

Research Article

Rapid Spectrophotometric Method for Determination of Imazethapyr Herbicide in Commercial Formulations and Environmental Samples

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Imazethapyr is an important and extensively used broad spectrum herbicide and due to its toxicity, it can adversely affect the environment and non-target organisms including human beings. So, it is of paramount importance to investigate its environmental pollution and behavior in soil. Therefore, a simple, sensitive and rapid method has been developed for its determination and to carry out the adsorption study of this herbicide on four Indian soils using batch equilibrium technique. In This method the amino group of imazethapyr is transformed to corresponding dithiocarbamate derivative (in aqueous acetonitrile) which react with copper(I) perchlorate to form yellow coloured complex, showing λ_{\max} at 375 nm. The method is quite sensitive and the molar absorptivity (ϵ) and Sandell's sensitivity were found to be $3.4 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0850 \mu\text{g cm}^{-2}$ respectively. The Groundwater Ubiquity Score (GUS), which indicates the leaching potential of a pesticide, was used to evaluate soil and ground water pollution. The GUS for imazethapyr ranges from 2.29 to 2.80, classifying it as a transition pesticide.

Keywords: Imazethapyr, Pollution and Health hazards, Spectrophotometric, Leaching, Soil Adsorption Study

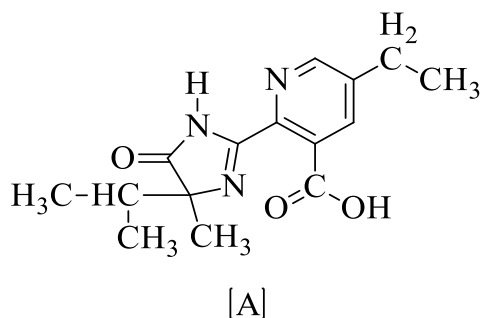
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Introduction

Weeds badly affects the agricultural produce by competing for nutrients, water, light, space and other growth requirements with crops and therefore reduce the crop yield to considerable extent. Chemical method of weed control is the cheapest and economically viable option, which offers good scope for harvesting a better crop [1-3]. Herbicides are a class of pesticides applied with the intention of killing, controlling or preventing the excessive growth of weeds or unwanted plants. Imazethapyr {5-ethyl-2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid}, (A), is an imidazolinone compound used as a broad spectrum, selective herbicide for the control of grassy broadleaf weeds in legumes, sunflowers, cereal grains and can also be used in the non-crop situations like forestry, rubber and oil palm plantation. [4-5]. Imazethapyr inhibit acetolactate synthase in plants, have high weed control efficacy and selectivity at low use rates and has flexibility in timing of application [4-7]. The farmers tend to increase the application of such herbicides to enhance the food crop production without taking into consideration the negative aspects associated with these chemicals.



By the excessive and repeated use of herbicides like imazethapyr, these may get accumulated as residues in the crops, soil, surface and ground water, thus severely damaging the environment, which ultimately may result in phytotoxicity to food crops, adverse effects on non-target organisms and ultimately severe health hazards to human and animals [8].

Imazethapyr is readily soluble in water, have low mammalian toxicity and does not adsorb strongly to soil [9-11]. Like other pesticides, the ultimate reservoir for this herbicide is the soil irrespective of its application target. Soils and sediments often called as geosorbents are important sinks for pesticides; the extent of adsorption on these geosorbents affects the various processes like bioactivity, mobility, persistence, toxicity, volatilization and bioaccumulation which operate on unadsorbed pesticide [12]. Therefore, from environmental point of view, thorough understanding of adsorption of Imazethapyr on soil is of paramount importance for the prediction of its movement in soils and aquifers. Also, its determination in residues on environmental samples for the purpose of monitoring pollution and health hazards is equally important. Aiming at these objectives it is therefore, inevitable to develop a simple, sensitive and reliable method based on a widely applicable technique for the analysis of imazethapyr.

