PHYTOREMEDIATION – A Novel Technology to Decontaminate Polluted Sites

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Phytoremediation

Phytoremediation is defined as the use of green plants and their associated microorganisms, soil amendments, and agronomic techniques to remove, degrade or detoxify harmful environmental pollutants.

Phytoremediation technologies:

I. Rhizosphere Enhanced Bioremediation (or Phytostimulation)
II. Phytodegradation (or Phytotransformation)
III. Phytostabilization
IV. Phytoextraction (or Phytoaccumulation)
V. Rhizofiltration
VI. Phytovolatilization
VII. Phytoexcretion (?)
Phytoremediation processes

**Phytovolatilization**: transfer of pollutants from the soil to the atmosphere.

**Phytoextraction**: transfer of pollutants from the soil and accumulation in the above ground parts of the plant.

**Phytodegradation**: enzymatic degradation of the pollutants in the plant tissue.

**Rhizofiltration**: transfer of pollutants from the soil and accumulation in the roots of the plant.

**Phytostabilization**: Stabilization of heavy metals in the soil/root surface and reduction of heavy metal mobility.

**Enhanced Bioremediation (or Phytostimulation)**: Enhancement of the microbial community and increase of biodegradation in the rhizosphere.

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Phytoremediation Research at TU-Crete

**General Project**: Phytoremediation of contaminated sites with heavy metals using Mediterranean plants.

**Specific aims**:
- Heavy metals: Lead (Pb), Cadmium (Cd) and their mixtures.
- Identification of Pb and Cd hyperaccumulators among Mediterranean plants.
- Focusing on salt-tolerant plants.
Why halophytes??

- Halophytes can be cultivated with saline irrigation water which is a desirable feature since often high-quality irrigation water is not available even for application to crops in arid and semi-arid regions.
- Salt-water irrigation is becoming an increasingly important practice because the quality of irrigation waters is decreasing as water supplies for agriculture become restricted due to urban needs and climate change.
- Salinity has been shown to be a key factor for
  - the increased bioavailability of metals in the soils due to reduced soil metal sorption
  - the translocation of metals from roots to the aerial parts of the plant - an important feature for phytoextraction applications

Salt-tolerant plants examined:

Plant #1: *Tamarix smyrnensis*

Plant #2: *Nerium oleander*

Plant #3: *Atriplex halimus*
**Tamarix – Experiments**

Pot experiments with plants grown in metal polluted soils in order to evaluate the effect of metals and soil salinity on the growth of plant

**Measurements:**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Total metals</td>
</tr>
<tr>
<td>Height</td>
<td>Plant available metals</td>
</tr>
<tr>
<td>Water content</td>
<td>pH</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>EC</td>
</tr>
<tr>
<td>Proteins</td>
<td>Organic matter</td>
</tr>
<tr>
<td>Peroxidase activity</td>
<td>Total CaCO₃</td>
</tr>
<tr>
<td>Metal content (in roots and shoots)</td>
<td></td>
</tr>
</tbody>
</table>

**Pot Experiments**

*T. smyrnensis* growing in contaminated soil with 800 ppm Pb and 16 ppm Cd

10 -15 cm cuttings of *T. smyrnensis*

Propagation period : 21 days

Adaptation period : 8 months

Experimental period : 10 weeks

**Experimental Conditions**

- **Temperature** : 19 – 47°C
- **Humidity** : 18 – 70%
- **Photoperiod** : 14 -15 h
Tamarix smyrnensis

Pb accumulation in the plant

![Graph showing Pb concentration in plant tissue](graph.png)

Cd Accumulation

Cd concentration in individual parts of *T. smyrnensis* at different soil salinities

<table>
<thead>
<tr>
<th>Salinity [%]</th>
<th>L/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.35</td>
</tr>
<tr>
<td>0.5</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
</tr>
</tbody>
</table>
**Tamarix smyrnensis**

Biomass (dry weight) Treatment with mixture of Pb & Cd at different salinities

Chlorophyll in the leaves Treatment with Cd at different salinities

**Tamarix: Salt crystals on leaves**

Droplets secreted by salt glands were crystallized on the leaves due to high Temperatures.
Cadmium excretion from leaf tissue of *T. smyrnensis* (pot experiment). Comparison of control plants and plant treated with 16 ppm Cd of dry weight of soil at two soil salinities (0% and 0.5%)

Metals excreted on the leaf surface of *T. smyrnensis* (pot experiment). Comparison of control plants and plant treated with 800 ppm Pb and 16 ppm Cd of dry weight of soil at two soil salinities (0% and 0.5%)
Heavy Metal Tolerance

Plant mechanisms of heavy metal tolerance:
   i.  Avoidance
   ii. Exclusion
   iii. Immobilization
   iv. Excretion
   v.  Mechanisms involving enzymatic changes

❖ The resistance of halophytes to salt stress is usually correlated with a more efficient antioxidant system (Zhu et al., 2004).
❖ Thus, halophytes may be more capable to cope with heavy metals stress than common plants since heavy metal stress induces oxidative stress to cellular structures.

