{Quantity adsorbed}}{\text{Quantity added}} \times 100$$
$$= \frac{4.37}{4.98} \times 100$$
$$= 87.75$$
Note: Quantity adsorbed = Added – Recovered</td></tr>
<tr><td rowspan="2">B</td><td colspan="2">For Kd and Koc</td></tr>
<tr><td>Equilibrium time 48 h, Soil solution ratio 1: 25
$$Kd = \frac{C_{soil}}{C_{water/\ aqueous\ phase}}$$
C_{soil} = 45.58% of applied µg in 1 g soil
$$= \frac{45.58 \times 12.5 \times 9.95 \times 1}{100}$$
$$= 56.69$$</td><td>$$C_{water/aqueous\ phase}\ (\mu g/ml) = \frac{2.71 + 2.71}{2}$$
$$= 2.71$$
$$Kd = \frac{56.69}{2.71} = 20.92$$
$$\text{Koc (cm3/g)} = \frac{100}{\%\ oc} \times Kd$$
$$= \frac{100}{1.30} \times 20.92$$
$$= 1609.23$$</td></tr>
</table>

The distribution co-efficient (K_d) for Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda soils was 20.92, 47.41, 18.95, 13.41 and 6.87, respectively (Table 2.6).

Table 2.6: Distribution co-efficient (K_d), Organic carbon normalised adsorption co-efficient (Koc) and apparent desorption co-efficient (K_{des}) for soils

Soil	Kd (µg/ml)	K_{OC} (cm^3/g)	K_{des} (cm^3/g)
Bardoli	20.92	1609.23	50.63
Nanivahiyal-amended	47.41	434.95	60.59
Phondaghat	18.95	631.67	30.25
Paria	13.41	536.40	31.17
Vansda	6.87	981.43	28.60

There was no insignificant adsorption (0.10%) of tebuconazole in test vessel (poly propylene centrifuge tube) and also control soils.

2.3.3.8.2 Desorption kinetics

Desorption kinetics of tebuconazole in the five soils was studied at 1:25 soil-solution ratio. The desorption equilibrium time was 48 h for all the soils. The desorption of tebuconazole at equilibrium for Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda soils was 33.06, 29.21, 45.25, 44.51 and 46.64%, respectively. The desorption kinetics of tebuconazole in all soils is given in Figure 2.6.

The apparent desorption co-efficient (K_{des}) [the ratio between the content of the test substance remaining in soil phase and the mass concentration of the desorbed substance in the aqueous phase] for Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda soils was 50.63, 60.59, 30.25, 31.17 and 28.60 cm^3/g, respectively .

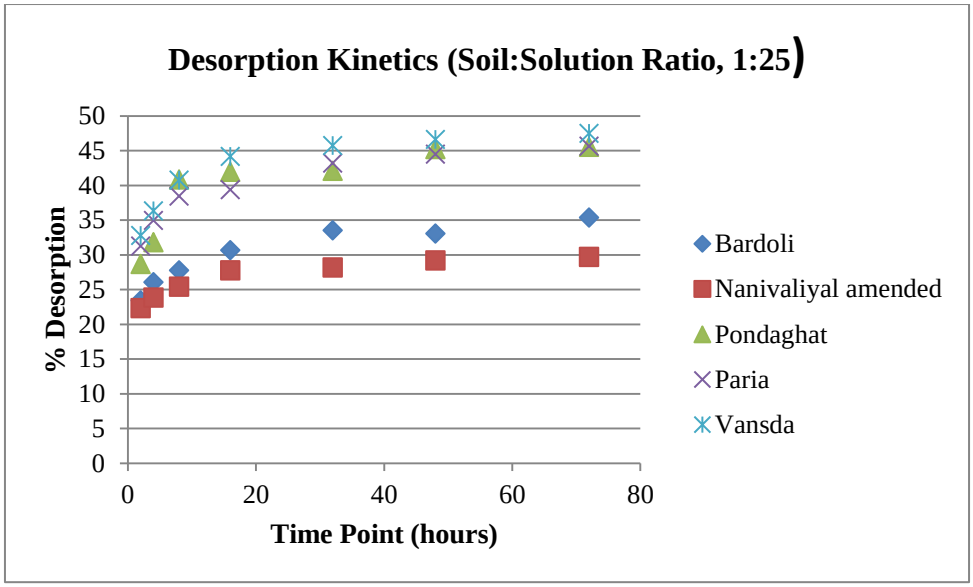

Figure 2.6: Graph of desorption kinetics of tebuconazole from different soils at soil - solution ratio of 1:25

