A Study towards the evaluation of leaching behavior of zineb fungicide

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ABSTRACT

The evaluation of leaching behavior of pesticides is one of the major steps to control environmental and health hazards associated with their uses. The pesticide leaching is operative only on unadsorbed fraction of the pesticide and influence the extent of surface and ground water contamination. In the present manuscript, leaching behavior of zineb fungicide in terms of ground ubiquity score (GUS) has been evaluated based on its adsorption on soils of different characteristics. To accomplish the above objective, a spectrophotometric methodology has been developed which is based on the measurement of yellow copper(I) bis-dithiocarbamate complex at 354 nm formed from the reaction of zineb with copper(I) perchlorate. The GUS values in the range 1.2-1.4 classify it as non-leacher fungicide. If used in recommended dose, it does not represent hazard to ground water contamination.

Keywords: Agro-environmental pollution, leaching, soil adsorption, zineb

1. Introduction

Pesticide chemicals are used worldwide to improve agricultural production by preventing crop losses before and after harvesting. The excessive use of pesticides in agriculture has resulted in pollution of soil and water due to their leaching and migration to water sources. These exogenous chemicals ultimately enter the living organisms through drinking water or food chains and lead to various health effects viz. carcinogenesis, mutagenesis, teratogenesis and various other developmental effects (Tuormaa, 1995). Concern about the environmental and health hazards of excessive use of pesticides has prompted research into their environmental fate which is mostly played out in the soil. Soil is an ultimate reservoir of pesticide chemicals irrespective of their application target i.e. whether applied directly to soils or received indirectly from spray drifts or with washing of remaining deposits on treated sites by rain or irrigation water. In soil, the pesticides get fractionated between soil solution phase (in free form) and soil solid phase through adsorption on clay and organic fractions (in bounded form) (Wauchope et al., 2002). Pesticide adsorption by the soil is a naturally occurring phenomenon which affects its volatilization, persistence, mobility and toxicity which in turn affect surface and groundwater contamination (Iraqi and Iraqi, 2000). The free detection of pesticides in surface and ground water has led to a number of experimental studies on their adsorption in soils for evaluation of their leaching behavior (Jaya et al., 2009; Vischetti et al., 2002; Kuisi, 2002; Kumar and Philip, 2006; Nemeth-Konda et al., 2002). Hence thorough understanding of pesticide adsorption in soil for predicting their movement into water bodies through leaching is of great importance to environmental regulation and pollution control.
Zineb is an agricultural fungicide used to control fungal diseases on fruits, vegetables, grains and nut crops. Toxicological studies have revealed that the fungicide has low acute toxicity with oral LD<sub>50</sub> values for rats ranges from 1850 to 8900 mg/Kg (Dikshit and Diwan, 2003). However, it has been reported to exhibit significant toxic effects on hepatic system, reproductive system and developmental processes (Karim, 1997; Soloneski et al., 2001; Jia and Mishra, 2007). Further, significant pathological changes in liver, kidney and spleen, heart congestions and brain hemorrhage have also been reported (Karim, 1997). Because of the wide range of uses and associated toxic effects of zineb, there is also a concern about its potential effects not only on workers who handle and often come in contact with this chemical, but also on general population from dietary exposure to residues left on food crops and drinking water. Of no less importance is the determination of this fungicide in residues on environmental samples for the purpose of monitoring pollution and health hazards.

Various analytical methods viz. chromatographic (Jongen et al., 1991; Lo et al., 1996), flame atomic absorption spectroscopic (Turker and Sezer, 2005), voltammetric (Lin et al., 1999), capillary electrophoretic (Aulakh et al., 2007), spectrophotometric methods (Rao and Verma, 1989; Kesari and Gupta, 1998; Agrawal et al., 1992; Malik et al., 2000; Saini et al., 2012; Kaur et al., 2009) have been reported in the literature for the determination of zineb. Spectrophotometric procedure can tolerate little interfering materials and the equipment being cheap and easily accessible in all the laboratories, it is extensively used for the determination of the fungicide. Some of the spectrophotometric procedures based on its acid hydrolysis followed by the measurement of evolved carbon disulphide. This carbon disulphide evolution method itself is time consuming, tedious and requires special apparatus and experimental skills. Further, in view of the difficulty of dispersing these water insoluble materials, the analysis is required to be repeated several times in order to obtain reproducible results. In the present study, a new, simple, rapid, selective and sensitive spectrophotometric method has been developed for the determination of zineb and subsequently validated to accomplish above objectives. The method is based on the reaction of zineb with copper(I) perchlorate in 1:2 molar ratio in DMSO-acetonitrile (1:4 v/v) media to form intense yellow copper(I) bis-dithiocarbamate complex, showing maximum absorbance (λ<sub>max</sub>) at 354 nm.

