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.

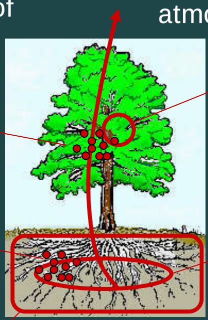
Phytoremediation technologies:

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.

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:

Plant

- Biomass
- Height
- Water content
- Chlorophyll
- Proteins
- Peroxidase activity
- Metal content (in roots and shoots)

Soil

- Total metals
- Plant available metals
- pH
- EC
- Organic matter
- Total $CaCO_3$

Pot Experiments

Experimental Conditions

***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

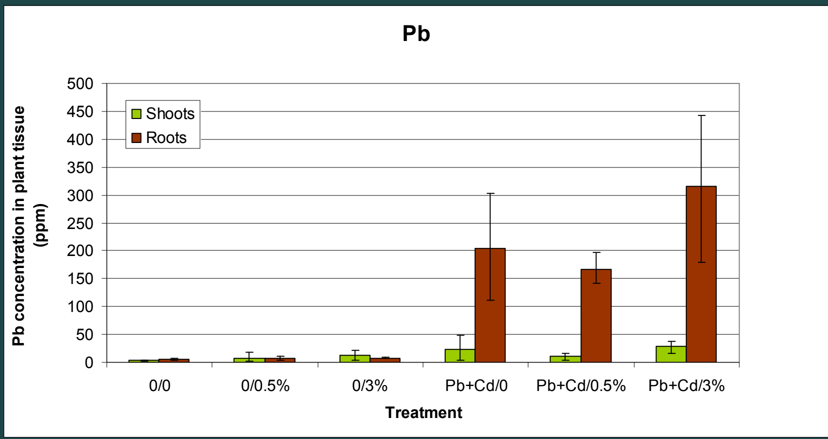
Temperature : 19 – 47°C

Humidity : 18 – 70%

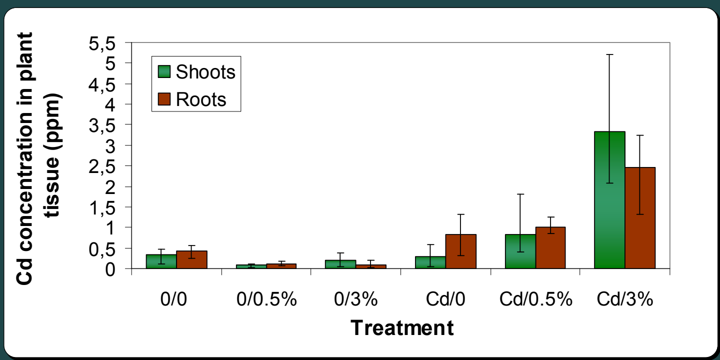
Photoperiod : 14 -15 h

Tamarix smyrnensis

Pb accumulation in the plant



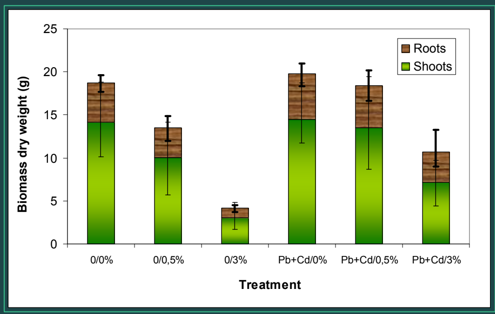
Cd Accumulation



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

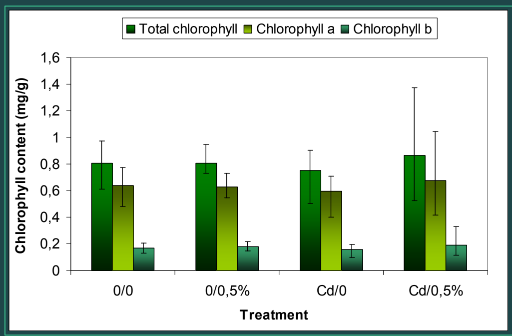
Salinity [%]	L/R
0	0.35
0.5	0.82
3	1.4

Tamarix smyrnensis

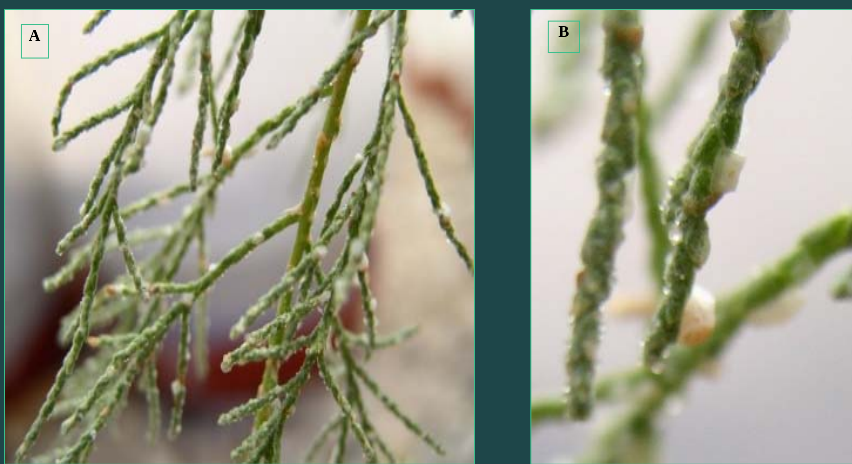


Biomass (dry weight)
Treatment with mixture of Pb & Cd at different salinities

