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Comparative Study of Sorption-Desorption Behavior of Benzimidazole Based Pesticides on Selected Soils

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Abstract

A commercially available fungicide, Carbendazim and two newly synthesized Benzimidazole fungicides 2-(4-fluorophenyl)-1*H*-benzimidazole (FBNZ) and N-(1*H*-benzimidazol-2-ylmethyl) acetamide (ABNZ were investigated for their sorption-desorption behavior on four different agricultural soils of Pakistan, involving batch equilibrium method. The data obtained in all tests showed linear adsorption isotherms. All three fungicides showed a greater degree of adsorption on soil samples. Average adsorption percentage for soil1 was 15.7%, 8.0% and 17.6%; for Soil 2 it was found to be 13.8%, 18.1% and 23.9%, for Soil 3 it was 34.3%, 29.7%, and 23.8% and for Soil 4 it was 33.4% and 35.5% for Carbendazim, FBNZ and ABNZ respectively. The Kd parameters are low indicating that the interaction between the soil particles and fungicides were low. The sorption parameter was low in soil1 and soil 2 as compared to soil 3 and soil 4 for all the fungicides. This is because of low clay content in soil 1 and soil 2 as compare to soil 3 and soil 4. Desorption studies reveal that the adsorbed fungicides were firmly retained by soil particles and their adsorption was almost irreversible. The results indicate that soil organic matter (SOM) and appropriate pH also play promising role in sorption capacity. Newly Synthesized FBNZ and ABNZ show great antifungal activity. FBNZ causes 35% inhibition of Aspergillusflavus while ABNZ causes 40% inhibition of Microsporumcanis and Fusariumsolani.

Keywords:

Carbendazim, Benzimidazole, Sorption, Desorption, physico chemical properties

1. Introduction

Adsorption and desorption studies of pesticides in soils are essential in understanding environmental fate, environmental behavior and environmental effects of pesticides [1, 2, 3, 4]. Moreover sorption processes of pesticides also impact their bio-efficacy and persistence [5]

The soil pesticide interaction (sorption process) is determined and influenced by number of factors such as physicochemical characteristics of the pesticides, soil components [9, 10] (most important are clay and organic matter) and various intermolecular interactions such as Vander-Waals forces [6,7,8].

Benzimidazole fungicides also known as MBC as these generate Methyl Benzimidazole Carbonate, these were introduced as systematic and broad spectrum fungicides

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capable of controlling a wide range of plant, crops and fruits diseases even after infection [12, 13, 14]. FBNZ and ABNZ show great antifungal activity causing 35% inhibition of Aspergillusflavus and 40% inhibition of Microsporumcanis and Fusariumsolani, respectively. Benzimidazole fungicides are also used in post-harvest food storage and as pre-planting treatments for seeds. As well as these are also found to be effective against some dominant pathogens, such as Pithomyces, Chartarum, Sclerotiniasclerotiorum S. minor and Podosphaeraleucotricha [15].

Pakistan is an agriculture country having ago based economy, therefore major areas of land are used for agricultural actives like cultivation of crops, fruits etc. Hence in order to protect these crops and fruits various pesticides are used frequently. Benzimdazole based pesticides are widely used in Pakistan on a variety of crops. For example Carbendazim, which is a systemic fungicide, is used widely on crops, vegetables and fruits like cotton, rice, wheat, rapeseed, tobacco, apples, oranges, to cure diseases like powdery mildew, blossom blight. Eyespot, leaf spot, stems for treating blossom blight in mangoes, damping of cotton, etc [16]. While pesticide use is quite prevalent in Pakistan, but there is limited of knowledge about the fate of these pesticides in terms of toxicity and persistence is limited. Therefore, present research is design to overcome this insufficient knowledge by the study of the effect and interactions of benzimidazole pesticides on selected Pakistani soils having range of physicochemical analysis.

In order to achieve the above objective it is necessary to understand the fundamental mechanisms that are involved in the binding of pesticides with soil. As mentioned earlier soil properties that have been known to affect the fate of the pesticides are; content of clay, organic matter and pH of the soil. However the relationship between sorption characteristics such as the distribution coefficients K_d or K_{oc} and the total clay content or total organic matter are not direct. In addition to this the local climate as well as soil organisms present in a particular each soil sample may also influence the fate of the pesticides in the soil [17, 18].

2. Experimental

2.1. Chemicals

Carbendazim is methyl 1- H- benzimidazole-2-carboxylate (MBC): Analytical standard of carbendazim was purchased from ACCU Standard USA. Benzimidazole fungicides: Benomyl (2-(4-fluorophenyl)-1-H- Benzimidazole, FBNZ), Analytical grade benomyl, 99% pure was purchased from ACCU Standard USA, and benlate (N-(1H-Benzimidazole -2-ylmethl) acetamide ABNZ) 50% was purchased locally. Chemical structure and basic properties of required chemicals are shown in Table 1.

2.2. Solvents

Acetone and Methanol used were 99.9 % pure from Merck, Germany.

2.3. Soil Samples

Four soils (0-10cm) were collected from different cultivated soil areas of Punjab and Khyber Pakhtunkhwa (KPK), with no recent history of pesticide application. Among them three were from the province of Punjab while one was from the province of Khyber Pakhtunkhwa (KPK). Soil 1 was taken from chak no. 136WB Tehsil Harappa District Multan, and soil 2 was from Tarnol District Rawalpindi both soil 1 and 2 were from Punjab. Soil 3 and 4 are forest soils collected from Murree, situated in district Rawalpindi, Punjab and Ayubia situated in District Abbotabad-KPK. Four soils having substantial differences representing a range of physical properties i.e. differences in level of clay organic matter and pH.

2.4. Soil Preparation

Sub samples of soils were mixed thoroughly, air dried at room temperature disaggregated manually using a marble mortar and a pestle. After this the soil was passed through a 2-mm screen sieve, and mixed manually to achieve homogeneity.

