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# SORPTION KINETICS OF ISOPROTURON AND ASSESSMENT OF ITS ECOTOXICITY ON *Lemna minor*

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## ABSTRACT

The study was carried out to investigate the ecotoxicity of Isoproturon on *Lemna minor* and the interaction of the $EC_{50}$ value of the chemical with sorption behaviour of the chemical in soil. The sorption isotherms ($K_f$ and $K_{OC}$) for Isoproturon were determined for three different soils having various organic carbon and clay content. $EC_{50}$ for *Lemna* was determined both with and without soil. Both the Freundlich adsorption coefficient ($K_f$) and normalized sorption coefficient ($K_{OC}$) values varied with different types of soil. There was moderate correlation between the log $K_f$ and log $K_{OC}$ existed. The regression study revealed a strong relationship between log $K_{OC}$ and organic carbon and between log $K_{OC}$ and soil clay content. There $EC_{50}$ value for *Lemna* grown with soil was higher than that grown without soil. However, the difference was statistically insignificant. Greater degree of inconsistency in various data suggests the reiteration of the study.

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## INTRODUCION

Isoproturon (IPU) is a widely used phenylurea derived systemic herbicide [3-(4-isopropylphenyl)-1, 1-dimethylurea] for the control of annual grasses and broad-leaved weeds in agricultural fields. This herbicide is specific for monocots and mainly inhibits the electron transport in photosystem II and thereby disrupts the photosynthetic pathway of the target plant (Arnaud *et al.*, 1994). It is one of the most common herbicide species, applied and to be found in water samples. Though IPU has a short environmental half-life (soil $DT_{50}$ 6–28 d) (Tomlin, 2001); reports on frequent finding of IPU in both surface and ground water at concentrations exceeding the European Union limit for drinking water (0.1 µg $liter^{-1}$) (Johnson *et al.*, 2001; Spliid and Køppen, 1998; Stangroom *et al.*, 1998) has arisen the issue of environmental concern.

Sorption is one of the soil phenomena which directly affects the retention, volatilization, biodegradation, transport of organic chemicals in soil and uptake by plants. Therefore, the sorption constant is an important parameter used in a variety of models for predicting the ecotoxicity of organic chemicals entering in the environment (Wu *et al.*, 2000; Blume and Ahlsdorf, 1993). Chiou *et al.* (1979) and Green and Karickhoff (1990) observed that the value of $K_{OC}$ for a compound measured in different soils approach towards a constant, and thus the $K_f$ value for any given compound is closely related to the organic carbon constant of the soil. Sorption behaviour of a chemical in soil may assist in predicting the toxicity in the natural environmet. Because, it help simulates the actual amount of exposure of an organism in the environment. Since IPU is a major herbicide found in the aquatic ecosystem, duckweed (*Lemna minor*) was selected as the test organism for assessing the IPU's ecotoxicity in the aquatic environment. The present research was undertaken to determine the toxicity ($EC_{50}$) of isoproturon on aquatic test plant duckweed (*Lemna minor*) as well as the influence of soil sorption of the herbicide on the same.

## MATERIALS AND METHODS

### Preparation of standard IPU solutions

From IPU stock solution (0.1014 mg A.I. $ml^{-1}$ in methanol), 5 different standard solutions (*viz.* 0.01, 0.03, 0.06, 0.1 and 0.2 mg $l^{-1}$) were prepared in Swedish Standard (SIS) *Lemna* growth medium as described in the OECD guideline 221 (OECD,2002).

### Setting IPU sorption experiment

The sorption of experiment was carried out using three different soils in a batch-slurry system with five sorbate concentrations (as mentioned above). The organic carbon and clay contents of the test soils are shown in Table 1. 10 g of air dried soil were weighed into a polypropylene centrifuge bottle and mixed with 20 ml standard IPU solutions. At least three replicates were run for the sorption experiment. A control was also carried out using the top concentration (*i.e.* 0.2 $mgl^{-1}$) without soil. The tubes were placed in end-over-end shaker for 16 hrs.

**Table 1.** Organic carbon and clay content of test soils

| Soil | OC content (%) | Clay content (%) |
|---|---|---|
| Soil A | 2.6 | 34 |
| Soil B | 3.4 | 59 |
| Soil C | 1.9 | 16 |
| Soil D | 4.1 | 65 |

**Sampling of the sorption study**

After shaking the centrifuge tubes were removed from the shaker and allowed to stand for about one hrs and then the liquid supernatant were decanted into glass sample tubes. For the ease of chemical analysis by HPLC, the control and the three lower concentrations were extracted and concentrated in methanol by Solid Phase Extraction (SPE) method using SPE cartridges and transferred to glass sample vial. The concentrations of IPU in the extracted sample and the two unextracted higher samples were then determined by HPLC. Using the peak area data obtained from HPLC concentrations in the test samples were calculated.