The analysis of this herbicide has been accomplished by chromatographic [13-18], enzymatic [19, 20], voltammetric [21] and spectrophotometric methods [22]. Our efforts have led to development of a simple and rapid spectrophotometric method for the determination of imazethapyr using copper(I) perchlorate in aqueous acetonitrile. The proposed method is based on the reaction of amino function of imidazolinone group in imazethapyr with an excess of carbon disulphide, pyridine and copper(I) perchlorate which results in the formation of yellowish copper(I) imazethapyr-dithiocarbamate complex showing maximum absorbance (λ_{max}) at 375nm. The proposed spectrophotometric method has been successfully applied to the analysis of imazethapyr in commercial herbicide formulation for the purpose of quality control and its recovery from water samples. The method has also been validated to study the adsorption of imazethapyr herbicide on four Indian soils of different soil characteristics and various adsorption parameters viz. distribution coefficient or soil-adsorption coefficient (K_d), soil organic carbon partition coefficient (K_{oc}) and Gibb's free energy (ΔG°) have been calculated. Further the leaching potential of imazethapyr herbicide has also been evaluated in terms of Groundwater Ubiquity Score (GUS).

Experimental

Apparatus and chemicals

Varian-Cary100BioUV-visible spectrophotometer was used for measurement. Acetonitrile (Merck Schuchardt OHG, Germany) was kept over phosphorus pentoxide (5gL^{-1}) and distilled twice. The analytical standard of imazethapyr (Sigma-Aldrich Loabochemikalien GmbH, Munich, Germany) was used, and its stock solution (10^{-3}mol L^{-1}) was prepared in distilled water. A formulation (Pursuit) of imazethapyr containing 10% active ingredient (SL) was procured from local authorized pesticide dealer. A standard solution of copper(I) perchlorate, 10^{-2}molL^{-1} was prepared as described earlier [23].

Soil samples

The soils samples from Solan and Lahaul & Spiti region of Himachal Pradesh were air-dried, crushed with wooden mortar and sifted through 2 mm sieve, dried and sieved soils samples were placed in sealed glass jars and stored at room temperature.

Preparation of calibration graph for imazethapyr

Aliquots (0.1-2.0 mL) of the standard solution (10^{-3}mol L^{-1} in water) of imazethapyr were taken in 10 mL measuring flasks and volume made to 2mL with water. Each solution was mixed with 1mL acetonitrile, a drop ($\sim 100\mu\text{L}$) each of carbon disulphide and pyridine and finally 1 mL of copper(I) perchlorate (10^{-2}mol L^{-1} in acetonitrile) was added. The flasks were kept in a 500 mL beaker containing 200 mL water and then placed in microwave irradiation for 50 seconds for the completion of the reaction. The final volume made to 10 mL with acetonitrile. The absorbance of the resulting yellow colour was measured at 375 nm for imazethapyr (**Figure 1**). The absorbance values were plotted against concentration of pure compound used and a calibration curve was prepared (**Figure 2**).

Formulation Analysis

A soluble granule formulation, containing 10% active ingredient, SL, pursuit, procured from local market was used. A single large sample of formulation equivalent to 5mg active ingredient was shaken with water and filtered. The residue was washed 2-3 times with 5 mL water. The filtrate and washings were diluted to a known volume (50 mL) with the same solvent. Suitable aliquots of the above solution were taken and processed for analysis as described above for the pure compound.