Samples of homogenized soils were analyzed for moisture content, organic matter percentage and pH. In order to find out the moisture content of the soils, the soil samples were dried at 105ºC until a constant weight was achieved and the moisture content was determined by the difference in the pre and post over weights. Organic matter content was determined by the loss on Ignition Method, where by soil samples were heated to a temperature of 400ºC in a Ney Vulcan burnout oven (or furnace) for 24 hours in order to oxidize any volatile organic matter present in the soil. Organic content was determined by the loss of weight after heating in the furnace.

Soil pH was measured by mixing 10 gm of dry soil and 10 ml of deionized water, after one hour of contact time the pH of the slurry was measured using Orion 420 plus pH meter equipped with a glass electrode. The relevant physicochemical properties of four soils are given in Table 2.

Table 1: Physiochemical Properties of Pesticides

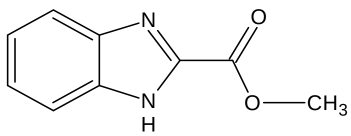
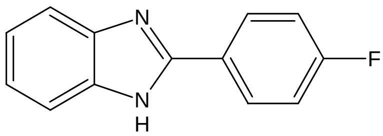
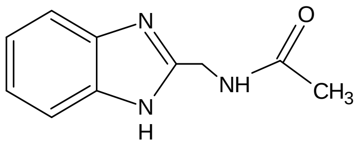
Pesticide	Colour and state	Structures and chemical names	Melting point
Carbendazim	White crystalline	 methyl 1*H*-benzimidazole-2-carboxylate	Decomposes at 250ºC
FBNZ	White crystalline	 2-(4-fluorophenyl)-1*H*-benzimidazole	261 ºC
ABNZ	Pale white crystalline	 *N*-(1*H*-benzimidazol-2-ylmethyl)acetamide	177 ºC

Table 2: Physiochemical properties of soils

Sample	Location	Soil Texture	OC (%)	Clay (%)	Sand (%)	Silt (%)	pH	C %	Primary Crops
Soil 1	Tarnol (Punjab)	Loamy & clayey	1.92	49	12	45	7.9	1.12	Wheat
Soil 2	Multan (Punjab)	Loamy & sandy	1.89	12	52	13	8.1	1.10	Wheat, Cotton
Soil 3	Murree (Punjab)	Loamy	1.97	36	14	33	8.2	1.15	Apples, Apricots
Soil 4	Ayubia (KPK)	Silt loam	6.51	44	24	41	7.6	3.79	Maize, French beans

2.5. Sorption of Benzimidazole on Minerals and Soils

All experiments have been performed under isothermal conditions at 25±1 ºC. Pesticide solutions were prepared in de ionized water and stored at 4 ºC.

For FBNZ and ABNZ different concentrations (0.25, 0.5, 0.75, 1.0, 2.5, 5.0 and 10 ppm) were prepared, whereas the limited solubility of Carbendazim allowed to prepare up to 5 ppm concentration. Sorbent/solution ratio was kept at 1:20. 10 mL of 0.1M NaCl was added as background electrolyte to simulate ionic strength close to natural soil solution. It was also added as an aqueous solvent phase in order to improve centrifugation and to minimize cation exchange.

Each sample consisted of 0.5g of soil or mineral mixed with 10mL of pesticide solution in 1:10 soil /solution ratio, placed in a 15mL Pyrex glass centrifuge tube, fitted with a screw cap. The tubes were continuously agitated on a Stuart Orbital Shaker at 90rpm for 24 h at 25 ºC in order to attain equilibrium. The adsorption experiment was done in duplicate for each concentration; also a blank sample containing only dissolved fungicides and 0.1 M NaCl was prepared to quantify the losses and to account for possible degradation during adsorption process.

The centrifuge tubes containing equilibrated material were centrifuged at 3000 rpm for 25 min at 25 ºC in a Hettich Zentrifugen EBA 20. Later the centrifuged tubes were decanted by filtering soil water suspension through 0.2 µm Nucleo-pore nylon membrane and clear aliquots analyzed by visible adsorption spectrophotometery by Hitachi U-2800 Spectrophotometer. The amount of the fungicides adsorbed (µg/g soil or mineral) was obtained by subtracting the obtained value from the blank and the amount remaining in the solution after equilibration using the relation:

$$X = V/m\ (C_B - C_e)$$

Where:
X= amount adsorbed,
V= solution volume,
m= grams of soil taken,
C_B= equilibrium concentration of blank and
C_e= equilibrium concentration.

2.6. Desorption studies

Desorption studies was conducted on the same fungicide soil solutions. After the sorption experiment, the supernatant was decanted and the tubes were reweighed. After, 9 mL of freshly prepared 0.01M $CaCl_2$ solution was added to the soil remaining in the centrifuge tubes and the samples were shaken for 24h at 90rpm, decanted and spectrophotometric measurements of the desorbed fungicides were made by visible adsorption spectrophotometry. Desorption, expressed as micrograms adsorbed/gram of soil (µg/g' soil), was obtained from the difference, taking into account the solution remaining in the soil after the supernatant was poured off.

2.7. Antifungal Bioassay

The invitro antifungal bioassay was carried out by Agar tube dilution protocol. Growth in the compound containing media was determined by measuring linear growth in millimeter. Concentration of each of the tested compound was 200µg/ml in DMSO. The antifungal bioassay was carried out against six species of fungi; *Trichophytonlongifusus, Candida albicans, Aspergillusfavus, Microsporumcanis, Fusariumsolani& Candida Glaberata.* The standard drug used was Miconazole for all the pathogens except for *Candida albicans* where Amphotecin-B was used.

2.8. Calculations

The adsorption values found were used to construct linear isotherm (C-type). [20,21]

$$X = K_d C_e \quad (1)$$

Where K_d is linear or sorption equilibrium distribution co-efficient in (mL ug^{-1}). X is the concentration of the pesticide adsorbed in (ug/g soil) and C_e is the pesticide concentration (ug/mL) at the equilibrium concentration.

