Dioxin pollution in Vietnamese hotspots and degradation activity of isolated bacteria in microcosms

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Formation and behavior of dioxins

- Pesticide manufacture
- Paper manufacture
- Waste incineration
- Forest fire

Behaviour of dioxins in soil
- Adsorbed on soils
- Very resistant in the environment
- Accumulate in fat tissues and concentrate in the food chain.

Effect of dioxins on human health
- Cause cancer, birth defects, reproductive, developmental and immunological problems.
Agent Orange and Dioxins in Vietnam

- Agent Orange (AO) defoliant: mixture 2,4 dichlorophenoxyacetic acid (2,4-D) and 2,4,5 trichlorophenoxyacetic acid (2,4,5-T)
- 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (2,3,7,8-TCDD) in Agent Orange: 0.05 to 50 mg/L
- 72 M liters of AO sprayed

2,3,7,8-TCDD the most toxic among PCDDs
Objectives

• Determine the dioxin residue in soils/sediments at hotpots.

• Examine the potential of microbial detoxification of dioxin-polluted soils/sediments sprayed with Agent Orange.
Sampling sites

- At heavily sprayed area, Cua of Quang Tri province: sediments of water reservoirs, ponds and top soils of paddy and upland fields.

- At Aluoi of Hue: soils of former military airbase Aso, soils of paddy and upland crop field, sediments of lake and ponds.

- Da Nang former military airbase

- Bien Hoa former military airbase
## Dioxin residues in soils/sediments at study sites

### A Luoi:

2,3,7,8-TCDD at former air-base:

- Topsoil in A Luoi air-base: ~ 900 pg/g
- Duck and fish fats: 40-60 pg/g
- Human blood: 14-40 pg/g

(Source: Dwernychuk & Hoang D.C, 2002)

### Da Nang and Bien Hoa former military air-bases pg/g

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample type</th>
<th>2,3,7,8-TCDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Da Nang</td>
<td>Sediment</td>
<td>271</td>
</tr>
<tr>
<td></td>
<td>Soil 01</td>
<td>633</td>
</tr>
<tr>
<td></td>
<td>Soil 02</td>
<td>7095</td>
</tr>
<tr>
<td>Bien Hoa</td>
<td>Sediment</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Soil 01</td>
<td>115</td>
</tr>
</tbody>
</table>
Microbial detoxification of dioxins

**Anaerobic condition**

- High chlorinated dioxins
- Less chlorinated or non-chlorodioxins

Reductive dechlorination done by *Dehalococcoides*:

Electron donor: organic acid → H₂

Electron acceptor:

\[
\begin{align*}
\text{Cl} & \quad \text{O} & \quad \text{O} & \quad \text{Cl} \\
\text{Cl} & \quad \text{O} & \quad \text{O} & \quad \text{Cl} \\
\end{align*}
\]

+ 2H → \[
\begin{align*}
\text{Cl} & \quad \text{O} & \quad \text{O} & \quad \text{Cl} \\
\text{Cl} & \quad \text{O} & \quad \text{O} & \quad \text{Cl} \\
\end{align*}
\]

**Aerobic condition**

- Non-, mono- or di-chlorodioxins
- \( \text{CO}_2 + \text{H}_2\text{O} \)

Degradation (breaking the aromatic ring)

Electron donor: organic acid → H₂

Electron acceptor: \( \text{rdh} \)
Reductive microbial dehalogenation of halogenated aromatic compounds

**Dehalococcoides with reductive dehalogenase genes**

Highly halogenated aromatic compounds

Less halogenated or non-halogen aromatic compounds

**Electron donor:** organic acids

Anaerobic condition

Function as electron acceptor
Examine 1,2,3,4-TCDD / 2,3-DCDD dechlorination:

spike soils/sediments with:

+ 1,2,3,4-TCDD or 2,3-DCDD

+ electron donors (lactate + butyrate + pyruvate + propionate)

Examine dechlorination of 2,3,7,8-TCDD:

Spike 2,3,7,8-TCDD to positive 2,3-DCDD dechlorinating microcosm.
Dechlorination activity after 4-month incubation
Lateral and angular dechlorination pathways