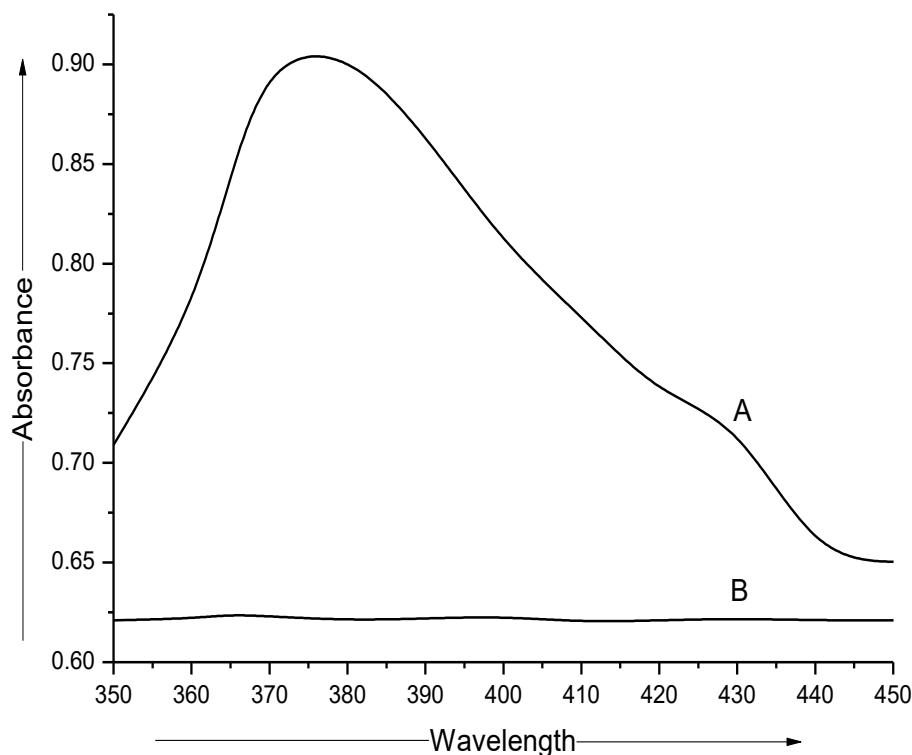


Figure 1 Absorption spectra of (A) Copper(I) imazethapyr (as its dithiocarbamate) complex (B) Reagent blank

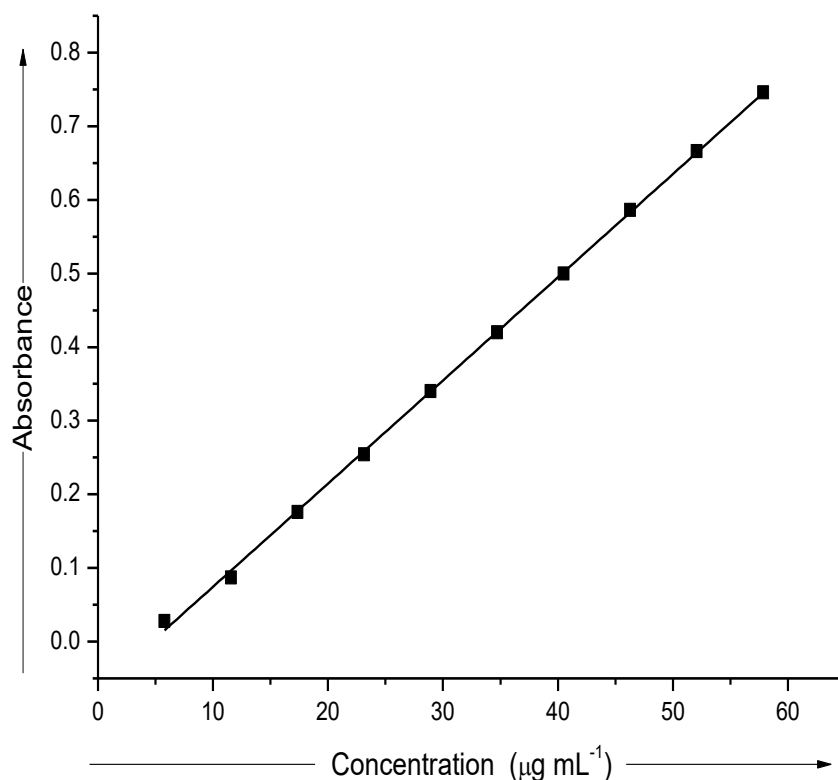


Figure 2 Relationship between absorbance and concentration (Calibration graph) for imazethapyr as coloured copper(I) dithiocarbamate complex

Determination of adsorption parameters

Imazethapyr adsorption isotherm on four Indian soils of different soil characteristics (**Table 1**) were obtained by the batch equilibration technique using 50 mL conical flask. Triplicate soil samples (2g) were equilibrated with imazethapyr solutions in the concentration range from 46.30-231.14 $\mu\text{g mL}^{-1}$ by shaking mechanically (150 rpm) at room temperature ($20 \pm 1^\circ\text{C}$) for 24h equilibration time.