Linear or sorption equilibrium distribution co-efficient, K_d, is related to soil OC and OM by following equations:

$$K_{OC} = 100\ K_d /\%OM$$

$$K_{OM} = 100\ K_d /\%OC$$

Since %OM/%OC=1.724, K_{OC} can be calculated K_{OC} (calc.) using K_{OM} and the factor 1.724. [22,23]

$$K_{OC}\ (calc) = 1.724\ K_{OM}$$

3. Results and discussion

In order to know the adsorption behavior with increasing concentration of the pesticides, the amounts of fungicide adsorbed were plotted against the equilibrium concentration of the fungicides in each soil sample. The adsorption isotherm thus obtained corresponded to a C-type isotherm. This type of isotherm is known to best describe soil sorption of hydrophobic organic contaminants which distribute themselves linearly between hydrophobic organic matter adsorbed on an inorganic mineral particle and solution. [19]

3.1. Carbendazim

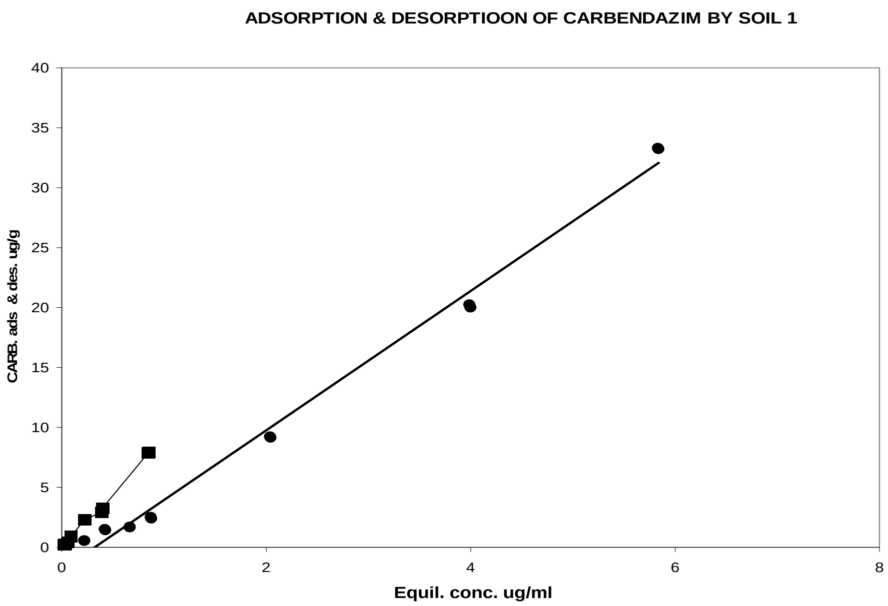


Fig. 1. Adsorption and desorption of Carbendazim by Soil

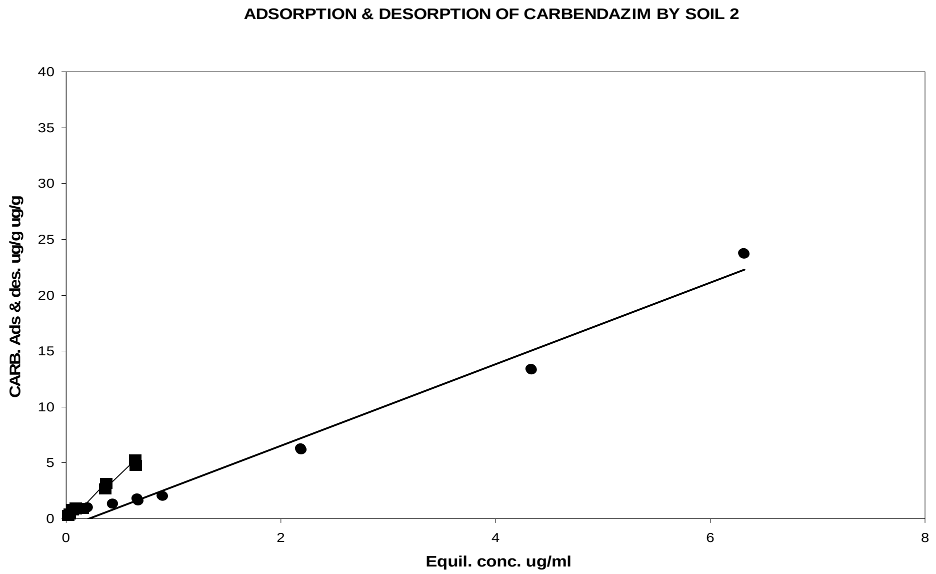


Fig. 2. Adsorption and desorption of Carbendazim by Soil 2

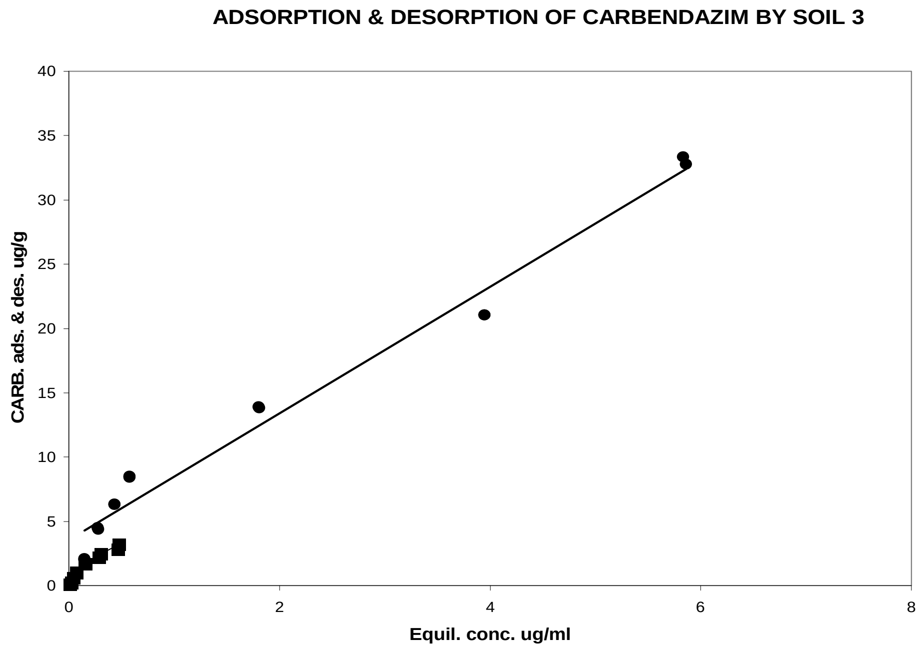


Fig. 3. Adsorption and desorption of Carbendazim by Soil3

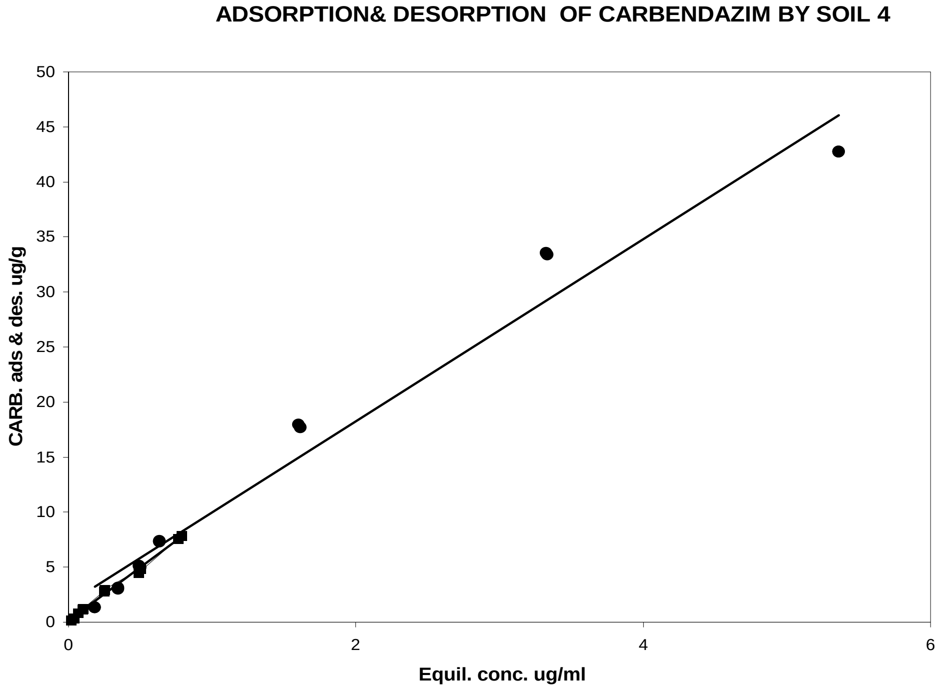


Fig. 4. Adsorption and desorption of Carbendazim by Soil 4

K_d, linear or sorption equilibrium distribution co-efficient in (mL ug^{-1}) was calculated by plotting concentration of the pesticide adsorbed in (ug/g soil) against C_e, which is the pesticide concentration (ug/mL) at the equilibrium concentration.

Table: 3 K_d values for adsorption and desorption of minerals and soils