The capacity of a soil to adsorb or retain a pesticide is the key parameter to know the extent to which it will leach through soil to groundwater and adsorption is related to soil characteristics viz. clay content, organic matter content, cation exchange capacity and pH. Therefore, proposed method has been validated to study the adsorption of zineb fungicide on two Indian soils of different soil characteristics to evaluate its leaching potential and surface and ground water contamination. The various adsorption parameters viz. distribution coefficient or soil-adsorption coefficient (K<sub>d</sub>), soil organic carbon partition coefficient (K<sub>oc</sub>) and Groundwater Ubiquity Score (GUS) for zineb adsorption have been calculated. The method has also been applied to the analysis of zineb in commercial fungicide formulation (for the purpose of quality control) and its recovery from water samples (to monitor health hazards). The formulation analysis is essential not only to ensure the quality of marketed products of the pesticide but also to get reliable residue/adsorption data.

2. Material and methods

Acetonitrile (Merck, Mumbai, India) was kept over phosphorus pentaoxide (5 g L<sup>-1</sup>) and distilled twice. The analytical standard of Zineb was supplied by Environmental Protection Agency N.C. U.S.A. Dimethylsulphoxide (DMSO) was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and was used as received. A standard solution of
copper(I) perchlorate, 0.01 M in acetonitrile was prepared by the method as described earlier (Verma et al., 1984). All the spectrophotometric measurements have been made with carry100Bio UV-Vis spectrophotometer. A shaking incubator (PT-422) manufactured by Popular Traders, Ambala, India was used in the soil adsorption study.

2.1 Preparation of Calibration Graph for Pure Compound

Aliquots (0.1-2.0 mL) of standard solution of zineb (5×10^{-4} M in DMSO) were taken in 10 mL measuring flasks and diluted to 5 mL with DMSO. To each solution was added 1 mL water and volume made 9 mL with acetonitrile. Each solution was mixed with 1 mL of copper(I) perchlorate (0.01 M in acetonitrile). The absorbance of yellow colored solution was measured at 354 nm against a reagent blank and the absorbance values were plotted against concentration of pure compound used and calibration curve was prepared (Figure 1).

![Figure 1: Spectrometric determination of Zine b: relationship between absorbent and concentration (Calibration graph)](image)

The calibration characteristics viz. Beer’s law range, molar absorptivity, Sandell’s sensitivity, slope and intercept values and correlation coefficient calculated from calibration graph are given in Table 1.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Optical Characteristics</th>
<th>Corresponding value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(\lambda_{\text{max}})</td>
<td>354 (nm)</td>
</tr>
<tr>
<td>2.</td>
<td>Beer’s law range</td>
<td>1.38-27.56 ((\mu g \text{ mL}^{-1}))</td>
</tr>
<tr>
<td>3.</td>
<td>Molar absorptivity ((\varepsilon))</td>
<td>0.77×10^{4}(\text{Lmol}^{-1}\text{ cm}^{-1})</td>
</tr>
<tr>
<td>4.</td>
<td>Sandell’s sensitivity</td>
<td>0.036 (\mu g \text{ cm}^{2})</td>
</tr>
<tr>
<td>5.</td>
<td>Stability of color</td>
<td>90 (min.)</td>
</tr>
<tr>
<td>6.</td>
<td>Slope</td>
<td>0.03214</td>
</tr>
<tr>
<td>7.</td>
<td>Intercept</td>
<td>0.00799</td>
</tr>
<tr>
<td>8.</td>
<td>Correlation coefficient (R)</td>
<td>0.99897</td>
</tr>
</tbody>
</table>
2.2 Formulation analysis

Dithane Z-78, a zineb commercial formulation containing 75% active ingredient (WP), procured from an authorized pesticide dealer was used. A single large sample of the formulation equivalent to 10 mg active ingredient was shaken with DMSO and filtered. The residues were washed 2-3 times with DMSO. The filtrate and washings were diluted to 50 mL with DMSO. Suitable aliquots (0.1-0.4 mL) of this solution were taken and processed for analysis as described above for the pure compound. Table 2 gives the assay results.