Typical Calculation:

<table>
<tr><td colspan="2">Intercept with y-axis (a) = 5726.93;
Slope of the line (b) = 225948.33
Correlation of coefficient (r) = 0.999
Refer Figure 2.7</td><td>Dilution Factor (D) = 1.0</td></tr>
<tr><td colspan="3">For Bardoli Soil (after 2h, sample-1R1</td></tr>
<tr><td>Recovered (µg/ml) = $\frac{\text{Peak Area - a}}{\text{b}}$ x D

= $\frac{252691 - 5726.93}{225948.33}$ x 1.0

= 1.09</td><td>M_{des}/m (µg) = Recovered x 25

= 1.09 x 25

= 27.25</td><td>M_{ads}/aq = Eq. Conc. x 25

= 2.94 x 25

= 73.50</td></tr>
<tr><td colspan="3">V_O = Initial volume of the aqueous phase in contact with the soil during the adsorption test (ml) = 25
V_R =Volume of the supernatant removed from the tube after the attainment of adsorption equilibrium and replaced by the same volume of 0.01M $CaCl_2$ (ml) = 20
V^i_R= 25 ml, Hence $\frac{V_0}{V_r^i}=1$
For further calculation of M A/aq and M des/aq mean values of M des/aq and m_m^{des} were taken.</td></tr>
</table>

M A/aq(µg) = M ads/aq $\left(\frac{V0 - V_R}{V_0}\right)$

= 73.25 x $\left(\frac{25 - 20}{25}\right)$

= 73.25 x 0.2

= 14.65

M des/aq (µg) = $M_m^{des}\left(\frac{v_0}{V_r^i}\right) - m_{aq}^A$

= 26.13 x 1 - 14.65

= 11.48

Desorption (%) = $\frac{\text{M des/aq (µg)}}{\text{Ads.(µg)}} \times 100$

= $\frac{11.48}{49.00} \times 100$

= 23.43

Kdes (cm^3/g) – Bardoli (Equilibrium time 48 h)

$$\text{Kdes (cm3/g)} = \frac{\text{M ads(s)} - \text{M des/a}}{\text{M des/aq}} \times \frac{V0}{m_{soil}}$$

$$= \frac{54.00 - 17.85}{17.85} \times \frac{25}{1.0}$$

= 50.63

K_{des} (cm^3/g) – Naniyahiyal-amended soil (Equilibrium time 48 h)

$$K_{des}(cm^3/g) = \frac{\text{M ads(s)} - \text{M des/aq}}{\text{M des/aq}} \times \frac{V_0}{m_{soil}}$$

$$= \frac{65.63 - 19.1}{19.17} \times \frac{25}{1.0}$$

= 60.59

K_{des}(cm^3/g) – Phondaghat (Equilibrium time 48 h)

$$K_{des}(cm^3/g) = \frac{\text{M ads(s)} - \text{M des/aq}}{\text{M des/aq}} \times \frac{V_0}{m_{soil}}$$

$$= \frac{45.75 - 20.70}{20.70} \times \frac{25}{1.0}$$

= 30.25

K_{des}(cm^3/g) – Paria (Equilibrium time 48h)

$$K_{des}(cm^3/g) = \frac{\text{M ads(s)} - \text{M des/aq}}{\text{M des/aq}} \times \frac{V_0}{m_{soil}}$$

$$= \frac{35.88 - 15.97}{15.97} \times \frac{25}{1.0}$$

= 31.17

K_{des}(cm^3/g) –Vansda (Equilibrium time 48 h)

$$K_{des}(cm^3/g) = \frac{\text{M ads(s)} - \text{M des/aq}}{\text{M des/aq}} \times \frac{V_0}{m_{soil}}$$