Soils	K_d					
	CARB		FBNZ		ABNZ	
	K_d ads	K_d des	K_d ads	K_d des	K_d ads	K_d des
Soil 1	5.81	9.13	1.16	9.28	2.67	10.9
Soil 2	0.12	7.43	2.61	11.49	2.95	11.73
Soil 3	4.91	6.21	3.51	8.69	4.59	6.66
Soil 4	11.6	9.67	10.49	7.38	a	a

MBC is highly adsorbed on forest soil 4 than other soils .The different sorptive behavior exhibited by MBC on soil (1-4) are probably caused by the general physical/chemical soil and solution properties. For example variation in sorptive behavior was studied with change in pH. Forest soil 4 has higher organic contents (9.2%) and lower pH (7.6) as compared to other soils so its higher adsorption might be due that the ionized molecules of the fungicides could be absorbed by the organic fraction.

3.2. Adsorption of FBNZ

The close similarity of the molecular structures of benzimidazoles may be responsible for the values of K_d showing the similar behavior in soils and minerals. Structural features, hydrophobicity, organic matter content of soil, solubility are the main factors for the K_d values of ABNZ being significantly higher. Other results of sorption of FBNZ a newly synthesized benzimidazole showing good fungicidal activity, shows the adsorption isotherms were linear also. The amount of fungicides adsorbed (%) on the silica was more than alumina.

FBNZ when applied to the soils of varying organic content indicate that the sorption is associated with organic content. The amount of fungicides adsorbed (%) was in the order soil4>soil3>soil2>soil1 again shows the relationship of adsorption with increase in the organic matter contents.