Table 1 Characteristics of the different soils used for adsorption study of imazethapyr.

Soil Sample	pH	Clay (%)	O C (%) [¥]	CEC [€] (meq/100g)
I	5.69	26.30	2.20	37.38
II	7.49	9.80	1.06	17.59
III	6.90	10.20	0.82	12.80
IV	6.80	9.80	0.64	11.50

OC[¥] = Organic carbon; CEC[€] = Cation exchange capacity

After equilibration, the suspensions were centrifuged and the equilibrium concentrations (C_e) were determined in supernatants by the method described above. Adsorption isotherms were evaluated by using Freundlich's adsorption equation which is written as:

$$X = K_f C_e^{n_f} \quad (1)$$

where X is the amount of pesticide adsorbed mg Kg^{-1} of the adsorbent; C_e is the equilibrium solution concentration (mgL^{-1}); K_f and n_f are adsorption coefficients which are calculated from the least square methods applied to the linear form of the Freundlich's adsorption equation. Other parameters for the adsorption process viz. distribution coefficient or soil adsorption coefficient (K_d), Gibb's free energy (ΔG°), soil organic carbon partition coefficient (K_{oc}) and Groundwater Ubiquity Score (GUS) have been calculated by using equations 2-5 respectively [24-26].

$$K_d = \frac{X}{C_e} \quad (2)$$

$$K_{oc} = K_d \times \left(\frac{100}{\%O.C.} \right) \quad (3)$$

$$\Delta G^\circ = -RT \ln K_d \quad (4)$$

$$GUS = \log t_{1/2} [4 - \log(K_{oc})] \quad (5)$$

where R = gas constant, T = absolute temperature, $t_{1/2}$ = pesticide persistence (half-life), OC = organic carbon content of soil. On the basis of GUS values, the pesticides can be classified as leacher ($GUS > 2.8$), transition ($2.8 > GUS < 1.8$) and non-leacher ($GUS < 1.8$) [26].

Result and Discussion

The smooth and quantitative transformation of herbicide to yellowish colored copper(I)-dithiocarbamate derivative (showing maximum absorbance at 375 nm) by the reaction of amino group of imazethapyr with copper(I) perchlorate in the presence of carbon disulphide and pyridine in aqueous acetonitrile medium forms the basis of present spectrophotometric method. Further the reaction rate is enhanced by the microwave heating which is quite different from conventional heating as it dissipates heat inside the medium and raises the energy of the molecules rapidly. The highly energized molecules usually result in higher reaction rates and greater selectivity [27]. Effect of time of microwave heating on the development and stability of colour was studied and it has been observed that maximum color intensity and stability was obtained corresponding to 50 second microwave heating. Moreover, the copper(I) perchlorate reagent forms a colorless solution in acetonitrile which is extremely stable. The kinetic stability of colored complex was studied with time and the color is found to be stable for at least 120 minutes (**Figure 3**). The excess of carbon disulphide and pyridine do not cause any interference. That the reaction proceeds in 1:1 molar ratio has also been established by photometric titration of imazethapyr with copper(I) perchlorate in aqueous acetonitrile. In this titration performed at 375nm (λ_{max} of coloured complex, Figure 1) the absorbance increases till imazethapyr to copper(I) molar ratio 1:1 is achieved indicating the formation of coloured complex (X) and thereafter it attains almost constant values indicating no more formation of coloured complex, an inverted L-shaped titration curve is obtained (**Figure 4**). On the basis of the above facts, the most plausible course of reaction for the formation of the yellowish coloured copper(I) imazethapyr-dithiocarbamate complex is:

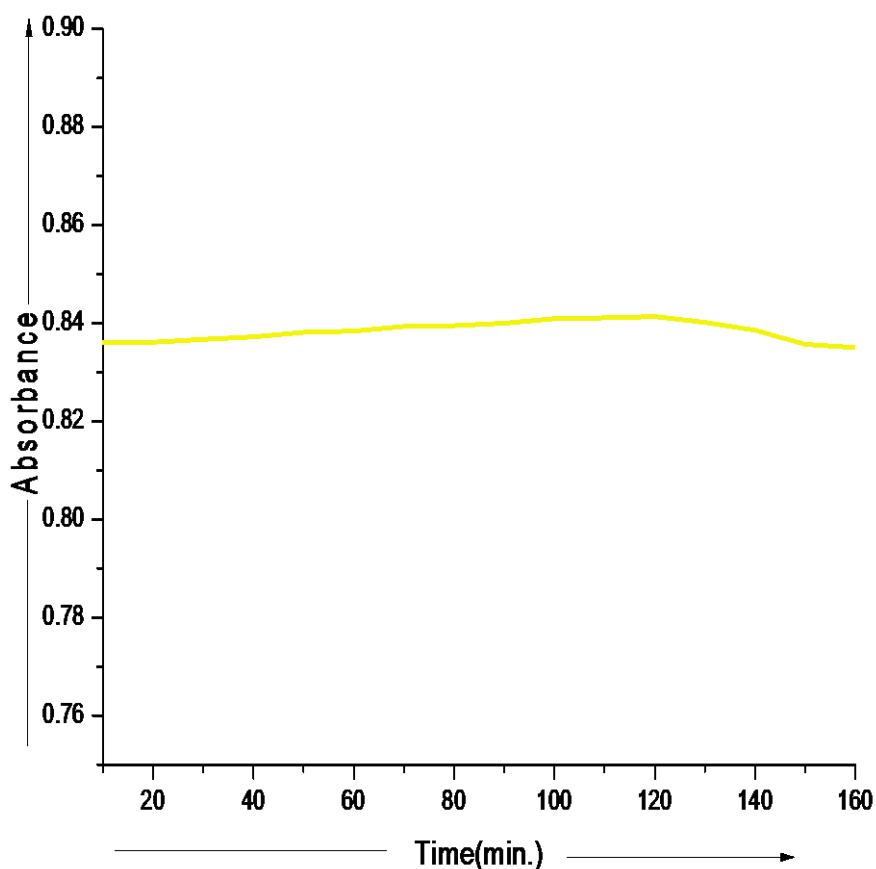
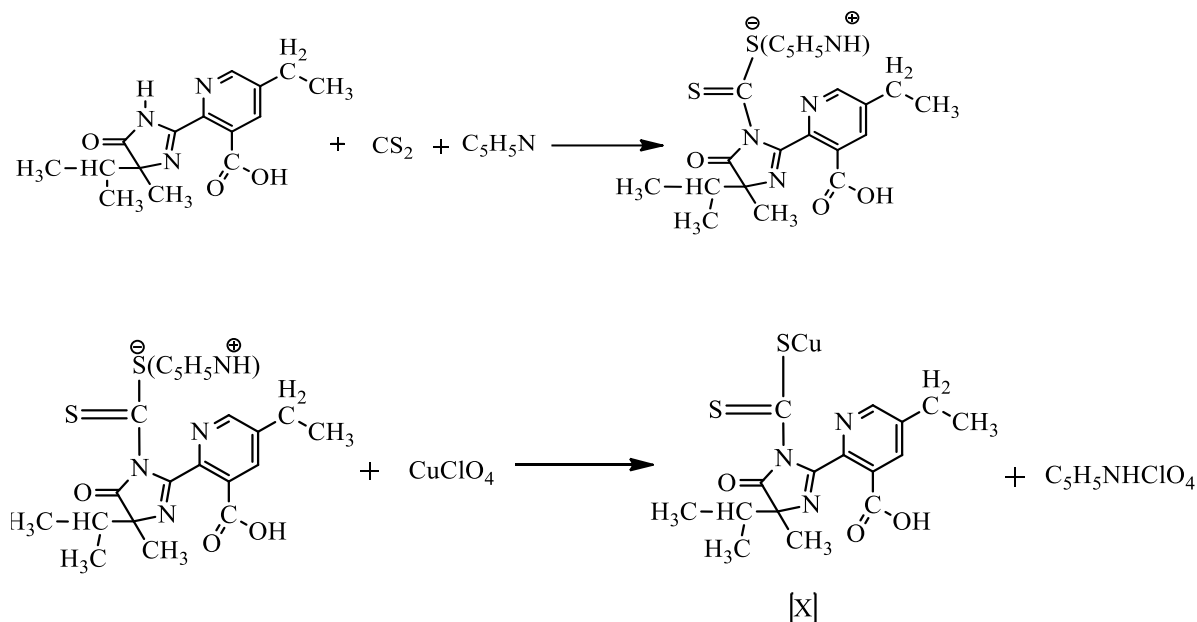


