Rehabilitation of heavy metal contaminated brownfields using woody plants and mycorrhizal symbiosis

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• The National Round Table on the Environment and the Economy (NRTEE) reported that there are as many as 30,000 brownfield sites across Canada (old railway yards, former gasoline stations, etc.)

• These brownfields disfigure neighbourhoods and may pose health and safety risks.

• In Montreal alone, some 1,600 brownfields have been identified. This represents almost 5% of the total area of the city.
Cleanup vs costs

• Cleanup costs are considered too expensive relative to income generated (> 1 M dollars ha\(^{-1}\)).

• Alternatives must be developed to provide the opportunity to treat these sites at lower cost.

• Green techniques using phytoremediation approaches can be an effective option.
Phytoremediation

The use of plants and their associated microorganisms to eliminate, limit or degrade soil or water contaminants.
Soil characteristics of urban brownfields

- Low level of organic matter content;
- Heavy and compacted soils;
- High pH;
- Poor drainage;
- Contamination of various origins (organic and inorganic);
- Very heterogeneous.
Characteristics essential for plants used in phytoremediation of heavy metal contaminated brownfields

- Facility of establishment;
- High yield in climatic and edaphic conditions of urban areas;
- Good root development capacity;
- Capacity to absorb large quantities of metals.
Potential of hyperaccumulator species for phytoremediation

- *Armeria maritima*  
  (Plumbaginaceae)
- *Brassica juncea*  
  (Brassicaceae)
- *Festuca arundinacea*  
  (Poaceae)
- *Minuartia verna*  
  (Caryophyllaceae)
- *Thlaspi caerulescens*  
  (Brassicaceae)
- *Vernonia petersii*  
  (Asteraceae)
General observations

- Difficult to establish in typical brownfield soil conditions.
- Hyperaccumulators tested generally had poor biomass production.
- Their low aboveground yield made them difficult to harvest and to manage.
Comparing diverse plants species for their phytoremediation potential

- Verify establishment and growth potential of willows and poplars on brownfields.
- Compare their metal accumulation with one of the highest-performing hyperaccumulating plant species.
- Verify the impact of chelating agent on growth parameters and metal accumulation.
Comparison of the phytoextraction potential of willow species with Indian mustard

Methods

- Three blocs 38.5 m²
- Three species (two willow clones and *B. juncea*)
- Two treatments: with and without EDTA¹ (20 mM)

¹ Disodium ethylenediamine tetraacetate dihydrate
Species studied

- Brassica juncea
- Salix viminalis (5027)
- Salix myabeana (SX67)
## Metal bioavailability

<table>
<thead>
<tr>
<th>Metals</th>
<th>Exchangeable</th>
<th>Carbonates</th>
<th>Oxides</th>
<th>Residues</th>
<th>Total metals</th>
<th>Criteria</th>
<th>Potential plant bioavailability mg/kg-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>x</td>
<td>38.4</td>
<td>96.2</td>
<td>282.2</td>
<td>416.8</td>
<td>B-C</td>
<td>38.4 to 134.6</td>
</tr>
<tr>
<td>Pb</td>
<td>x</td>
<td>162</td>
<td>311</td>
<td>384</td>
<td>857</td>
<td>B-C</td>
<td>162 to 473</td>
</tr>
<tr>
<td>Zn</td>
<td>0.6</td>
<td>149.4</td>
<td>419.6</td>
<td>329.8</td>
<td>899.4</td>
<td>B</td>
<td>149.4 to 569.6</td>
</tr>
</tbody>
</table>
Comparison of Cu content (µg) in plants

- EDTA induced a significant effect on the accumulation of Cu in above ground tissues of *B. juncea*.
- EDTA had no impact on willows.
- Significantly more Cu in willow roots.
Comparison of Pb content (µg) in plants

• EDTA induced a significant effect on the accumulation of Pb in above ground tissues of *B. juncea*.
• EDTA had no impact on willows.
• Significantly more Pb in above ground tissues of *B. juncea*.
• No difference in roots of the three species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Above Ground Tissues</th>
<th>Root Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. viminalis</em></td>
<td>2168</td>
<td>289</td>
</tr>
<tr>
<td><em>S. myabeana</em></td>
<td>270</td>
<td>41</td>
</tr>
<tr>
<td><em>B. juncea</em></td>
<td>3016</td>
<td>324</td>
</tr>
</tbody>
</table>
Comparison of Zn content (µg) in plants

- EDTA had no effect either on above ground or root tissue Zn content in the three species.