$$= \frac{27.38 - 12.77}{12.77} \times \frac{25}{1.0}$$

= 28.60

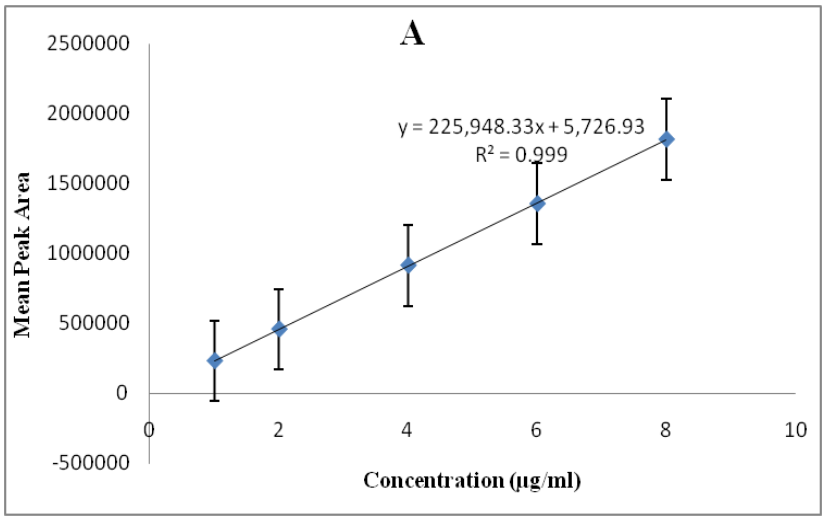

Figure 2.7: Linear dynamic curve of tebuconazole standard for determination of content in Desorption kinetics

2.3.3.8.3 Adsorption isotherm

The adsorption isotherm of tebuconazole was studied at concentrations, 1.0, 2.0, 4.0 6.0 and 8.0 µg/ml in all the five soils (Figure 2.8). The quantity of tebuconazole adsorbed in Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda soils ranged between 12.35 to 74.05, 17.60 to 108.80, 10.85 to 78.30, 10.85 to 60.80 and 6.35 to 41.80 µg/g, respectively (Table 2.7).

Table 2.7: Adsorption of tebuconazole at different concentrations in soils

Concentration (µg/ml)	Quantity Adsorbed (µg/g) in Soil, (Soil-Solution Ratio 1:25)				
	Bardoli	Nanivahiyal-amended	Phondaghat	Paria	Vansda
1	12.35	17.60	10.85	10.85	6.35
2	21.20	33.45	19.95	18.45	11.45
4	40.65	62.90	43.15	33.15	22.65
6	62.10	85.10	58.85	44.60	29.60
8	74.05	108.80	78.30	60.80	41.80

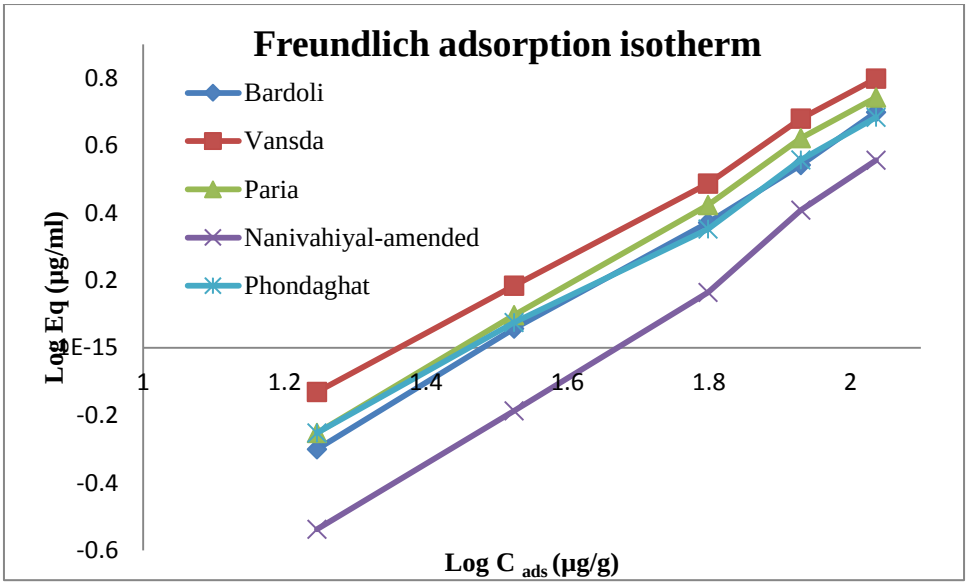

Figure 2.8: Graph of Freundlich adsorption isotherm in soils

Typical Calculation:

Total µg in solution = Mean Eq. Conc. (µg/ml) x 25

Added (µg) = Stock Solution Added (ml) x 9.94

Intercept with y-axis (a) = 11687.72; Slope of the line (b) = 217800.78 Correlation of coefficient (r) = 0.999 Refer Figure 2.9	Dilution Factor (D) = 1.0
For Test Concentration (1.0 µg/ml), Bardoli Soil Sample-1R1 & Bardoli Soil Sample-1R2	
Eq. Conc. (µg/ml) (1BAIR1) = (Area - a) / b x D = (121983 – 11687.72) / 217800.78 x 1.0 = 0.51	µg Absorbed = Added µg - Total µg in solution = 24.85 – 12.50 = 12.35

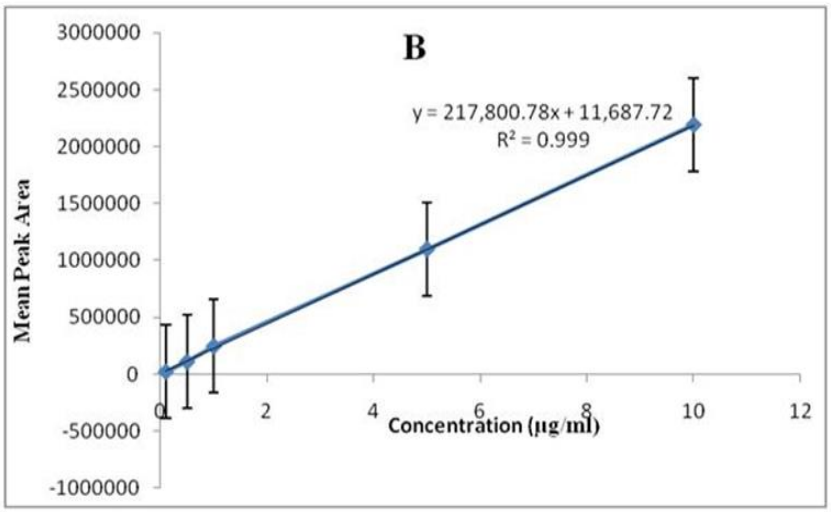

Figure 2.9: Linear dynamic curve of tebuconazole standard for determination of content in different soils

The slope and intercept of Freundlich adsorption isotherm for Bardoli soils was 0.8116 and 1.3161, for Nanivahiyal-amended soils was 0.7196 and 1.6488, for Phondaghat soils was 0.9326 and 1.2626, for Paria soils was 0.7414 and 1.2091 and for Vansda soils was 0.8688 and 0.9111, respectively (Table 2.8).

Table 2.8: Slope, intercept and r values for freundlich adsorption isotherm curves in different soils

Soil	Slope ($1/n_{ads}$)	Intercept (Kf_{ads})	*$Kf_{ads}(1/n_{ads})$	r
Bardoli	0.8116	1.3161	1.0681	0.9960
Nanivahiyal-amended	0.7196	1.6488	1.1865	0.9979
Phondaghat	0.9326	1.2626	1.1775	0.9967
Paria	0.7414	1.2091	0.8964	0.9980
Vansda	0.8688	0.9111	0.7916	0.9979

* = The correlation between adsorption constants (taken from table 2.8) and soil properties (taken from table 2.5) was calculated here. The correlation between tebuconazole adsorption constants $K_{ads}(1/n_{ads})$ and soil organic carbon, pH (as 0.01M $CaCl_2$) and clay contents was found 0.736, 0.475 and 0.366. The best correlation (r = 0.736) between soils OC contents and the adsorption constants $K_{ads}(1/n_{ads})$ for tebuconazole was found.