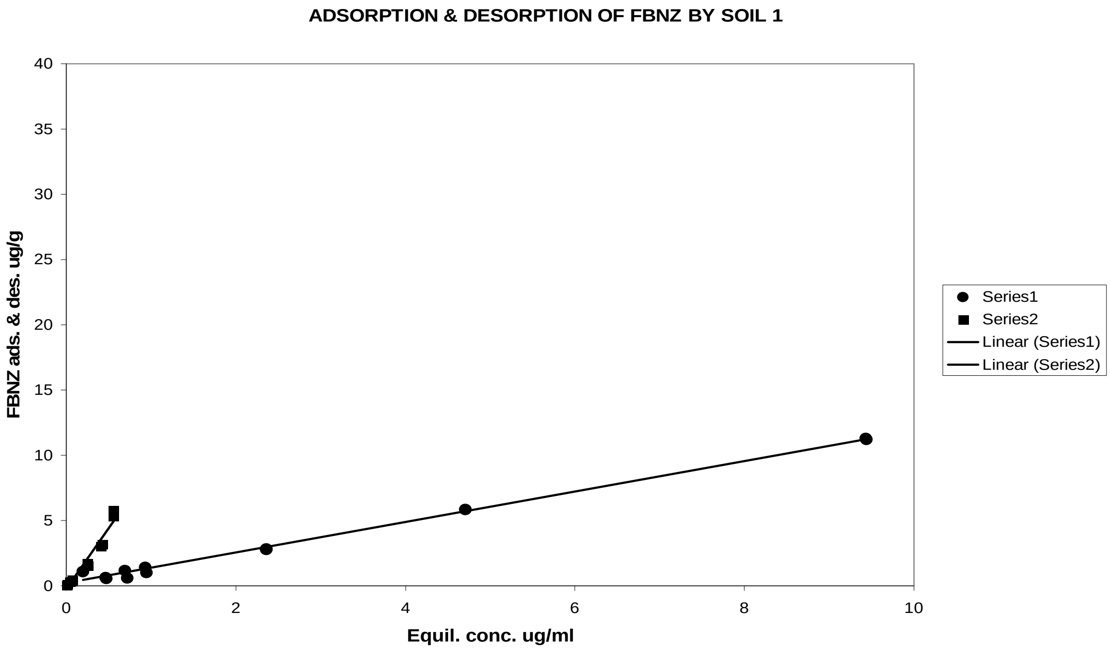


Fig 5. Adsorption and desorption of FBNZ by Soil 1

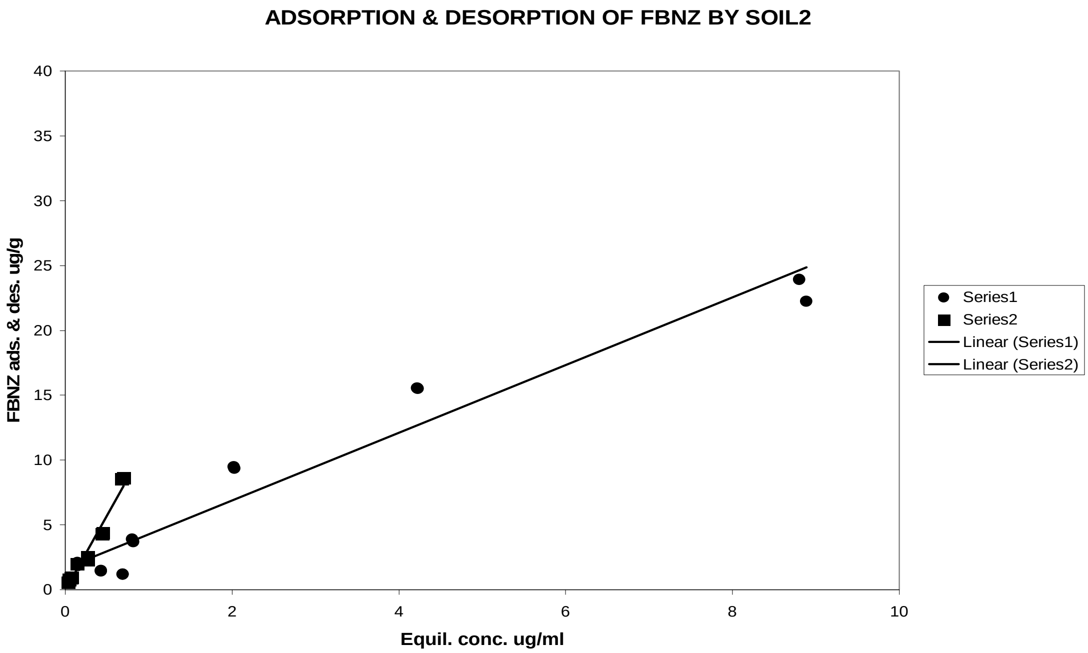


Fig 6. Adsorption and desorption of FBNZ by Soil 2

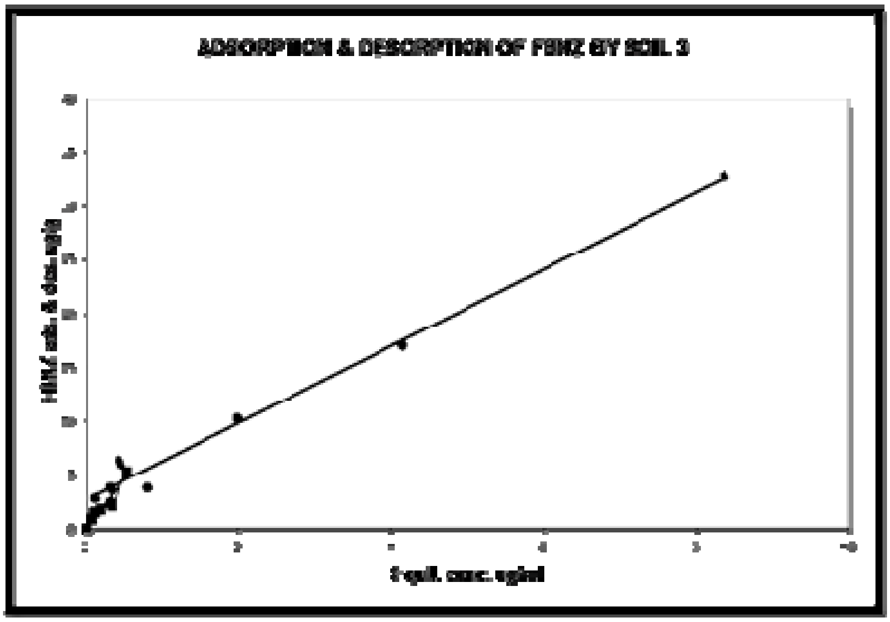


Fig 7. Adsorption and desorption of FBNZ by Soil 3

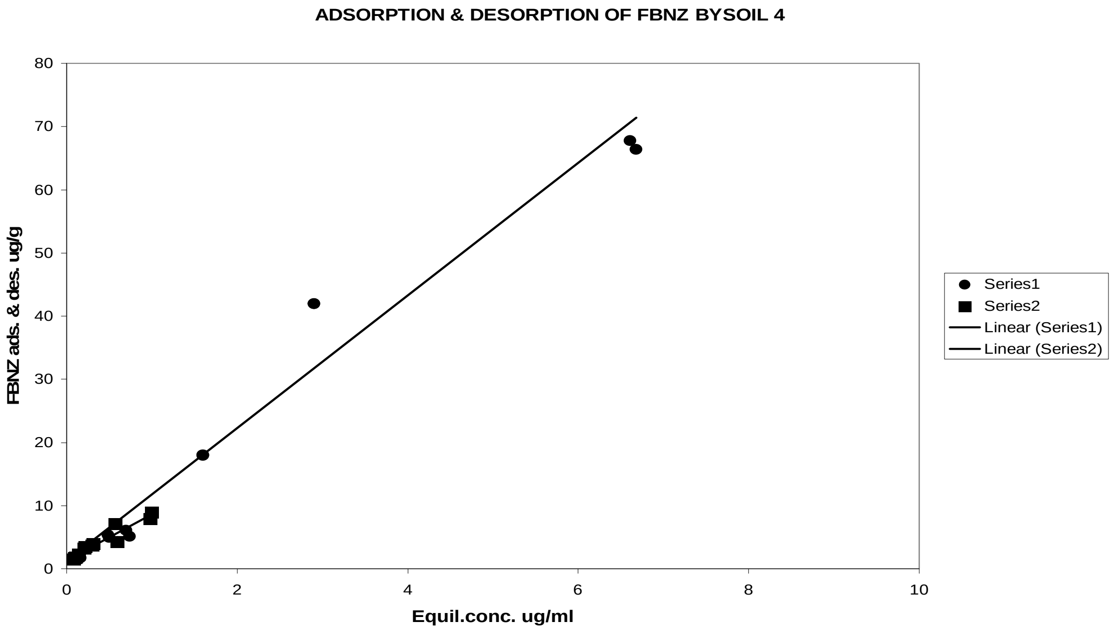


Fig 8. Adsorption and desorption of FBNZ by Soil 4

3.3. Adsorption of ABNZ

Adsorption of ABNZ showed very high value for montmorillonite as compared to rest of the minerals showing tight adsorption of ABNZ on montmorillonite than muscovite and silica. In soils the order of adsorption is same as FBNZ, soils (1-3) shows similar values of K_d.