Chlorophyll in the leaves
Treatment with Cd at different salinities

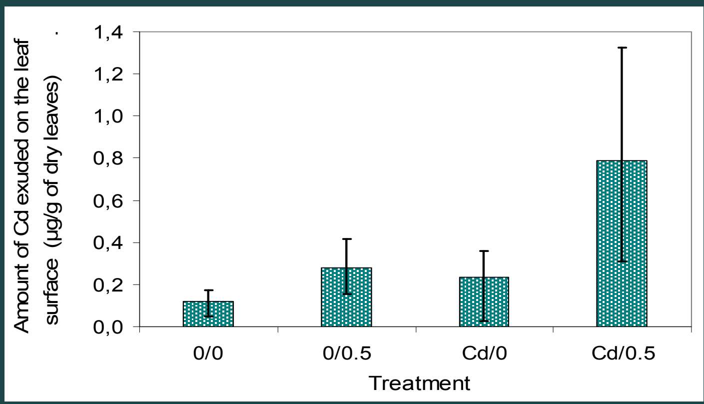


Tamarix: Salt crystals on leaves



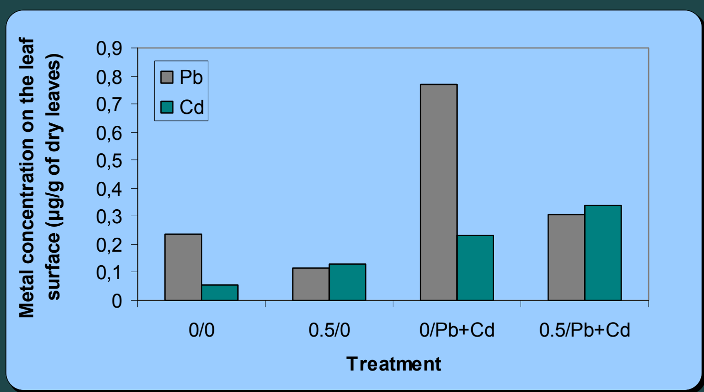
Droplets secreted by salt glands were crystallized on the leaves due to high Temperatures.

Cd Excretion by the Leaves



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%)

Pb & Cd Excretion by the Leaves



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

Tamarix smyrnensis

- 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.

Tamarix smyrnensis

Transverse section of the leaf of *T. smyrnensis* with salt gland

Salt crystals on leaf tissue of *T. smyrnensis* at different soil salinities

0% salinity

0.5% salinity

Hydroponic experiment

Experimental Conditions

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

Nutrient solution (mg/l):

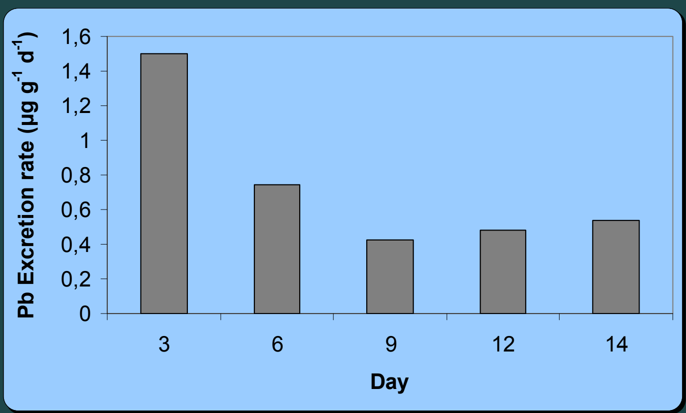
143.0 $Ca(NO_3)_2$	2.86 H_3BO_3
35.75 KNO_3	1.86 $MnCl_2.4H_2O$
17.75 KCl	0.22 $ZnSO_4.7H_2O$
35.75 KH_2PO_4	0.079 $CuSO_4.5H_2O$
35.75 $MgSO_4$	0.6 $FeSO_4.7H_2O$