It is evident from Figure 2.8 that all adsorption data fitted very well to Freundlich adsorption isotherm, as evident by the correlation coefficient values (r > 0.99). The adsorption constants $K\text{f}_{ads}$ and $1/n_{ads}$ were calculated from Freundlich equation and it was presented in Table 2.8. $K\text{f}_{ads}$, which represents the amount of fungicide adsorbed at an equilibrium concentration 5μg/ml, gives an estimate of the extent of adsorption at this concentration, while $1/n_{ads}$ represents the variation in adsorption with concentration of tebuconazole. In general, $1/n_{ads}$ values for pesticides were near to 1 or less than 1, indicating C- or L-type of adsorption isotherms (Giles, C. H., et al., 1960). L-type adsorption isotherms are characterized by the strong interaction between adsorbent and adsorbate. The adsorption decreases as the aqueous concentration of pesticide increases. However, in a few cases (in Bardoli and Vansada soils), adsorption isotherms were S-type. These types of isotherms indicate the interaction of the organic compounds having polar groups with the soil and their components. In the present study, tebuconazole which contains a hydroxyl group shows L-type adsorption isotherm in Nanivahiyal-amended soils followed by Pondagat and Paria soil while in Bardoli and Vansada soil S-type of adsorption isotherms were obtained. The nonlinearity of the adsorption isotherm is probably due to the progressive saturation of the solid surface by interaction of hydroxyl group of tebuconazole with the SOC or clay mineral fraction of the soil. $K\text{f}_{ads}$ values ranged between 0.9111 and 1.6488 for tebuconazole. The highest $K\text{f}_{ads}$ was obtained with soil containing relatively high OC content (Nanivahiyal-amended, Phondaghat and Paria soil). Tebuconazole was sorbed more efficiently on the Bardoli soil (1.3161 $\mu g g^{-1}$; r = 0.996) compared to the Phondaghat soil (1.2626 $\mu g\ g^{-1}$; r = 0.9967), even though the Bardoli soil is lower in soil organic carbon (SOC) contents. However, the Bardoli soil contained higher amount of clay content that could support tebuconazole sorption.

The higher sorption ability of the Bardoli soil was probably due to the higher clay content. Even though SOC is considered to be the most important factor influencing pesticide sorption, our data show that other factors also played an important role as well. Singh N., reported the lower sorption in a soil that is higher in SOM in comparison to other soils and concluded that the type/nature of the clay content can play an important role in sorption of azole-based fungicides (Singh N., 2005). It is not obvious which of the mentioned affects the most during tebuconazole sorption.

The correlation of adsorption constant $K\mathrm{f}_{ads}$ with soil properties can be used to predict the adsorption of pesticide in different soils or in determining the factors responsible for adsorption. But $1/n_{ads}$ is also an important coefficient for description of adsorption isotherms. Isotherms can change greatly with change of $1/n_{ads}$. Therefore, as suggested by earlier workers (Celis R., et al., 1999), $K_{ads}(1/n_{ads})$, was selected as a parameter of adsorption. The simple correlation between $K_{ads}(1/n_{ads})$ and the soil properties were calculated. A best positive correlation (r = 0.736) was observed between soil OC content and the adsorption constants $K_{ads}(1/n_{ads})$ for tebuconazole (Table 2.8). Present study indicates that soil OC and clay content is the soil components affect the adsorption of tebuconazole. Adsorption of the tebuconazole was poorly correlated (r = 0.475) with the soil pH and clay contents (Table 2.8). Čadková E. et al., is also found that the tebuconazole is adsorbed in soils with high organic matter and clay minerals contents (Čadková E. et al., 2013). Our results correlate well to findings reported by other authors Cadkova E., et al. and Rodrıguez-Cruz M.S. et al. found that content and quality of soil organic matter played an important role during the sorption of azole-based fungicides (Cadkova E., et al., 2011 and Rodrıguez-Cruz M.S. et al., 2006). Kaiser K., et al. reported that hydrophobic neutral organic matter can significantly support the interactions with

hydrophobic organic pollutants, including tebuconazole (Kaiser K., et al., 2001). It is in good agreement with the sorption trend found in the Nanivahiyal-amended soil that contains the highest amount of SOC. However, other authors have shown a significant role of clay minerals and other soil components (e.g., Fe-, and Mn-oxyhydroxides) in fungicide sorption (Sukop M. et al., 1992; Koutsopoulou E., et al., 2010). Again, present study confirms these findings.