D4 sediment (QT) microcosm

1,2,3,4-TCDD
TrCDD (1,2,3 & 1,2,4-TrCDD)
DCDD (1,2 + 1,3 + 1,4 & 2,3-DCDD)
MCDD (1 & 2-MCDD)
Dibenzo-p-dioxin

molar %

month of incubation

1,2,3,4-TCDD
TrCDD (1,2,3 & 1,2,4-TrCDD)
DCDD (1,2 + 1,3 + 1,4 & 2,3-DCDD)
MCDD (1 & 2-MCDD)
Dibenzo-p-dioxin
2,3,7,8-TCDD dechlorination by positive 2,3-DCDD dechlorinating community

![Graph showing molar percentage changes over time for 2,3-DCDD and 2-MCDD](image1)

![Graph showing molar percentage changes over time for 2,3,7,8-TCDD and 2,3,7-trCDD](image2)
Enhancing dechlorination of PCDDs in Danang sediment by anaerobic incubation

![Chart showing molar percentage of DD, MCDD, DCDD, 2,3,7-TrCDD, and 2,3,7,8-TCDD in Kill control and Live microcosm, 7 months.](image-url)
Isolation of Dibenzofuran-degrading aerobic bacteria

Soil/sediment sample

Soil extraction

Several times

ASO 002 (0 - 30 cm) 
Rajew strain

- Soil extraction + DF 
3rd transfer
3 days of incubation

+ Soil extraction + DF 
3rd transfer
3 days of incubation

- +
### Aerobic dibenzofuran degradation of isolates

<table>
<thead>
<tr>
<th>Isolating site/19 samples</th>
<th>Time getting turbid in liquid media, days</th>
<th>Media color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Đất lúa HươngLâm (R1)</td>
<td>5</td>
<td>white</td>
</tr>
<tr>
<td>Đất rẫy HươngLâm (S7)</td>
<td>5</td>
<td>yellow</td>
</tr>
<tr>
<td>Đất sân bay Aso (ĐAS)</td>
<td>20</td>
<td>yellow</td>
</tr>
<tr>
<td>Bùn Mai Đàn (BMĐ)</td>
<td>16</td>
<td>yellow</td>
</tr>
<tr>
<td>Đất lúa Mai Lộc (ĐML)</td>
<td>14</td>
<td>yellow</td>
</tr>
</tbody>
</table>

![Graph showing turbidity and DF remained over time](image_url)

- **DF remained** (1000 ppm at beginning)
Aerobic dibenzo-\(p\)-dioxin degradation of isolates

<table>
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<tr>
<th>Isolating site/19 samples</th>
<th>Time getting turbid in liquid media, days</th>
<th>Media color</th>
</tr>
</thead>
<tbody>
<tr>
<td>rice field, A Luoi (R1)</td>
<td>60</td>
<td>white</td>
</tr>
<tr>
<td>A Luoi (S7)</td>
<td>80</td>
<td>white</td>
</tr>
<tr>
<td>Cua, Mai Đàn (BMĐ)</td>
<td>20</td>
<td>white</td>
</tr>
<tr>
<td>Aso, stock place (BAS)</td>
<td>30</td>
<td>white</td>
</tr>
<tr>
<td>Cua, Mai Lộc (ĐML)</td>
<td>35</td>
<td>white</td>
</tr>
</tbody>
</table>

% DD remained (300 ppm at beginning)
Conclusions

- After more than 40 years, the residues of dioxins in soils/sediments in some hotspots are still high.

- Both reductively polychlorinated dibenzo-\(p\)-dioxin (PCDD) - dechlorinating and aerobic Dibenzofuran-degrading bacteria appear to be ubiquitous in soils/sediments after 40-year contamination by spraying with Agent Orange.

- Activities of PCDD reductive dechlorination and dibenzofuran degradation in microcosms show a potential of using indigenous bacteria to detoxify dioxins and the need to create appropriate environmental conditions for enhancing microbial detoxification of dioxins.
Acknowledgements

Mr. Nguyen Viet Hung, EPA of Thua Thien Hue