Figure 3 The Kinetic stability of copper (I) imazethapyr as dithiocarbamate complex

The proposed spectrophotometric method obeys Beer's law in the range of 5.8-57.9 $\mu\text{g mL}^{-1}$ of imazethapyr. The method is quite sensitive and the molar absorptivity (ϵ) and Sandell's sensitivity were found to be $3.4 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0850 \mu\text{g cm}^{-2}$ respectively at 375 nm. The method has successfully been applied to the analysis of a commercial formulation of imazethapyr for its active ingredient content and for the determination of herbicide in spiked water samples. The formulation analysis is essential not only for the purpose of quality control but also to get reliable adsorption data. The assay results of formulation ranges from 98.4-99.2%, with RSDs in the ranged 0.74-0.92% while the recoveries from water samples ranged from 92.6-94.7%, with RSDs 0.90-1.06% (**Table 2**).

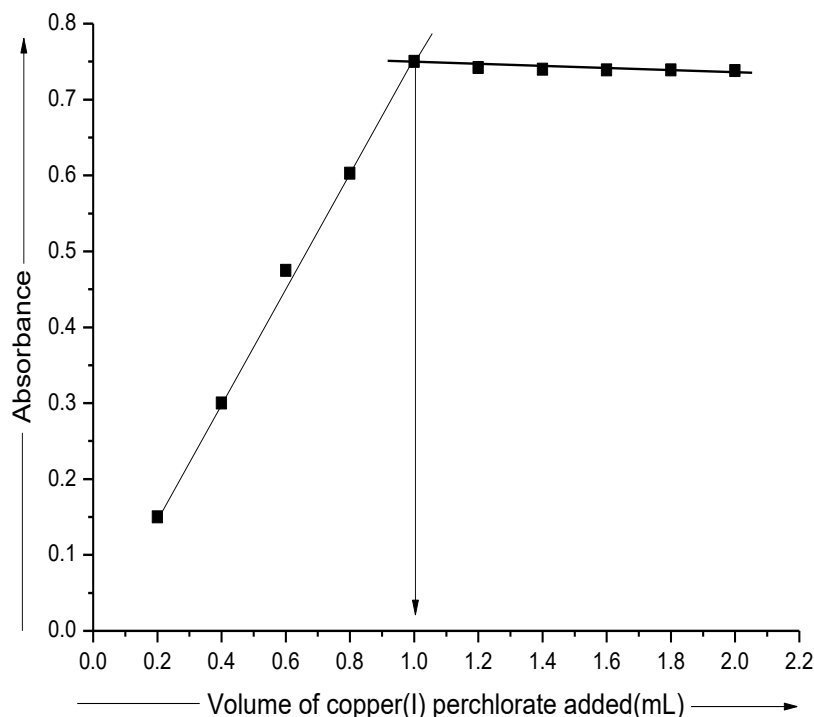


Figure 4 Photometric titration curve for imazethapyr as dithiocarbamate with copper(I) perchlorate

Table 2 Assay of a commercial formulation of imazethapyr containing 10% active ingredient and recovery from water samples

Active ingredient taken (μg)	Values are mean of three determinations with standard deviations (\pm)	
	Formulation Analysis	Recovery (%) from water samples
10.0	98.8 ± 0.86	92.6 ± 0.94
20.0	99.2 ± 0.83	93.2 ± 0.90
30.0	98.7 ± 0.92	94.7 ± 1.06
40.0	98.4 ± 0.74	93.8 ± 1.02