Tebuconazole being lipophilic exhibits a high affinity for SOC. As mentioned earlier, tebuconazole is considered slightly mobile and should not be found in deeper soil profiles. Tebuconazole is mainly accumulated in the top soil layer (0 –10 cm) and higher retention was recorded in soils amended with organic materials (Čadková E. et al., 2013). Nevertheless, tebuconazole was identified in deeper horizons, especially after increased application doses and also with high organic material amendments (Herrero-Hernandez E., et al., 2011). This implies that excessive organic matter addition may enhance tebuconazole mobility, possibly due the competition of dissolved organic matter for the sorption sites, and highlights the importance of SOM quality (i.e., content of humic and fulvic compounds). Other azole-based fungicides, such as penconazole, hexaconazole, propiconazole, and triadimefon, were also more mobile in soils higher in SOM content. Despite the fact that penconazole and hexaconazole moved through the soil profile down to 10–15 cm, the majority was retained in the top 5 cm. Propiconazole was found in soil profiles down to 20 cm (Singh, N., 2005). The mobility of propiconazole as well as tebuconazole is also influenced by the vegetation cover, which reduces the mobility of these fungicides (Gardner D.S. et al., 2001; Dousset S., et al., 2010). Based on our results and results obtained by other researchers, it can be assumed that SOM content and quality (in terms of hydrophobic neutral organic matter content and content of humic, fulvic, and

hydrophilic acids) are among the main factors influencing tebuconazole sorption, mobility, and persistence in the environment, but the contribution of clay minerals cannot be ignored. There are also other important factors involved in this process at field scale, such as the rate of applied fungicide, vegetation cover, temperature, soil moisture, and other environmental conditions.

2.3.3.8.4 Desorption isotherm

The Freundlich desorption isotherm of tebuconazole was studied at 1.0, 2.0, 4.0, 6.0 and 8.0 µg/ml concentrations in all the five soils and it was presented in figure 2.10. The desorbed concentration of tebuconazole in Bardoli, Nanivahiyal-amended, Phondaghat, Paria and Vansda location soils were found in the ranged between 2.25 to 19.95, 2.55 to 27.52, 3.08 to 29.78, 3.08 to 24.65 and 1.45 to 16.25 µg/g, respectively.

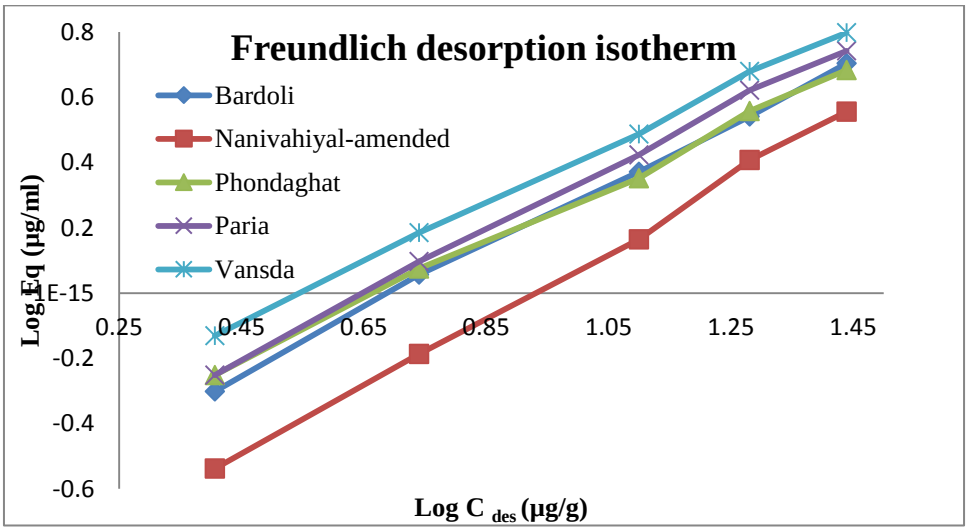

Figure 2.10: Graph of Freundlich desorption isotherm from soils

Typical Calculation:

<table>
<tr><td>Intercept with y-axis (a) = 11687.72;
Slope of the line (b) = 217800.78
Correlation of coefficient (r) = 0.999
Refer Figure 2.9</td><td>Dilution Factor (D) = 1.0</td></tr>
<tr><td colspan="2">For Test Concentration (1.0 µg/ml), Bardoli Soil Sample-1R1 & Bardoli Soil Sample-1R2</td></tr>
<tr><td>$M\ A/aq\ (\mu g) = M\ ads/aq \times \left(\frac{V_0 - V_R}{V_0}\right)$
$= M\ ads/aq \times \left(\frac{25 - 20}{25}\right)$
$= 12.50 \times 0.2$
$= 2.50$</td><td>$\text{Mean µg in Des. Sol} = \frac{0.19 + 0.19}{2} \times 25$
$= 4.75$</td></tr>
<tr><td colspan="2">µg Desorbed from 1.0 g soil = Mean µg in Des. Sol. – µg M A/aq
= 4.75 – 2.50 = 2.25</td></tr>
</table>

The slope and intercept of Freundlich desorption isotherm for Bardoli soil was 0.9615 and 0.6601, for Nanivahiyal-amended soil was 0.9389 and 0.9175, for Phondaghat soil was 1.0615 and 0.7594, for Paria soil was 0.8914 and 0.7111 and for Vansda soil was 1.0987 and 0.3007, respectively (Table 2.9).

Table 2.9: Slope, intercept and r values of Freundlich desorption isotherm curves for different soils

Soil	Slope ($1/n_{des}$)	Intercept (Kf_{des})	r
Bardoli	0.9615	0.6601	0.9968
Nanivahiyal-amended	0.9389	0.9175	0.9991
Phondaghat	1.0615	0.7594	0.9984
Paria	0.8914	0.7111	0.999
Vansda	1.0987	0.3007	0.9958

Freundlich constants for desorption of tebuconazole are presented in Table 2.9. Reversibility of adsorption plays an important role in determining the mobility of the pesticides in the soil profile. The amount desorbed was quantified and it was expressed as the percent of the amount sorbed. Adsorption of tebuconazole was completely reversible in Vansada, Phondaghat and Paria soils, and nearly 29-48% of the sorbed fungicide was desorbed. On the other hand, only nearly 22- 35% of tebuconazole was desorbed from the other two soils (Bardoli and Nanivahiyal-amended). There was no significant difference in the amount of tebuconazole desorbed from Bardoli and Nanivahiyal-amended soils. However, Vansada, Phondaghat and Paria soils behaved differently, as desorption was nearly reversible to adsorption. It is evident from the soil properties that Vansada soil has very low OC content while Paria and Phondaghat have low clay mineral contents. In soils having very low OC content and mineral fraction plays a significant important role. The Freundlich $1/n_{des}$ values take into account the nonlinearity in the desorption isotherms. In the present study, in general, $1/n_{des}$ values were higher than the $1/n_{ads}$ values, indicating that the rate of desorption is higher than the rate of adsorption showing hysteresis. The hysteresis (H) was quantified using $(1/n_{des})$ / $(1/n_{ads})$ ratio. Hysteresis is negative when the $(1/n_{des})/(1/n_{ads})$ ratio is more than 1, while it is positive when the $(1/n_{des})/ (1/n_{ads})$ ratio is less than one. Generally, desorption for tebuconazole showed negative hysteresis in all types of soil under study.

Finally, the adsorption-desorption studies of tebuconazole indicate that the tebuconazole is moderately sorbed in the low OC contents Indian soils. The isotherms fitted the Freundlich equation well. Linear regression analysis of adsorption constants and soil properties showed that the product of $K_{ads}(1/n_{ads})$ has a good correlation with OC compare to other soil parameters (Table 2.8); suggesting that OC is the main soil

parameter that dominates the adsorption process. Tebuconazole, shows more adsorption and less desorption in Nanivahiyal-amended and Bardoli soils while Vansada, Phondaghat and Paria soils showed less sorption and more desorption, indicating tebuconazole is showing moderate mobility of tebuconazole in three soils but overall it can be considered a safe fungicide for the Indian environments.

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