- *S. viminalis* had significantly more Zn in above ground and root tissues.
Conclusions

Fast growing species such as willows are:

• Establish easily.
• Compete well in open fields.
• Efficiently absorb diverse contaminants and cumulate them in their tissues.
• Produce significant biomass, potentially with high contaminant content.
• Provide immediate visual impact (rapid growth).
• Facilitate harvest and treatment.
• Generate biomass that can be harvested and used for diverse applications.
A new experimental set up in 2006

Objectives

• Compare both growth and capacity of two woody plant clones belonging to the genera *Salix* and *Populus* when inoculated or not with a commercial AM fungal inoculum containing *G. intraradices*, in heavy metal (Cd, Zn, Cu and Pb) contaminated brownfields;

• Evaluate the longer term HM phytoextraction potential of these clones.
Experimental design

• 2 plant species
  – *Salix viminalis* (5027)
  – *Populus x generosa*
    A Henry “Unal”

• 2 treatments
  – With or without inoculation with a commercial AM fungal (G. *intraradices*)

• Five blocks (replicates)

At plantation, 1620 ml of mycorrhizal inoculum containing 500 spores per 100 ml were mixed thoroughly into the first 5 cm of the soil surface.
Methods

In September 2007 (at the end of the second growing season following establishment:

- Samples of leaves, stems and roots were taken.
- Zn, Cu, Cd and Pb concentrations were determined.
Dry leaf and stem biomass production per plant of *P. × generosa* and *S. viminalis* clones, non-inoculated (Ctrl) or inoculated (Gi) with *G. intraradices*, measured after two growing seasons.

N.B. Within each tissue, columns with a different letter are significantly different at p < 0.05; there was no significant difference between inoculation treatments (n= 40). Bars represent standard deviation.
Heavy metal concentrations measured in *P. × generosa* and *S. viminalis* tissues, inoculated (Gi) or non-inoculated (Ctrl) with *G. intraradices*, at the end of the second year of growth.

**ANOVA comparisons**

<table>
<thead>
<tr>
<th>Metals</th>
<th>Leaves</th>
<th>Stems</th>
<th>Surface roots</th>
<th>Deep roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>Sv=Pg</td>
<td>Sv&gt;Pg***</td>
<td>Gi: Sv=Pg**</td>
<td>Sv&gt;Pg**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ctrl: Sv&gt;Pg**</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>Sv=Pg</td>
<td>Sv&gt;Pg***</td>
<td>Sv&gt;Pg*</td>
<td>Sv&gt;Pg**</td>
</tr>
<tr>
<td>Cu</td>
<td>Sv&gt;Pg**</td>
<td>Sv&gt;Pg***</td>
<td>Sv&gt;Pg*</td>
<td>Sv&gt;Pg*</td>
</tr>
<tr>
<td>Pb</td>
<td>Sv&gt;Pg***</td>
<td>Sv=Pg</td>
<td>Sv=Pg</td>
<td>Sv=Pg</td>
</tr>
</tbody>
</table>
Heavy metal concentrations measured in *P. × generosa* and *S. viminalis* tissues, inoculated (Gi) or non-inoculated (Ctrl) with *G. intraradices*, at the end of the second year of growth.

### ANOVA comparisons

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<td>Gi: Sv=Pg**</td>
<td>Sv&gt;Pg**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ctrl: Sv&gt;Pg**</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>Sv=Pg</td>
<td>Sv&gt;Pg***</td>
<td>Sv&gt;Pg*</td>
<td>Sv&gt;Pg**</td>
</tr>
<tr>
<td>Cu</td>
<td>Sv&gt;Pg**</td>
<td>Sv&gt;Pg***</td>
<td>Sv&gt;Pg*</td>
<td>Sv&gt;Pg*</td>
</tr>
<tr>
<td>Pb</td>
<td>Sv&gt;Pg***</td>
<td>Sv=Pg</td>
<td>Sv=Pg</td>
<td>Sv=Pg</td>
</tr>
</tbody>
</table>
Mean biological concentration factors (BCF) in the leaves and the stems of *P. × generosa* (Pg) and *S. viminalis* (Sv) clones, inoculated (Gi) or non-inoculated (Ctrl) with *G. intraradices*, at the end of the second growing season.