The method has also been applied with success to the adsorption studies of this herbicide on four Indian soils. The adsorption isotherms of imazethapyr herbicide on four Indian soils of different soil characteristics (Table 1) are shown in **Figure 5**. According to the Gile's classification the adsorption isotherms imazethapyr herbicide may be classified as L-type [28, 29]. This type of isotherm indicates that the solid surface has an average affinity for the pesticide and molecules of the pesticide are most likely to be adsorbed in a flat position and they do not suffer a strong competition from solvent molecules. K_f and n_f are Freundlich constants related to the adsorption capacity and adsorption intensity respectively which are calculated from the plot of $\log X$ versus $\log C_e$ (**Figure 6**) and results are presented in **Table 3**. The observed value of $n_f < 1$ for the adsorption of imazethapyr herbicide indicates a typical chemisorptions process. It means that with the increase in the concentration of pesticide the adsorption decreases which might be due to the fact that at higher concentration, there is an increased difficulty to access the adsorption site [30]. The K_f values for four soil types used are in the order Soil I > Soil II > Soil III > Soil IV which might be related with the higher OC, clay and CEC values of soil I.

Other parameters viz. K_d , K_{oc} and GUS for the adsorption of imazethapyr were also calculated using equations 2-5 and are presented in Table 3. The pesticide soil adsorption coefficient, K_d , and the soil organic carbon adsorption coefficient, K_{oc} , are the important parameters used for describing the adsorption behaviour and environmental fate of pesticides as these quantify the strength of pesticide adsorption and their mobility in soil and adsorbents. The soil adsorption coefficient (K_d) values for imazethapyr were observed 1.26, 1.20, 1.10 and 1.0 for soil I, soil II soil III and soil IV respectively. From the k_d values it can be inferred that imazethapyr is adsorbed maximally in case of soil I (with higher k_d) owing to its higher CEC, OC and clay content, but the overall adsorption of this herbicide is less in the all soil types. The introduction of (K_{oc}) which is calculated by normalizing adsorption coefficient (K_d) with the organic carbon (OC) content of the soil, eliminates the effect of organic content in the soils on adsorption [31]. The K_{oc} values ranges from 57-149 for the four soil types. The lower values of k_d and K_{oc} for imazethapyr indicates lesser adsorption and weak binding of this herbicide onto soil thus making it more mobile in soils, the same has been also reported by many workers [17, 32-33].

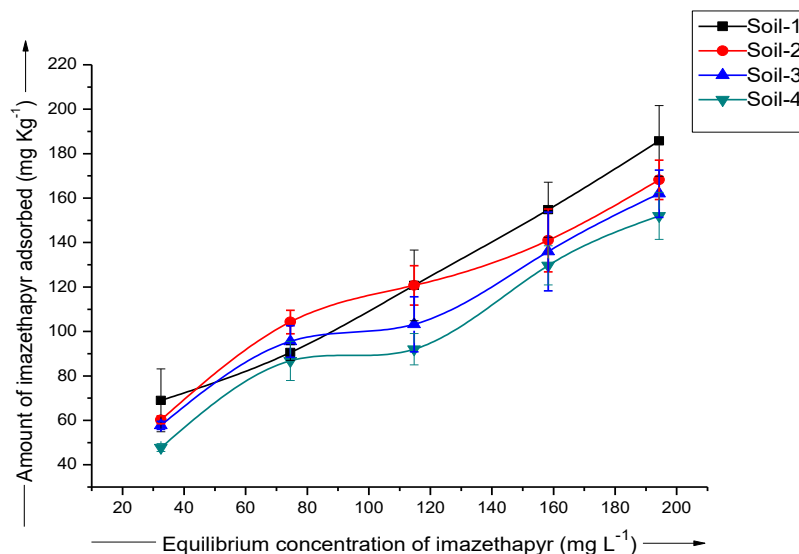


Figure 5 Adsorption isotherm of imazethapyr on four soils (error bars represent the standard deviation of three replicates)