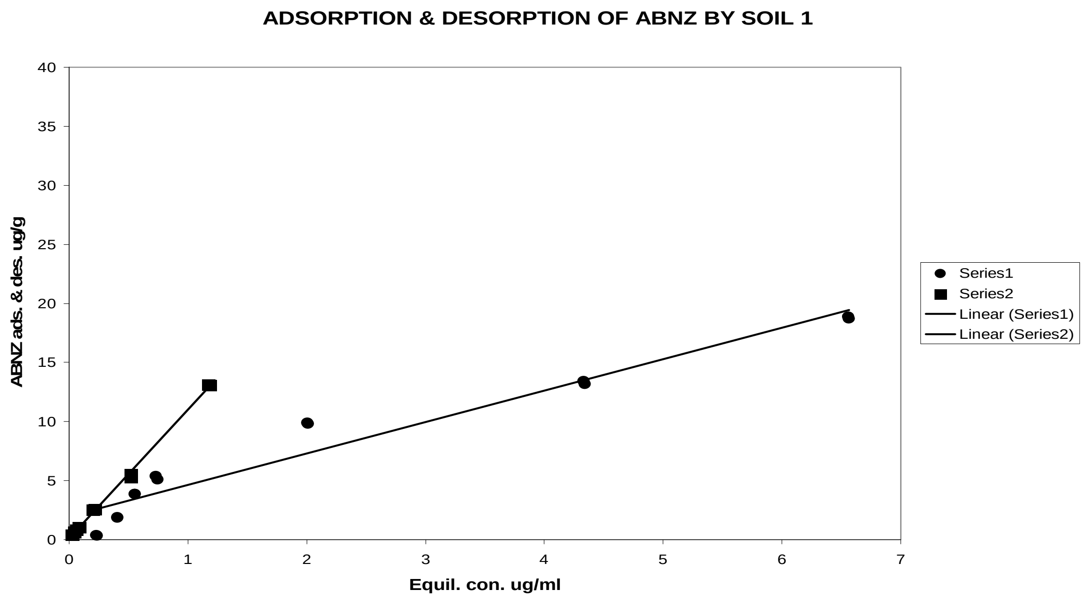


Fig 9. Adsorption and desorption of ABNZ by Soil

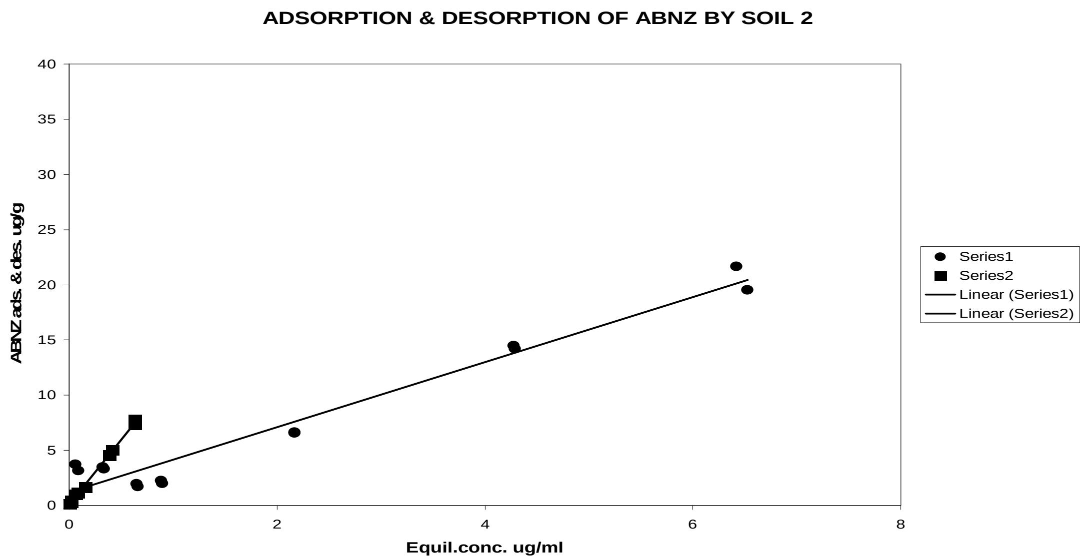


Fig 10. Adsorption and desorption of ABNZ by Soil 2

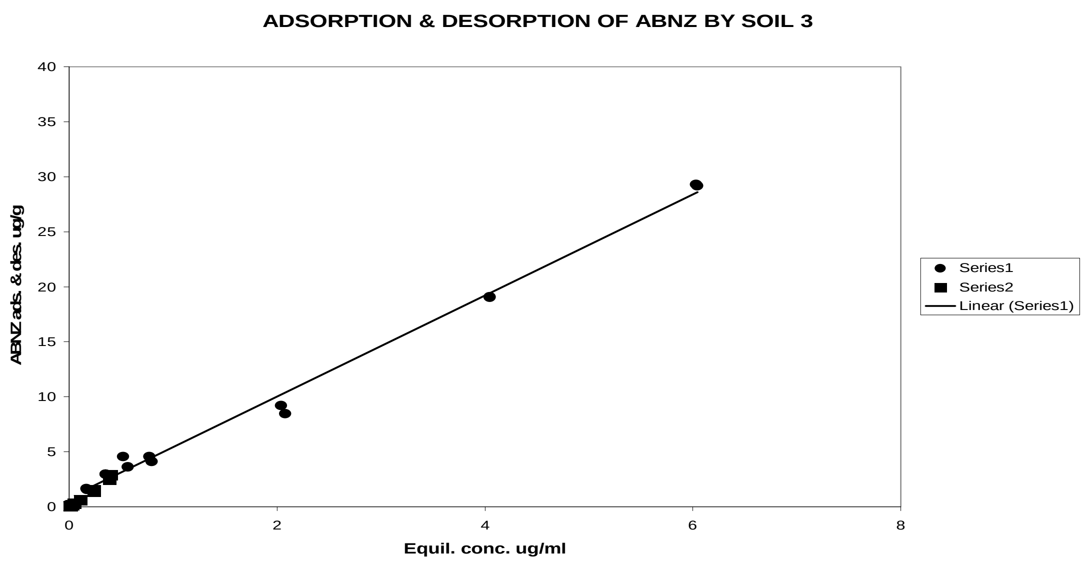


Fig 11. Adsorption and desorption of ABNZ by Soil 3

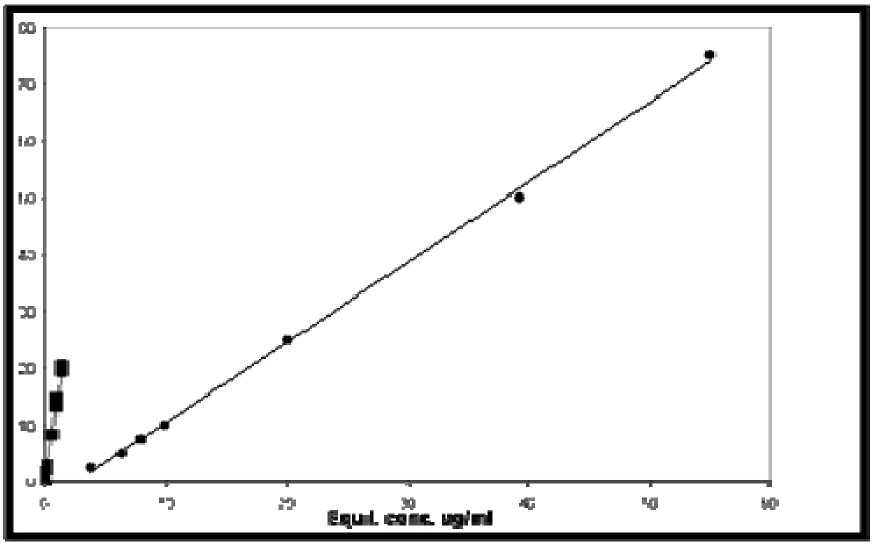


Fig 12. Adsorption and desorption of ABNZ by Soil 4

In all soil adsorption increases with increasing the concentration and no limiting adsorption was observed within the concentration range studied. The amount of fungicides adsorbed (%) was in the order of carbendazim >ABNZ > FBNZ in soil 1 from Tarnol. In the soil 2 from Multan, this order was ABNZ > FBNA > carbendazim. In red soil 3 from Murree this order was ABNZ > carbendazim > FBNZ, while the forest soil 4 from Ayubia has shown the order as carbendazim > FBNZ.

3.4. Desorption studies

Desorption isotherm represents the the microgram of the fungicide still adsorbed per gram of soil as a function of equilibrium concentration after one adsorption cycle.

Desorption studies showed that the Tarnol soil 1 lost nearly 54.49%, 35.78%, 68.75% of the carbendazim, FBNZ and ABNZ and soil 2 from Multan lost nearly 36.69%, 43.06%, 55.30% of the carbendazim, FBNZ and ABNZ and red soil 3 from N.W.F.P. lost nearly 47.14%, 15.06%, 16.95% of the carbendazim, FBNZ and ABNZ and forest soil 4 lost nearly

75.63%, 71.29% carbendazim and FBNZ after one desorption. The high percentage of desorption was found in soil 4 shows that adsorbed fungicides were not firmly retained by the soil particles.

The relative high value of $K_{d\ (des)}$ compared with $K_{d\ (ads)}$ for soils (1-3) shows adsorption was reversible. The $K_{d\ (des)}$ were lower in soil 4 indicates they are more prone to release the adsorbed fungicides.

3.5. Antifungal Activity

The sorption and transport behaviour of Benzimidazole derivatives and their possible impact on the environment was carried out in order to explain and compare differences in sorption behavior of FBNZ) and (ABNZ) with commercially available (MBC) to soil minerals of different ecosystems. Anti-fungal activity of these Benzimidazole derivatives was compared with the MBC to find out their effectiveness as fungicides which is shown in table 2.

Table 4: Anti-fungal activity of newly synthesized Benzimidazole derivatives

CARB	FBNZ
30%	35%
20%	-
30%	5%

(Concentration taken was 200 µg mL^{-1} at 27 ºC with incubation time of 7 days.)