Hydroponic experiment

Measurements

- **Excretion rates of the metals** were measured by cleaning residues off leaf surfaces:
  The area bellow the plant was covered by weighted tissue paper. In the 3rd, 6th, 9th, 12th and 14th day the leaves were washed with 0.1% v/v HNO_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

Pb Excretion Rates



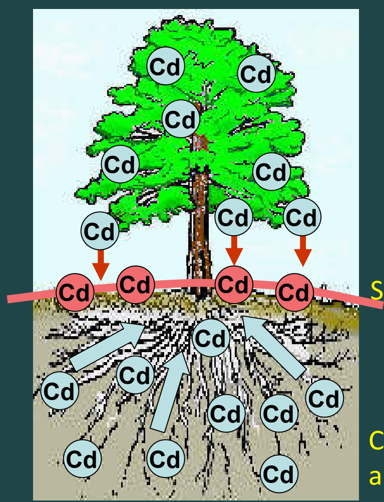
Pb excretion from leaf tissue of *T. smyrnensis* exposed to 100 ppm Pb and 5 ppm Cd (hydroponic experiment)

<u>Phytoextraction</u> of contaminated soils with heavy metals

- ❑ Problems of Phytoextraction
  - Contaminated crop disposal
  - Remediation time required
- ❑ Phytoexcretion process <u>should be kept in mind</u>
  - If not properly addressed, it reduces the effectiveness of other phytoremediation processes

Phytoextraction + Phytoexcretion

Opportunity to intervene (?)



Surface accumulation

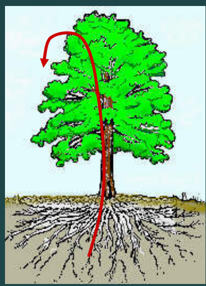
Capture and remove on appropriate media

Phytoremediation processes:

Phytoexcretion:
Excretion of heavy metals from the leaves

Phytovolatilization

Phytoextraction



Phytodegradation

Rhizofiltration

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)

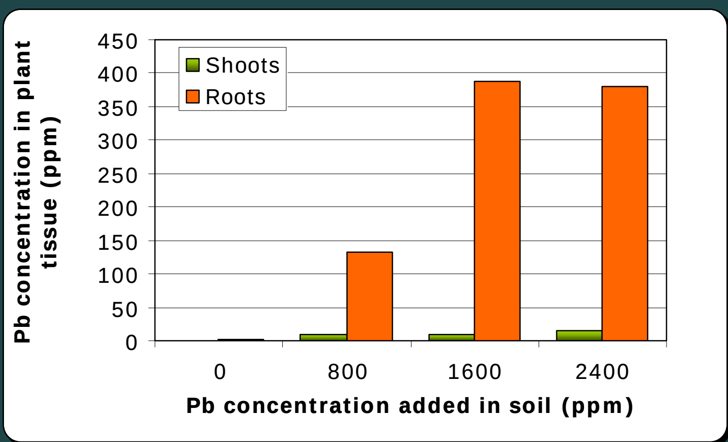
- Cd (0, 0.5, 3% NaCl)
- Pb (0, 0.5, 3% NaCl)
- Pb & Cd (0, 0.5, 3% NaCl)
- Pb increasing concentrations (0, 0.5, 3% NaCl)

Hydroponic experiments (2-4 weeks)

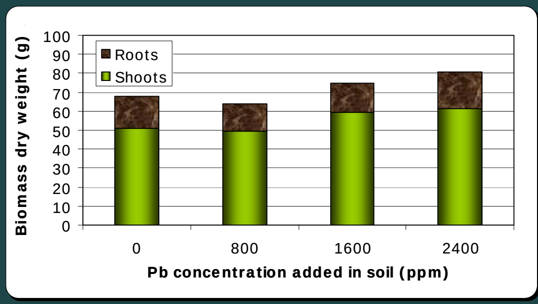
- Pb increasing concentrations
- Cd increasing concentrations