<table>
<thead>
<tr>
<th>Amount taken (µg)</th>
<th>Recovery (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Present method</th>
<th>Comparison method&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>97.6±0.68</td>
<td>98.8±0.77</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>98.3±0.92</td>
<td>97.9±0.86</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>95.7±0.55</td>
<td>89.9±1.03</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>98.9±1.01</td>
<td>94.7±1.19</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean±standard deviation for 5 determinations.
<sup>b</sup>Rao and Verma, 1989.

2.3 Determination of Zineb in grains (Wheat and Rice) and apple fruit samples

Zineb standard solution was prepared by dissolving 6 mg fungicide in 50 mL DMSO; this solution was further diluted 10 times with DMSO for a final concentration of 12 µg mL<sup>-1</sup> zineb. Suitable aliquots (5.0-8.0 mL) of this standard solution were added to 5 g of grains (wheat, rice) and fruit (apple) samples. The samples were well mixed and then extracted with 6 mL of DMSO and then two installations of 5 mL of acetonitrile and finally volume made 20 mL with acetonitrile in each case. The extracts were taken and processed for analyzing concentration of zineb using the procedure described above. The results are given in the Table 3.

Table 3: Recovery of zineb from grains (wheat and rice) and apple fruit samples

<table>
<thead>
<tr>
<th>Active ingredient added, µg</th>
<th>Recovery of active ingredient&lt;sup&gt;a&lt;/sup&gt;, %</th>
<th>Present method</th>
<th>Comparison method&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Rice</td>
<td>Apple</td>
</tr>
<tr>
<td>60</td>
<td>96.4±0.64</td>
<td>95.2±0.98</td>
<td>93.3±0.56</td>
</tr>
<tr>
<td>72</td>
<td>95.9±0.57</td>
<td>93.3±0.60</td>
<td>90.7±0.97</td>
</tr>
<tr>
<td>84</td>
<td>90.6±0.81</td>
<td>88.2±0.84</td>
<td>90.3±0.76</td>
</tr>
<tr>
<td>96</td>
<td>89.7±0.96</td>
<td>79.9±1.01</td>
<td>85.4±0.94</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean±standard deviation for 5 determinations.
<sup>b</sup>Rao and Verma, 1989

2.4 Soil adsorption study

Adsorption studies were conducted on two different soils collected from Solan district of Himachal Pradesh, India. Bulk samples of soils were air dried and passed through 40 mesh
sieves to remove stones and large particles. The soil characteristics viz. pH, clay, organic carbon and cation exchange capacity were determined by a reported method (Jackson, 1967) and are summarized in Table 4.

Table 4: Characteristics of the Indian soils used in the adsorption study of zineb

<table>
<thead>
<tr>
<th>Soil Sample</th>
<th>pH</th>
<th>Clay (%)</th>
<th>Organic carbon (%)</th>
<th>Cation Exchange Capacity (meq/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6.5</td>
<td>20.0</td>
<td>1.5</td>
<td>11.0</td>
</tr>
<tr>
<td>II</td>
<td>7.2</td>
<td>32.6</td>
<td>0.8</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Zineb adsorption isotherms on two Indian soils of different soil characteristics were obtained by the batch equilibration technique using 50 mL conical flask at definite temperature, i.e. 20°C. Triplicate soil samples (2 g) were equilibrated with zineb solutions in the concentration range from 27.5-82.5 μg mL⁻¹ on the shaker at 150 rpm at definite temperature for 6 h equilibrium time. After equilibration, the suspensions were centrifuged and the equilibrium concentrations (Cₑ) were determined in supernatants by the spectrophotometric method described above. Adsorption parameters K₅, Kₒₛ, ∆Gₒ and GUS index have been calculated by using equations 1-4 respectively (Papa et al., 2004; Bhardwaj et al., 2007).

\[ K_d = \frac{X}{C_e} \] (1)

\[ K_{ₒₛ} = K_d \times \left( \frac{100}{%O.C.} \right) \] (2)

\[ ∆Gₒ = -RT \ln K_d \] (3)

\[ GUS = \log t_{1/2} \left[ 4 - \log(K_{ₒₛ}) \right] \] (4)

Where X is the amount of pesticide adsorbed mg Kg⁻¹ of the adsorbent; Cₑ is the equilibrium solution concentration (mg L⁻¹); t₁/₂ is pesticide persistence (half life), OC is organic carbon content of soil. The GUS score is used to study the leaching behavior of pesticides and these can be classified as leacher (GUS>2.8), transition (2.8>GUS<1.8) and non-leacher (GUS<1.8) (Papa et al., 2004). All these parameters for the adsorption of zineb on two Indian soils of different soil characteristics were calculated and the results are shown in Table 5.