Excretion mechanism

❖ Salt secretion through salt glands is considered as an adaptive strategy to regulate plant tissue ion concentration
❖ An important mechanism which contributes to the resistance of all plants to increased salinity levels.
❖ Halophytes are adapted to saline environments:
   ➔ salt avoidance
   ➔ salt tolerance
   ➔ salt evasion
❖ The main function of salt glands is the secretion of excess stress-inducing ions that invade the plant
Species of the genus *Tamarix* are well known as salt-tolerant plants with the ability to excrete excess salt as salt droplets through salt glands on their leaf surface.

There is evidence that the salt glands of *Tamarix sp.* secrete with minimal selectivity a variety of different ions and that the composition of the secreted salts is related to the composition in the rhizosphere.
Salt crystals on leaf tissue of *T. smyrnensis* at different soil salinities

0% salinity  0.5% salinity

**Hydroponic experiment**

Hydroponic growth with exposure to 100 ppm Pb and 5 ppm Cd

- **Age of plants:** 10 months
- **Experimental period:** 2 weeks
- **Temperature:** 19 – 24°C
- **Humidity:** 57 – 66%
- **Photoperiod:** 12 h

**Experimental Conditions**

Nutrient solution (mg/l):

- $143.0\ \text{Ca(NO}_3\text{)}_2$
- $2.86\ \text{H}_3\text{BO}_3$
- $35.75\ \text{KNO}_3$
- $1.86\ \text{MnCl}_2\cdot4\text{H}_2\text{O}$
- $17.75\ \text{KCl}$
- $0.22\ \text{ZnSO}_4\cdot7\text{H}_2\text{O}$
- $35.75\ \text{KH}_2\text{PO}_4$
- $0.079\ \text{CuSO}_4\cdot5\text{H}_2\text{O}$
- $35.75\ \text{MgSO}_4$
- $0.6\ \text{FeSO}_4\cdot7\text{H}_2\text{O}$
**Excretion rates of the metals** were measured by cleaning residues off leaf surfaces:
The area below the plant was covered by weighted tissue paper. In the 3\textsuperscript{rd}, 6\textsuperscript{th}, 9\textsuperscript{th}, 12\textsuperscript{th} and 14\textsuperscript{th} day the leaves were washed with 0.1% v/v HNO\textsubscript{3} and the resulting solution was absorbed by the paper.

**Metal content analysis in the paper wipes** was performed by ICP according to modified method of Soon

**Metal content analysis in the plant tissue** was performed by ICP spectroscopy according to modified method of Soon

**Determination of Pb and Cd content in the nutrient medium** was performed by ICP spectroscopy

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**Pb Excretion Rates**

![Graph showing Pb excretion rates from leaf tissue of *T. smyrnensis* exposed to 100 ppm Pb and 5 ppm Cd (hydroponic experiment)]
**Phytoextraction of contaminated soils with heavy metals**

- Problems of Phytoextraction
  - Contaminated crop disposal
  - Remediation time required

- Phytoexcretion process should be kept in mind
  - If not properly addressed, it reduces the effectiveness of other phytoremediation processes

**Phytoextraction + Phytoexcretion**

Opportunity to intervene (?)

Surface accumulation

Capture and remove on appropriate media
Phytoremediation processes:

**Phytoexcretion:**
Excretion of heavy metals from the leaves

**Phytoextraction**

**Phytodegradation**

**Phytostabilization**

Enhanced Bioremediation (or Phytostimulation)

**Phytoexcretion:**

**A Novel Approach of Phytoremediation (?)**

- **“Phyto-Excretion”:**
  - The plant can be viewed as a “biological pump” for heavy metals
  - Intervening and capturing the droplets on suitable media before they are recycled onto the top soil

- **Advantages:**
  - The frequency of tree pruning and uprooting is lowered
    - lower costs
    - faster remediation times
    - possibility of recovery of metals
  - Coupled to phytoextraction
Planning of Experimental part