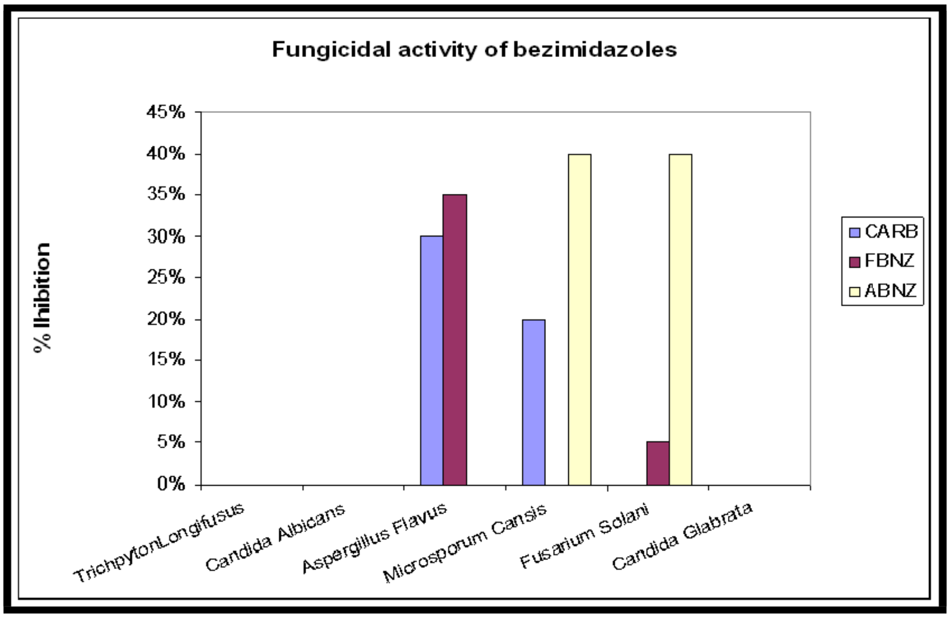


Fig. 13. Graphical measure of fungicidal activity of Benzimidazole derivatives

The FBNZ and ABNZ have shown good fungicidal activity against *Aspergillusflavus,* Microsporumcansisand solani as compared to MBC.

3.6. Linear Regression Analysis

Using linear regression analysis the effect of soil physicochemical properties on the adsorption was further investigated taking into consideration the properties and K_d values of selected soil.

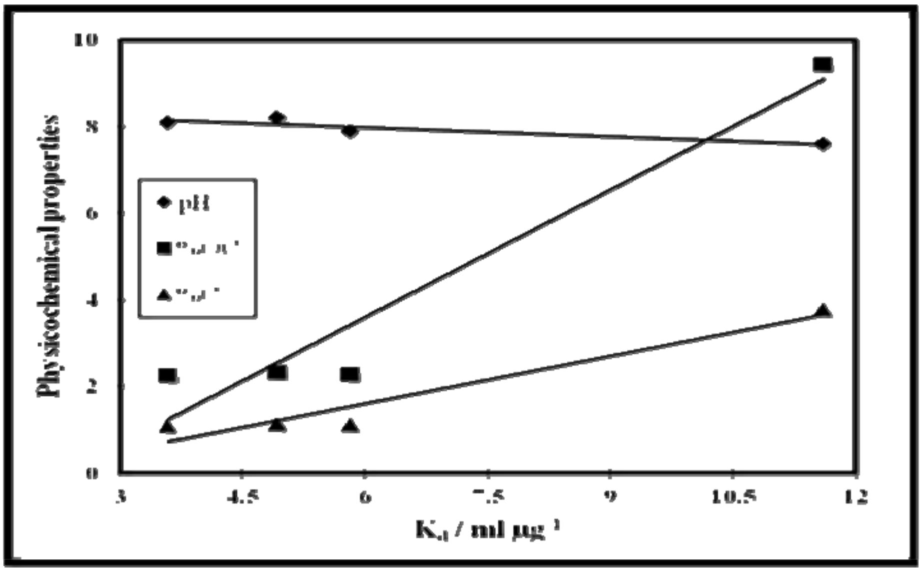


Fig. 14. Effect of pH, % OC, % C values on adsorption of three pesticides in studied soils

Table 5: Linear regression analysis for sorption properties of selected soils

Sorption coefficient	Property (x)	Correlation coefficient (r)	Prob. level (p)	Intercept (a)	Slope (b)
	pH	-0.960	0.033	8.396	-0.068
K_d	OC	0.875	0.125	-2.262	0.979
	OM	0.875	0.125	-0.577	0.365

A negative correlation was observed between K_d and pH which indicated the enhanced extent of MBC adsorption in soil samples with lowering pH. For whole soil the pH values are responsible for the dissociation or protonation processes of both the MBC and the adsorbent surfaces (soils). In contrast, a fairly strong positive correlation between the sorption coefficient (K_d) and percent of organic matter was observed as indicative of an increased adsorption of MBC with increasing OM. In addition a moderate positive correlation between soil moisture and K_d also suggested the influence of soil moisture content on adsorption of MBC.

4. Conclusion

In all soils adsorption increased with the increasing concentration of the pesticides and no limiting adsorption was observed within the concentration range studied.

The order of carbendazim > ABNZ > FBNZ in all soils indicated that carbendazim was adsorbed more in all of them and it is dependent on different properties of the pesticide such as its tendency for binding to soil, its structural features, hyrophobicity ,its vapor pressure, its water solubility, and its resistance to being broken down over time.Sorption was reversible and the order of adsorption was soil 4 > soil 3 > soil 2 >Soil l.

Highest adsorption on soil 4 is dependent on factors in the soil, such as its texture, its ability to retain water, and the amount of organic matter contained in it, its pH etc. Similarly the desorption values were constantly higher than those for adsorption in rest of the soils.Soil 4. having higher organic matter and low pH gave low value of adsorption as compared to desorption indicated that it is reluctant to release the fungicide and it will influence the uptake and metabolism of plant or microorganisms or other bioactivities in soil.

Data limitations are still the major obstacle towards establishing clear environmental trends in Pakistan. The environmental regulatory authorities of Pakistan can greatly benefited from these preliminary studies to conduct such studies on larger scales.

Extensive educational programs are required for capacity building of farmers as low level of knowledge of farmers requires the need for their proper awareness and training to mitigate health and environmental risks associated with the proper selection, usage and handling of pesticides.

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