**Lemna Growth Inhibition Test**

The *Lemna* growth inhibition experiment was conducted in two batches with five replications. The first set was prepared taking only the standard IPU solutions (20ml) in a 250 ml conical flask (i.e. without soil). The second set of ecotoxicity test was undertaken by taking 15 g of soil D in 60ml of each standard IPU solutions and then stirred to mix the soil and solution well. In both batches of conical flasks 2-3 *Lemna* colonies were placed and photographed before incubation in a plant growth cabinet for 2 week (20°C, 18 hrs light, 8 hrs dark). After 2 weeks, photographs for the batches without soil were taken. For the rest, frond numbers were counted to determine the growth of *Lemna*.

**Table 2.** Mean $K_f$ , nf and $K_{OC}$ values for different soils

| Soil | $K_f$ (ml $g^{-1}$) | nf (-) | $K_{OC}$ |
|---|---|---|---|
| Soil A | 1.20 | 0.68 | 46 |
| Soil B | 0.94 | 0.87 | 28 |
| Soil C | 1.17 | 0.75 | 61 |
| Soil D | 1.85 | - | 59 |

**Growth rate estimation**

Growth of *Lemna* was determined using both area of fronds and change in frond numbers. The areas of colonies were determined using the image analysis software *Image J*. It was done after converting the images at 8-Bits in a binary format. The area (in $cm^2$) covered by the fronds was measured by analyzing particles using a fixed length (rular). Then inhibition rate (*I*) was calculated using the following formula,

$$I = \frac{A_c - A_t}{A_c} \times 100$$

Where,

Ac = ln (final area of the control) –ln (starting area of the control)

At = ln (final area of the treated *Lemna*) –ln (starting area of the treated *Lemna*)

*I* = % inhibition in growth

The computation of the growth rate using the frond number was done by the following equation,

$$GR = \frac{\ln(N_j) - \ln(N_i)}{t_j - t_i}$$

Where,

*GR* = Average specific growth rate

$N_i$ = No. of fronds determined at time i

$N_j$ = No. of fronds determined at time j

$t_i$ = Moment time for start of the period

$t_j$ = Moment time for end of the period

The inhibition data was then used in the Probit programme to develop concentration-response relationship. The $EC_{50}$ of IPU was determined both excluding and including soil data set.

### Measurement of sorption parameters

The Freundlich adsorptions constant ($K_f$) was calculated by putting the soil sorption data in the solver function in the MS Excel and using the following equation:

$$C_s = K_f C_e^{1/n}$$

Where,
$C_s$ is the concentration on soil at the end of experiment (mg/g);
$C_e$ is concentration in the liquid phase at the end of experiment (mg/ml);
$K_f$ is the soil-water partition coefficient (ml/g); and
*1/n* is the Freundlich coefficient.

### Measurement of $K_{OC}$ values

$K_{oc}$ values for Soil A, B and C were calculated using the following formula,

$$K_{oc} = \frac{K_f \times 100}{\% \, OC}$$

Where,
$K_{OC}$ is the normalized sorption coefficient;
$K_f$ is the Freundlich sorption coefficient; and
*OC* is organic matter content of soil (%).
From the mean $K_{OC}$ value of soil A, B and C, the $K_f$ for soil D was also estimated (Table 2).

### Statistical analysis

The collected data for $EC_{50}$ values and the sorption data at the end were analyzed statistically using t-Test with the help of SPSS data analysis software.

## RESULTS AND DISCUSSION

### Sorption coefficients

The values of $K_f$, nf and $K_{OC}$ for IPU in three different soils is given in Table 2. The $K_f$ value ranges from 0.94 to 1.2. While the $K_{OC}$ values varied widely, ranges between 28 and 61. In all three cases the nf value is less than 1. This indicated that the relationship between $K_f$ and $K_{OC}$ is nonlinear; it is rather exponential.

The $K_f$ value indicates the sorption behaviour of organic chemicals in soil system. Here the $K_f$ for different soil is not dispersed widely and the value is comparatively low. It indicates the less tendency of Isoproturon to be sorbed with the soil. Isoproturon is not considered to be sorbed to soil to a large extent compared with other herbicides as reported by other investigators (Singh *et al.*, 2001). However, the reports on the $K_f$ for Isoproturon is much greater and can be as high as 3.84 (Johannesen *et al.*, 2003). Inconsistent values obtained from different replicated soil samples may be contributed toward this low value of $K_f$. The lower range of $K_f$ indicates that it differs less from soil to soil and may be used to calculate the sorption behaviour of a chemical in a different soil with less uncertainty.

On the other hand, the $K_{OC}$ value had a greater range and varied with the soil organic carbon content. The greater range $K_{OC}$ may make it unpredictable to assess the sorption behaviour of a chemical in a separate soil. Actually, the organic matter appears to be a function of a partition medium for organic chemicals in solution. Hence it varies very sharply for the variation in organic carbon content.

The regression studies between different sorption coefficients ($K_f$ and $K_{OC}$) and OC and clay content of soil as shown in Figure 1 (a) – (d) revealed a strong correlation between log $K_{OC}$ and % OC ($R^2$ = 0.986) as well as $K_{OC}$ and % clay ($R^2$ = 0.996). On the other hand, the relationship was moderate between log $K_f$ and log $K_{OC}$.($R^2$ = 0.643). This is, rather inconsistent with the results demonstrated by Wu *et al.* (2000) and Johannesen *et al.* (2003). They reported a strong relationship between the $K_f$ and $K_{OC}$ values of IPU.