Table 5: Adsorption parameters for the adsorption of zineb on two Indian soils

<table>
<thead>
<tr>
<th>Adsorption parameters</th>
<th>Soil I</th>
<th>Soil II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil-adsorption coefficient (K₅)</td>
<td>9.66</td>
<td>8.15</td>
</tr>
<tr>
<td>soil organic carbon partition coefficient (Kₒₛ)</td>
<td>644.2</td>
<td>1018.5</td>
</tr>
<tr>
<td>Gibb’s free energy (∆Gₒ in KJ mol⁻¹)</td>
<td>-5.62</td>
<td>-5.19</td>
</tr>
<tr>
<td>Groundwater Ubiquity Score (GUS)</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

3. Results and discussion

In the proposed method the use of tetraacetonitrilocopper(I) perchlorate (CuClO₄.4CH₃CN) in acetonitrile, a colorless reagent which is extremely stable (Verma et al., 1984; 1985), has been described for the spectrophotometric determination of zineb. The method is based on the formation of intense yellow colored copper(I) bis-dithiocarbamate complex which the reagent forms with the bis-dithiocarbamate moiety of the fungicide in 2:1 molar ratio in DMSO-
acetonitrile (1:4 v/v) media. The analysis is accomplished by measuring the absorbance of the color at 354 nm. The most plausible course of reaction is:

\[
\text{H}_2\text{C} = \text{N} - \text{C} - \text{S}^\text{Zn} + 2\text{Cu}^+ \rightarrow \text{H}_2\text{C} = \text{N} - \text{C} - \text{SCu} + \text{Zn}^{2+}
\]

That bisdithiocarbamates form copper complexes is quite well known (Verma et al., 1984; 1985). The color reaction is instantaneous and the color is stable for at least 90 minutes. The Beer’s law is obeyed up to 27.56 µg mL\(^{-1}\) of zineb solution. The molar absorptivity (\(\varepsilon\)) and Sandell’s sensitivity were found to be 0.77×10\(^4\) L mol\(^{-1}\) cm\(^{-1}\) and 0.036 µg cm\(^{-2}\) at 354 nm. Sandell’s sensitivity represents the number of micrograms of the determinand per mL of a solution having absorbance of 0.001 for a path length of 1 cm (Agnihotri et al., 1999). The method is quite sensitive and can be used for the determination of as little as 1.38 µg mL\(^{-1}\) fungicide solution. The method has successfully been applied to the analysis of a commercial formulation of zineb for the purpose of quality control. The recoveries of active ingredient were in the range 95.7-98.9%, with RSDs in the range 0.68-1.01% (Table 2). The maker’s specification of the formulation has been established by an independent method (Rao and Verma, 1989). Recoveries of the fungicide from fortified grains (wheat and rice) and apple fruit samples were good, ranging from 79.9-96.4%, with RSDs ranging from 0.56-1.01% (Table 3).

The simplicity and rapidity of the color forming reaction between the fungicide and copper(I) reagent, sufficient stability of the color formed and copper(I) reagent and well established stoichiometry of the reaction encouraged us to develop a photometric titration procedure for the determination of zineb in its formulation. Using this procedure zineb in the range 2.76-27.56 µg could be determined with a maximum RSD of 0.71%. The high accuracy and precision obtained in photometric titrations of zineb with copper(I) perchlorate leads to support the stoichiometry of the color reaction. In these titrations performed at 354 nm (\(\lambda_{\text{max}}\) of colored complex) the absorbance increases till zineb to copper(I) molar ratio 1:2 was achieved (indicating the formation of colored complex) and thereafter, it attains almost constant values indicating no more formation of colored complex. An inverted L-shaped titration curve is obtained (Figure 2).

![Figure 2: Photometric titration of Zineb (1 ml 0.0005M) with 0.0005 copper (I) perchlorate](image-url)
To assess the validity of the proposed method in soil adsorption study for the evaluation of the leaching behavior and ground water contamination of zineb, the effect of various common ions on the determination of this fungicide has been studied. The proposed method has been found to be free from interferences due to Na\(^+\), Ca\(^{2+}\), Al\(^{3+}\), Fe\(^{2+}\), Zn\(^{2+}\), Mn\(^{2+}\), PO\(_4^{3-}\), SO\(_4^{2-}\), NO\(_2^-\), NO\(_3^-\), CO\(_3^{2-}\), Cl\(^-\) present in soil.