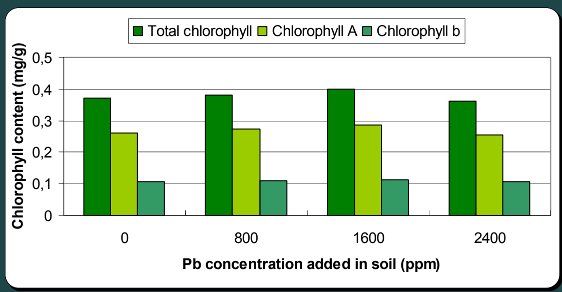
Nerium oleander

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



Biomass (dry weight) of *Nerium oleander*

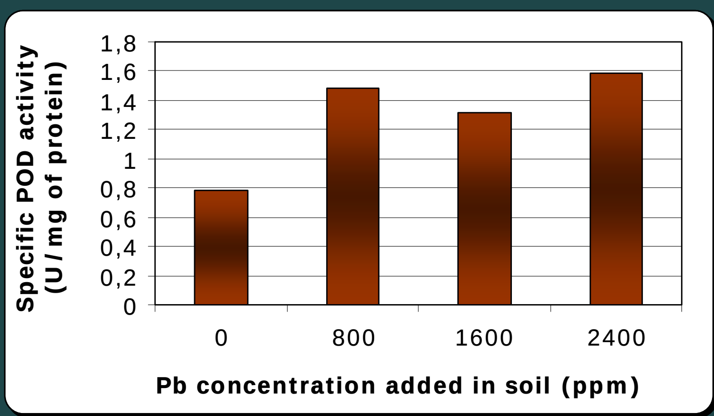




Chlorophyll contents of *Nerium oleander*

Is the plant under stress??

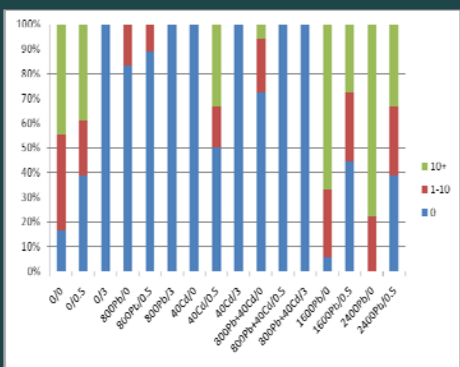
Effect of Pb on peroxidase activity of *Nerium oleander*



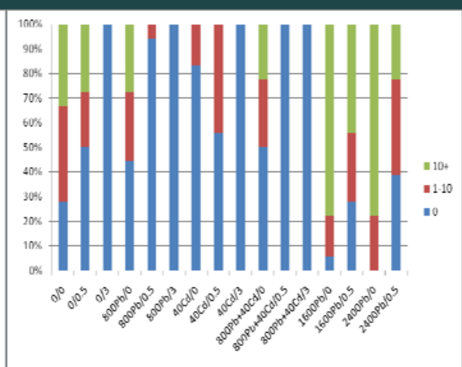
Interactions between Aphids (*Aphis nerii*) and Oleander Growing on Pb and Cd Contaminated Soil

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)



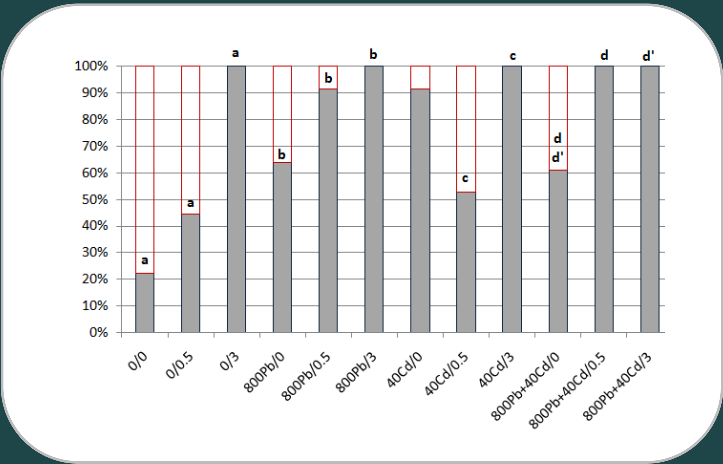
Weeks 5 to 7



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:

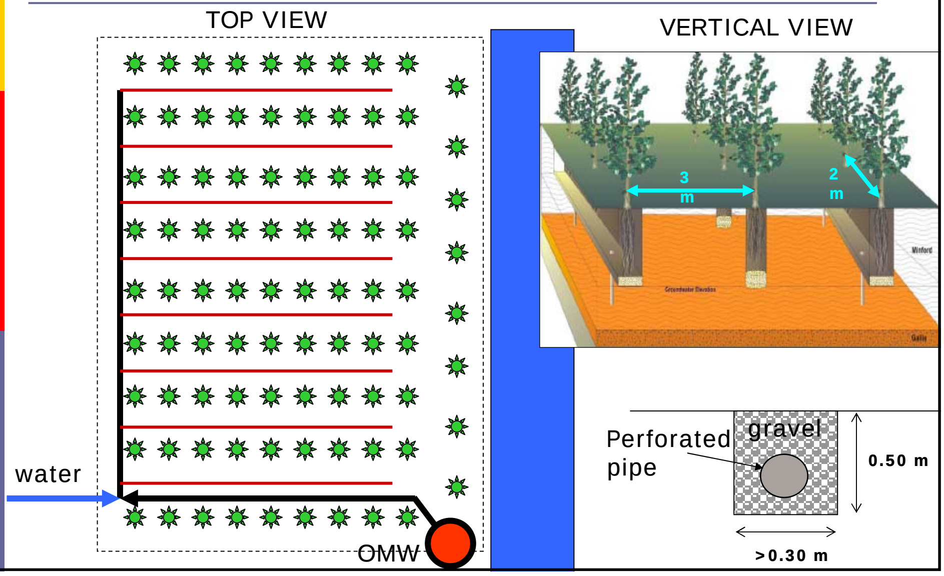
A new Pb-hyperaccumulator (?)

Kadukova, J., and N. Kalogerakis, "Lead accumulation from non-saline and saline environment by *Tamarix smyrnensis* Bunge", *European Journal of Soil Biology*, 43, 216-223 (2007).

Manousaki, E., J. Kadukova, N. Papadantonakis and N. Kalogerakis, "Phytoextraction and Phytoexcretion of Cd by the Leaves of *Tamarix Smyrnensis* Growing on Contaminated Non Saline and Saline Soils", *Environmental Research*, 106, 326-332 (2008).

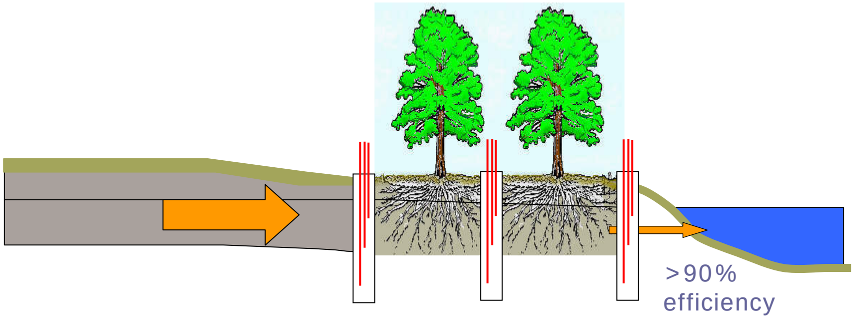
Kadukova, J., E. Manousaki and N. Kalogerakis, "Pb and Cd Accumulation and Excretion by Salt Cedar (*Tamarix smyrnensis* Bunge)", *International Journal of Phytoremediation*, 10, 31–46 (2008).

Phytoremediation of organics (OMW)



Protecting the river (riparian zone)

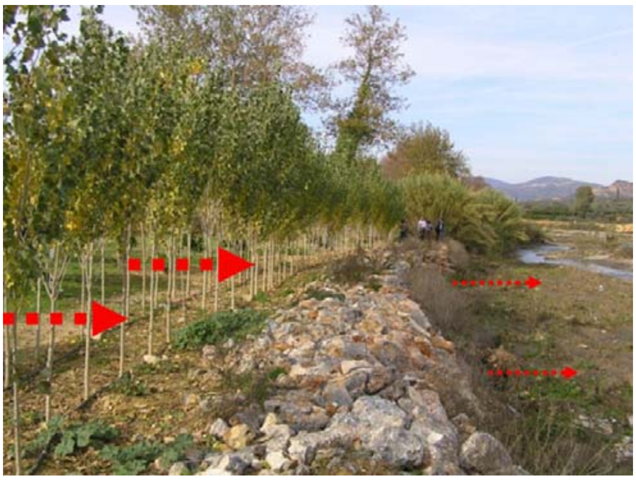
- Aim: To stop the pollutant plume and degrade contaminants that have been extracted by the plants
- Monitoring through multilevel wells



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

ENVIFriendly

LIFE
Environment



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, 2001; 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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