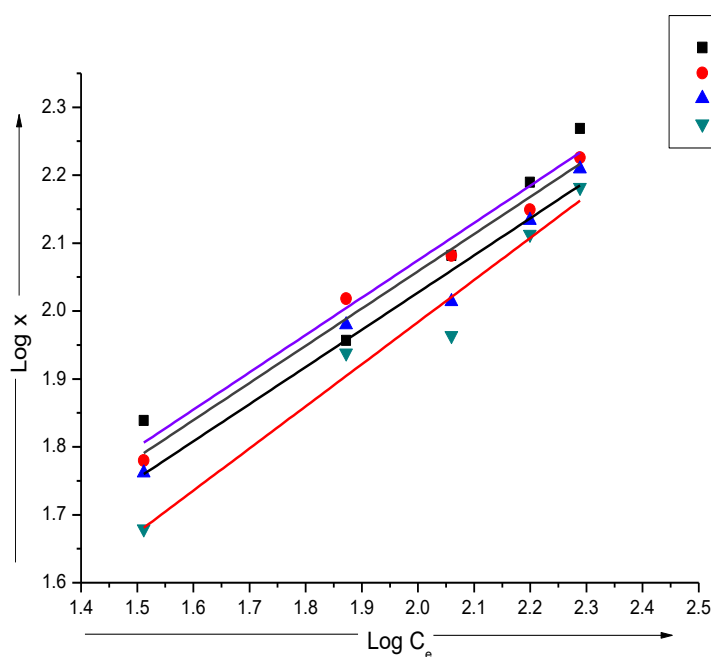


Figure 6 Plot of $\log X$ versus $\log C_e$ for the evaluation of Freundlich's adsorption coefficients K_f and n_f for imazethapyr

Table 3 Adsorption parameters of imazethapyr on four Indian soils at $20 \pm 1^\circ\text{C}$

Soil Type	K_f	n_f	K_d	K_{oc}	GUS	ΔG ($\text{kJ K}^{-1} \text{mol}^{-1}$)	Adsorption isotherm
I	9.44	0.55	1.26	57.50	2.80	-0.56	L-type
II	8.95	0.56	1.20	113.03	2.44	-0.44	L-type
III	8.13	0.56	1.09	133.85	2.53	-0.21	L-type
IV	4.78	0.64	1.01	148.96	2.29	-0.02	L-type

The organic matter content (OC), clay content and cation exchange capacity (CEC) of soils are the important factors of soils which affects the adsorption of pesticide. It has been reported that the CEC and clay content are more important for the adsorption of imazethapyr which has also been supported in the present study [33]. The value of Gibb's free energy (ΔG°) for the adsorption of imazethapyr herbicide was observed negative in all the cases suggesting the energetically favorable adsorption process. As indicated from the low values of K_d and K_{oc} , imazethapyr is weakly adsorbed in all four tested soils, consequently, this herbicide can leach into ground water,

similar studies has also been reported in literature [34]. Further, the leaching potential of this herbicide is calculated on the basis of Groundwater Ubiquity Score (GUS), the most commonly used model, which relate pesticide persistence (half-life) and adsorption in soil (K_{oc}) of the pesticide. The leaching potential of imazethapyr in terms of GUS index was determined by using experimentally observed K_{oc} value for each soil sample and its literature reported half-life of 18 days of imazethapyr [35]. The observed GUS score for imazethapyr ranges from 2.29 to 2.80 for the four soils used which classifies it as a transition herbicide [26].

Conclusion

The proposed spectrophotometric method for the determination of imazethapyr possesses advantage in terms of the instantaneous development of the colour with a colorless copper(I) reagent, sufficient stability of the colour as well as reagent solution, non-extraction of the coloured product (a time-consuming feature, common in colorimetric analysis), well established stoichiometry of the colour reaction and above all simplicity and rapidity of the procedure. The adsorption isotherm of imazethapyr has been found to be L-type. The K_{oc} and K_d values suggest lesser adsorption of this herbicide in soil types. On the basis of GUS values of imazethapyr, it can be classified as transition herbicide. So, the weaker adsorption, higher GUS value and the higher solubility of imazethapyr in water might increase its movement in soil resulting in leaching of this herbicide and which can cause ground water contamination. Use of organic amendments and adjusting the application dose according to soil properties might be an effective management practice for controlling potential pollution.

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