Plant: *Nerium oleander*

- Pot experiments (10 weeks)
  - Cd (0, 0.5, 3% NaCl)
  - Pb (0, 0.5, 3% NaCl)
  - Pb & Cd (0, 0.5, 3% NaCl)
  - Pb increasing concentrations (0, 0.5, 3% NaCl)

- Hydroponic experiments (2-4 weeks)
  - Pb increasing concentrations
  - Cd increasing concentrations

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*Nerium oleander*

Pb concentration (mg kg$^{-1}$ dry weight) in individual plants parts

![Graph showing Pb concentration added in soil (ppm) vs. Pb concentration in plant tissue (ppm) for Shoots and Roots.](image)
**Nerium oleander**

**Biomass (dry weight) of Nerium oleander**

![Biomass graph](image)

**Chlorophyll contents of Nerium oleander**

![Chlorophyll graph](image)

**Is the plant under stress??**

**Effect of Pb on peroxidase activity of Nerium oleander**

![Peroxidase activity graph](image)
Interactions between Aphids (*Aphis nerii*) and Oleander Growing on Pb and Cd Contaminated Soil

Portion of plants *N. oleander* infested by aphids during weeks 5 to 7 and 8 to 10 are almost identical for all treatments.

(Plants infestation recording: no presence of aphids, plant infested by number of aphids from 1 – 10 and plant infested by >10 aphids)

Weeks 5 to 7

Weeks 8 to 10
Portion of plants *N. oleander* not infested by aphids \((P(X=0))\) for various treatments with lead and cadmium as a function of salinity. Portions marked with the same letter are significantly different with each other (corresponding to different saline concentrations) at least at 5% level of significance.

**Overview of experimental results**

*Tamarix smyrnensis:*

Suitable for phytoextraction in environments with increased salinity.

*Nerium oleander:*

A very good choice for phytostabilization.

*Atriplex halimus:*

A new Pb-hyperaccumulator (?)


Phytoremediation of organics (OMW)

TOP VIEW

VERTICAL VIEW

water

Perforated pipe

gravel

OMW

Subsurface disposal area of OMW
Protecting the river (riparian zone)

- Aim: To stop the pollutant plume and degrade contaminants that have been extracted by the plants
- Monitoring through multilevel wells

**APPLICATION:** Poplars to control the flow of nitrates from the agricultural land next to Evrotas river.
Remediation of saline soils

• *Salinization* is one of the most serious problems confronting sustainable agriculture in irrigated production lands in semi-arid and arid regions. UN-EP estimates that ~20% of agricultural land and 50% of cropland in the world is salt stressed (Ravindran et al., 2007)

• Soils need proper amendments as a source of calcium (Ca\(^{2+}\)) to replace sodium (Na\(^{+}\)) from the cation exchange sites. The displaced Na\(^{+}\) is leached from the root zone through excess irrigation (Qadir et al., 2003). [Chemical remediation – Potential aquifer problems?]

• Can phytoremediation help?

Phytoremediation of saline soils by halophytes

• Phytoremediation desalination approach #1
  – Cultivation of certain salt tolerant plant species with the ability to increase the dissolution of soil calcite (CaCO\(_3\)) in the rhizosphere to provide Ca\(^{2+}\) that can be exchanged with Na\(^{+}\) at cation exchange sites. Displaced Na\(^{+}\) can be leached out of the soil with irrigation water. (Qadir and Oster, 2002; Qadir et al., 2003; Qadir et al., 2004; Gerhardt et al., 2006) [Aquifer problems?]

• Phytoremediation desalination approach #2
  – Halophytes could be grown on salt-affected soils to remove significant amounts of salt and Na\(^{+}\) through their aerial parts. Salt is removed from the soil to the extent that soil can be returned to agricultural productivity (Chaudhri et al., 1964; Gritsenko and Gritsenko, 1999; Owens, 2003; Keiffer and Ungar, 2002; Gerhardt et al., 2006; Ravindran et al., 2007).
CONCLUDING REMARKS

There is a group of plants (halophytes) that have the capability to excrete heavy metals from their leaves as a detoxification mechanism.

In this case, the plant becomes a “biological pump” for heavy metals. “Phyto-excretion” is an alternative phytoremediation process that should be further explored.

The use of halophytes for phytoremediation applications should be further explored:

- Rhizodegradation of organic contaminants [they can deal better with stress]
- Rhizosphere enhanced bioremediation of mixed pollutants (metals + organics) [by removing the metals the microbes work better]
- Soil desalination [a low cost long term remediation approach]

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