<table>
<thead>
<tr>
<th>Clones</th>
<th>Tissues</th>
<th>Inoculation</th>
<th>Cd</th>
<th>Zn</th>
<th>Cu</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. × generosa</em></td>
<td>Leaves</td>
<td>Gi</td>
<td>2.56</td>
<td>1.24</td>
<td>0.15</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>3.29</td>
<td>1.35</td>
<td>0.21</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>Gi</td>
<td>1.87</td>
<td>0.44</td>
<td>0.09</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>2.31</td>
<td>0.46</td>
<td>0.12</td>
<td>0.038</td>
</tr>
<tr>
<td><em>S. viminalis</em></td>
<td>Leaves</td>
<td>Gi</td>
<td>2.97</td>
<td>1.52</td>
<td>0.22</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>4.41</td>
<td>1.76</td>
<td>0.29</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>Gi</td>
<td>3.30</td>
<td>1.12</td>
<td>0.19</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>5.50</td>
<td>1.35</td>
<td>0.24</td>
<td>0.081</td>
</tr>
</tbody>
</table>

\(a\) Biological concentration factor = tissue concentration / soil concentration (n=20).  
\(b\) Values in bold are active bioaccumulation (>1).

**Calibration**

- **Biological concentration factor** (BCF) indicates the degree of metal bioaccumulation in plant tissues.
- **Inoculation** refers to the treatment with *G. intraradices*.
- **Control (Ctrl)** samples were non-inoculated.
- **Metal concentrations** (Cd, Zn, Cu, Pb) are presented in bold for active bioaccumulation (>1).

**Important Considerations**

- **Leaf and Stem Comparison**: BCF values for leaves are generally lower than stems, suggesting a lower bioaccumulation in leaves.
- **Inoculation Impact**: Inoculation with *G. intraradices* shows a trend towards higher BCF, indicating increased bioaccumulation in inoculated plants.
- **Metal Distribution**: Differences in metal concentrations between inoculated and control plants highlight the influence of inoculation on metal bioaccumulation.
Percentage of root length bearing AM fungi structures of the *S. viminalis* and *P. × generosa* clones, inoculated (Gi) or non-inoculated (Ctrl) with *G. intraradices* at planting, in the surface (0-20 cm) and deep (20-40 cm) soil layers, on the second year of the field trial.

<table>
<thead>
<tr>
<th>Clones</th>
<th>Inoculation</th>
<th>Mycorrhizal root colonization (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Surface (0-20 cm)</td>
<td>Deep (20-40 cm)</td>
</tr>
<tr>
<td><em>P. × generosa</em></td>
<td>Gi</td>
<td>48.9 a</td>
<td>40.0 a</td>
</tr>
<tr>
<td></td>
<td>Ctrl</td>
<td>44.6 a</td>
<td>36.6 a</td>
</tr>
<tr>
<td><em>S. viminalis</em></td>
<td>Gi</td>
<td>5.8 b</td>
<td>2.8 b</td>
</tr>
<tr>
<td></td>
<td>Ctrl</td>
<td>4.8 b</td>
<td>2.6 b</td>
</tr>
</tbody>
</table>
Results of paired t-tests comparing *S. viminalis* and *P. x generosa* aboveground plant heavy metal concentration in leaves and stems of inoculated (Gi) and non-inoculated (Ctrl) plants between the first (1) and second (2) year of growth in the field.

<table>
<thead>
<tr>
<th>Clones</th>
<th>Tissues</th>
<th>Inoculation</th>
<th>Metal concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cd</td>
</tr>
<tr>
<td><em>P. x generosa</em></td>
<td>Leaves</td>
<td>Gi</td>
<td>1=2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>1=2</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>Gi</td>
<td>1=2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>1=2</td>
</tr>
<tr>
<td><em>S. viminalis</em></td>
<td>Leaves</td>
<td>Gi</td>
<td>1=2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>1=2</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>Gi</td>
<td>1&lt;2**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ctrl</td>
<td>1&lt;2*</td>
</tr>
</tbody>
</table>
Conclusions

• Willows and poplars constitute wonderful tools to restore brownfields or polluted sites in urban areas;

• Their rapid establishment and growth allow them to quickly create a green cover with positive environmental, economic and social impact…
Conclusions cont’d…

- Willows in particular showed interesting ability to tolerate and absorb large quantities of trace metals (notably Zn and Cd);
- Inoculation with arbuscular mycorrhizal is possible and constitutes an interesting approach to increase the establishment, but their impact on absorption capacity has not been demonstrated in the studies conducted.
Acknowledgements

- Fonds des priorités gouvernementales en sciences et en technologies du ministère de l’Environnement du Québec
  - Montréal Botanical Garden
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  - Southwest Borough of the City of Montreal
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