**Table 3.** Mean and standard deviation for $EC_{50}$ values in presence and absence of soil D

| Treatment | Mean | Standard deviation |
|---|---|---|
| Without soil | 0.12 | 0.073 |
| With soil | 0.17 | 0.053 |

In the *Lemna* growth study, for most cases highest inhibition in growth was observed highest at the highest concentration and the lowest at control and 0.01 mg $l^{-1}$ level (see Appendix). The $EC_{50}$ value for *Lemna* using soil or without soil was found 0.171 and 0.121 mg $l^{-1}$ (Table 3). It is noticeable that the value is greater in with soil treatment than the treatment lacks soil. However, statistical analysis revealed no significant difference between the two data sets. The reason behind may be the values dispersed very widely.

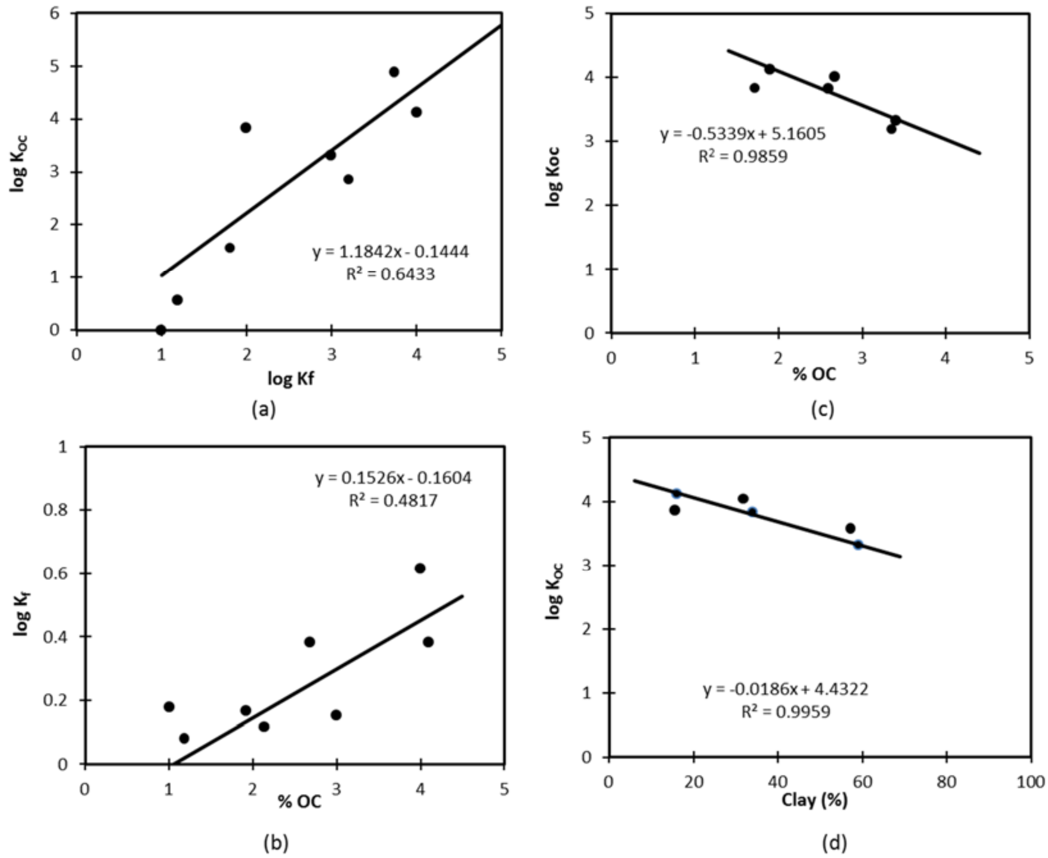


**Figure 1.** Regression for (a) soil sorption data (log $K_{OC}$ values) on the Freundlich sorption coefficient (log $K_f$ values) for Isoproturon in different soils; (b) soil organic carbon (OC) and Log $K_f$, (c) OC and Log $K_{OC}$; and (d) soil clay content and log $K_{OC}$. Ecotoxicity assessment of IPU on *Lemna*

The comparatively lower mean $EC_{50}$ value in treatment lacking soil is presumable. Because it is assumed that some portion of the IPU will be adsorbed to the soil adsorption sites (organic matter and clays). Hence, the toxicity of the herbicide towards *Lemna* will be buffered to some extent. Because organic matter is a major sorbent of organic chemicals (Chiou, 1989 and Luthy *et al.*, 1997).

A fair degree of irregularity was observed in the various data sets. And this strongly indicated the errors might have been occurred during the conduction of the study. A second attempt may be undertaken to assess the results of the present study.

## COMPETING INTEREST

The authors declare that they have no competing interests.

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