Spatial variability of pesticide degradation in soil: mechanisms and implications

Gary D. Bending

Warwick HRI, University of Warwick, Wellesbourne, Warwick UK

Pesticide degradation in the environment

Key questions

• How variable are pesticide degradation rates within individual fields?

• What are the mechanisms underlying within-field spatial variability of degradation rate?
Isoproturon
(3-(4-isopropylphenyl)-1,1-dimethylurea)

- Phenyl-urea herbicide
- used for control of weeds in cereal crops
- slowly degraded and moderately mobile in soil

Pesticides use in Great Britain (2003)

<table>
<thead>
<tr>
<th>Use</th>
<th>Active ingredient</th>
<th>Area treated (10^3 ha)</th>
<th>Amount used (t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide</td>
<td>Isoproturon</td>
<td>2,661</td>
<td>2,730</td>
</tr>
<tr>
<td></td>
<td>Glyphosate</td>
<td>1,473</td>
<td>1,285</td>
</tr>
<tr>
<td></td>
<td>All herbicides</td>
<td>14,006</td>
<td>8,520</td>
</tr>
<tr>
<td>Fungicide</td>
<td>Epoxiconazole</td>
<td>3,434</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Chlorothalonil</td>
<td>1,619</td>
<td>799</td>
</tr>
<tr>
<td></td>
<td>All fungicides</td>
<td>14,503</td>
<td>3,566</td>
</tr>
<tr>
<td>Insecticide</td>
<td>Cypermethrin</td>
<td>2,105</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>All insecticides</td>
<td>3,809</td>
<td>434</td>
</tr>
</tbody>
</table>
Pesticides most commonly exceeding 0.1 µg l⁻¹ in surface freshwater (England and Wales, 2002)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>% samples &gt;0.1 µg l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproturon</td>
<td>10.4</td>
</tr>
<tr>
<td>Mecoprop</td>
<td>10.6</td>
</tr>
<tr>
<td>Diuron</td>
<td>11.5</td>
</tr>
<tr>
<td>MCPA</td>
<td>8.7</td>
</tr>
<tr>
<td>2,4 D</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Degradation of isoproturon

- Degradation is microbially mediated
- Considerable spatial variability in degradation rates between and within fields
Degradation of isoproturon across the Wellesbourne farm

% isoproturon remaining after 2 weeks

Distance (m)

0 200 400 600 800 1000 1200 1400

Distance (m)

Degradation of isoproturon across the Wellesbourne farm

% isoproturon remaining after 2 weeks

Distance (m)

0 200 400 600 800 1000 1200 1400

Distance (m)
Isoproturon degradation in Deep Slade field after 21 days

% isoproturon remaining

- >60
- 50 - 60
- 40 - 50
- 30 - 40
- 20 - 30
- 10 - 20
- <10

Northings (m)

Eastings (m)

Sampling Pattern

'Fast' sites

'Slow' sites
Degradation of isoproturon in soil from Deep Slade field

Dynamics of isoproturon degrading organisms
a. Fast degrading sites
Dynamics of isoproturon degrading organisms
b. Slow degrading sites

Denaturing Gradient Gel Electrophoresis (DGGE)

- PCR amplification of bacterial community 16S rRNA genes
- Separation on formamide / urea gradient gel

- Provides information on
  - microbial community structure
  - identity of organisms
  - non-culturable organisms
Denaturing gradient gel electrophoresis

Time
0 4 hrs 12 hrs
Species A B A B A B
Increasing concentration of denaturant

Bacterial community DGGE profile from fast degrading sites

Muyzer (1993)
190 bp 16S rRNA fragment

Control Treated
M 1 2 3 4 5 1 2 3 4 5 M
High Low
**Isolation of isoproturon degrading bacteria**

- Fast degrading soil inoculated into MSM plus IPU (MSI)
- After complete degradation, culture re-enriched into MSI
- Following complete degradation, spread onto MS-IPU agar
- Single colonies checked for degradation
Degradation of isoproturon by isolates SRS2 and 782

\[(\text{CH}_3)_2\text{HC} \overset{\text{NHCON(\text{CH}_3)_2}}{\longrightarrow} \text{Isoproturon}\]
\[(\text{CH}_3)_2\text{HC} \overset{\text{NHCONHCH}_3}{\longrightarrow} \]
\[(\text{CH}_3)_2\text{HC} \overset{\text{NHCONH}_2}{\longrightarrow} \]
\[(\text{CH}_3)_2\text{HC} \overset{\text{NH}_2}{\longrightarrow} \text{Isopropyl aniline}\]

16S rRNA from DGGE bands and isolates

Caulobacter leidyi
Sphing. adhaesiva
Sphing. mali
Sphing. asaccharolytica

Sphing. rosarosa
Sphing. adhaesiva
Sphing. mali
Sphing. sanguinis
Sphing. parapaucimobilis

Sphing. subarctica
Sphing. yanoikuyae
Caulobacter subvibrioides

Sphing. flava
Sphing. sp.
Sphing. sp.
Erythrobacter longus

Sphing. subarctica
Sphing. sp.
Sphing. sp.
Porphyrobacter sp.
Porphyrobacter sp.

Porphyrobacter sp.
Porphyrobacter sp.
Porphyrobacter sp.
Sphing. trueperi
Sphing. rosarosa
Sphing. adhaesiva
Sphing. mali
Sphing. sanguinis
Sphing. parapaucimobilis

High DGGE band
Low DGGE band

Isolate SRS2
Isolate 782
Soil factors influencing microbial activity

Soil Properties
- Organic matter
- pH
- Nutrient status
- Mineralogy

Environment
- Temperature
- Moisture content
- Aeration

Pesticide degradation

Relationship between isoproturon degradation and soil pH
Degradation of isoproturon by SRS2 in pure culture

Specific mineralization rate (d^{-1})

Degradation of isoproturon by SRS2 in pH 6.5 soil

% isoproturon remaining

Time (days)
Degradation of isoproturon by SRS2 in pH 7.5 soil

Further questions

- What is responsible for isoproturon degradation at low pH sites?
- Does isolate 782 play a role in degradation?
16S PCR DGGE using *Sphingomonas* sp. specific primers

- 360 bp fragment
- separation on 20-60 % urea/formamide gradient

*Sphingomonas* sp. DGGE community profile at isoproturon DT90

Fast degrading (high pH) sites

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IPU treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRS2 A B C D E F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomonas sp. CFDS-1</td>
<td>99 %</td>
<td></td>
</tr>
<tr>
<td>Sphingomonas sp. D12</td>
<td>96 %</td>
<td></td>
</tr>
<tr>
<td>Sphingomonas sp. SRS2</td>
<td>100 %</td>
<td></td>
</tr>
</tbody>
</table>
**Sphingomonas sp. DGGE community profile at isoproturon DT90**

Fast degrading (high pH) sites

Slow degrading (low pH) sites

**Conclusions**

- Strains isolated using enrichment procedures may not represent those acting *in situ*

- Diverse closely related strains can adapt to degrade IPU within a single field

- Spatial variability in IPU catabolism is the result of interaction between pH and degradative *Sphingomonas* spp
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