Adsorption of a pesticide is generally evaluated using adsorption isotherms which represent a functional relationship between the amount of pesticide adsorbed (X, mg Kg\(^{-1}\)) and the pesticide concentration in solution at equilibrium (C\(_e\), in mg L\(^{-1}\)) (Kuisi, 2002). The values of ‘X’ and ‘C\(_e\)’ have been determined by the proposed spectrophotometric method. The adsorption isotherms of zineb fungicide may be classified as L-type of Gile’s classification according to the initial portion of the isotherms (Figure 3) (Somasundaran and Wang, 2006).

![Figure 3: Adsorption isotherm zineb on soil I and soil II](image)

The L-types of isotherms represent a system where the solid surface has an average affinity for the pesticide and the solvent is relatively inert: there is no strong competition from the solvent for adsorption sites (Fernandez-Perez et al., 2004). The adsorption coefficients K\(_d\) and K\(_{oc}\) of the pesticide are important parameters used to measure the strength of adsorption of pesticide to soils and are directly related to its mobility and persistence. The value of K\(_d\) represents the extent of adsorption and in general, higher the value of K\(_d\), greater is the adsorption of pesticide (Jaya et al., 2009). K\(_d\) values for zineb have been found to be 9.66 and 8.15 (Table 5) indicating that adsorption of zineb increased with increasing organic carbon in the two soils studied as these provide soils with increased numbers of adsorption sites onto which fungicide molecules can bind. The K\(_d\) for a pesticide is soil-specific and varies with soil texture and its organic matter content but the K\(_{oc}\) is less soil specific (Chen et al., 2004) and is calculated by normalizing adsorption coefficient (K\(_d\)) with the organic carbon (OC) content of the soil. Higher K\(_{oc}\) values (644.2-1018.5) suggesting strong adsorption of zineb on soils (Tiryaki and Temur, 2010). The value of K\(_{oc}\) for zineb on two soil types decreased with increased fraction of OC, indicating that adsorption is not only due to organic matter of soil but also influenced through some other factors (Jaya et al., 2009). The cation exchange capacity (CEC) is another parameter that influences the adsorption of pesticides. The value of CEC is directly proportional to the hydrophobic nature of adsorbent: the greater the value of CEC of soil, the more hydrophobic its surface will be, consequently higher affinity for hydrophobic (water repelling or insoluble) pesticides and vice versa (Pal and Vanjara, 2001).
The amount of pesticide adsorbed is also dependent on pH of the soil. In general, the adsorption of pesticide is less in alkaline than in acidic soils. However at quite high and low pH of the soil, adsorption of pesticide decreases due to change in clay mineralogy (Kumar and Philip, 2006).

Similar behavior has also been observed in the present study. The value of ΔG° for adsorption of fungicide was observed negative, suggesting the energetically favorable adsorption process. The Groundwater Ubiquity Score (GUS) is the most commonly used model which relates pesticide persistence (half life) and adsorption in soil (Koc). The leaching potential of the fungicide in terms of the GUS index was determined by using the experimentally observed Koc value for each soil sample and the reported half life of zineb (Vogue et al., 1994). The GUS score for zineb has been observed in the range 1.2-1.4 which classifies it as a non-leacher fungicide. Consequently it does not represent hazard to surface and ground water contamination if used judiciously in right amount at right time. Further from above study, it has been observed that zineb has higher adsorption for soils with high organic and clay contents. Hence, soils where the fungicide is used indiscriminately can also be amended with additives of high organic content viz. manure, compost, cow dung etc. in order to increase its retention and consequently to reduce the mobility. This addition will also improve the fertility and health of the soil by serving as source of soil nutrients.

4. Conclusion

The applied pesticide irrespective of its application target ultimately enters soil and then to aquatic environment through leaching and resulting into surface and groundwater contamination. The evaluation of leaching behavior of zineb in terms of GUS index which is a measure of contamination risk to aquatic environment has been evaluated based on its adsorption on two soils of different characteristics. High values of Koc suggest strong adsorption of zineb on both soil types. GUS values in the range 1.2-1.4 classify it as non-leacher and does not represent hazard to ground water contamination. However, excessive use of fungicide can pose a threat to aquatic environment. The proposed spectrophotometric methodology developed to accomplish the objectives of the study is simple, sensitive, rapid and free from interferences of common ions present in soil. The instantaneous development of the yellow color with a colorless copper (I) reagent, stability and well established stoichiometry of the color reaction and non-extraction of the colored product are other salient features of the method.

5. References


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