**Session 2: Wastewater construction - materials and optimisation of efficiency**

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CONSTRUCTED WETLAND TECHNOLOGY AND ITS WORLDWIDE PRACTICE AND APPLICATION

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Constructed wetlands are engineered systems designed and constructed to utilize the natural processes such as vegetation, soil/substrate, and their associated microbial assemblages. They are designed to take advantage of the same processes occurring in natural wetland environments to treat polluted waters. The mechanisms that are available for that purpose therefore numerous and often interrelated:

- settling of suspended particulate matter
- filtration and chemical precipitation through contact of the water with the substrate and litter
- chemical transformation
- adsorption and ion exchange on the surfaces of plants, substrate, sediment and litter
- breakdown, and transformation and uptake, of pollutants and nutrients by microorganisms and plants
- predation and natural die-off of pathogens.

**Constructed wetlands** are a cost-effective and technically feasible approach to the treatment of wastewater and runoff for several reasons:

- wetlands can often be less expensive to build than other treatment options
- operation and maintenance expenses (energy and supplies) are low
- operation and maintenance require only periodic, rather than continuous, on-site labour
- wetlands are able to tolerate fluctuations in flow
- wetlands are able to treat wastewaters with very different constituents and concentration
- they facilitate water reuse and recycling.

In addition:

- they provide habitat for many wetland organisms
- they can be built to fit harmoniously into the landscape
- they provide numerous benefits in addition to water quality improvement, such as wildlife habitat and the aesthetic enhancement of open spaces
- they are an environmentally sensitive approach that is viewed with favour by the general public.

Wetland treatment systems use watertolerant plant species and shallow, flooded or saturated soil conditions to provide various types of wastewater treatment. The two basic types of constructed wetland treatment systems include **surface flow (SF) wetlands**, and **subsurface (SSF) wetlands**.

Surface flow wetlands (SF) are densely vegated by a variety of plant species and typically have water depths less than 0.4 m. Open water areas can be incorporated into a design to provide for the optimization of hydraulics and for wildlife habitat enhancement. Typical
hydraulic loading rates are between 0.7 and 5.0 cm d\(^{-1}\) (between 2 and 14 ha per 1000 m\(^3\) d\(^{-1}\)) in constructed surface flow treatment wetlands (SF).

Subsurface flow wetlands (SSF) use a bed of soil or gravel as a substrate for the growth of rooted emergent wetland plants. Pretreated wastewater flows by gravity, horizontally or vertically, through the bed substrate, where it contacts a mixture of facultative microbes living in association with the substrate and plant roots. The bed depth in SSF wetlands is typically between 0.6 and 1.0 m, and the bottom of the bed is sloped to minimize water flow overland.

Most frequently used species in SSF constructed wetlands are common reed (*Phragmites australis*), cattail (*Typha spp.*), bulrush (*Scirpus spp.*), reed canarygrass (*Phalaris arundinacea*) and sweet mannagrass (*Glyceria maxima*). Some oxygen enters the bed substrate by direct atmospheric diffusion and some through the plant, resulting in a mixture of aerobic and anaerobic zones. Most of the saturated bed is anoxic or anaerobic under most wastewater design loadings. Typical hydraulic loading rates in SSF wetlands range from 2 to 20 cm d\(^{-1}\) (from 0.5 to 5 ha Dm\(^{-3}\) d\(^{-1}\)).

The “IWA (International Water Association) - Specialist Group on use of macrophytes in water pollution control” is organizing a Specialised Conference every two years. The last took place in Florida in 2000, it was the 7\(^{th}\) in this series. The most important outcome of this conference was that constructed wetlands have established themselves as a viable option for water treatment and that there is the huge potential for a world wide application.

In the oral presentation this will be proofed by several selected examples around the world (in developing and in developed countries) demonstrating the wide applicability of constructed wetlands, eg for the removal of different water constituents (carbon, nitrogen, phosphorus, pathogens, trace organics, anorganic substances) from domestic, industrial, agricultural wastewaters, from stormwater and from polluted surfacewaters. Surface flow wetland systems will be covered as well as subsurface flow ones, ecological considerations as well as optimisation and modelling.
Clay and Bentonite Technology in Constructed Wetlands

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Constructed wetlands for waste water treatment always, and artificial wetlands sometimes demand a sealing to protect the groundwater from impacts arising from polluted water, or to retain surface water for a technical or biological purpose.
Construction technologies preferably use concrete or plastic coating. Nonetheless, old technologies using clay or silt in wetland construction have many other advantages but being just natural.
The formation of clay is depending on the climatic situation. The water saturated clay of temperate Northern Europe is in use in bricks, tiles or layers with magnitudes of 0,50 m up to several m.
In Mediterranean countries the clay mineral ‘bentonite’, is in trade as dry powder. Mixed with soil the product provides an earthen sealing when compressed to a particular density. Using the bentonite technology avoids some of the major problems arising in wetland construction with water saturated clay: the swelling and shrinking of clay according to the availability of water and its state of hydration.
Wetland plants have a special affinity towards clay and silt. Here, too, a bentonite soil layer is less affected by rhizome growth than saturated clay layers. Contrarily, bentonite without compressing is a top mineral nutrient to support plant growth.
THE FUNCTIONAL ROLES OF MACROPHYTES IN DIFFERENT TYPES OF CONSTRUCTED WETLANDS

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Macrophytes are an indispensable component of constructed wetland systems that are used to treat different types of wastewaters. However, the functional roles of the macrophytes have been questioned because the treatment processes in constructed wetlands, as in more conventional treatment technologies, mainly rely on physical/chemical and microbial activities. The lecture will summarize the functional roles of macrophytes in different types of constructed wetland systems, and identify research needs.

The most important functions of the macrophytes in relation to the treatment of wastewater are the physical effects the presence of the plants gives rise to. The macrophytes stabilise the soil surface, counteract erosion, provide good conditions for physical filtration, prevent vertical flow systems from clogging, insulate the surface against frost during winter, and provide a huge surface area for attached microbial growth. Contrary to earlier belief, the growth of macrophytes does not increase the hydraulic conductivity of the substrate in soil-based subsurface flow constructed wetlands. The metabolism of the macrophytes affects the treatment processes to different extents depending on the type of constructed wetland. Plant uptake of nutrients (and heavy metals) is only of quantitative importance in low-loaded systems (surface flow systems). Macrophyte mediated transfer of oxygen to the rhizosphere by leakage from roots enhance aerobic degradation of organic matter and nitrification. However, the rates are generally too low compared to the oxygen demand at the loading rates usually applied. Some types of constructed wetland systems relies on all or the majority of the wastewater to be removed by evapotranspiration in the system. Hence, there is no effluent of water from such systems. Release of low molecular weight organic compound from roots might stimulate denitrification, but to what extent is still not known. It has also been reported that some plants excrete antibiotic substances, which might help remove pathogenic bacteria from water. One of the unresolved questions is to what extent the macrophytes are able to take up and metabolise aesthetically pleasing xenobiotic substances from the surroundings, and if so, if the rates are adequate for water treatment purposes. The macrophytes have additional site-specific values by providing habitat for wildlife and making wastewater treatment systems
Optimisation of constructed wetlands for treatment of petrochemical waste waters in Hungary

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In the field of water management and environmental protection the solution of purification of petrochemical wastewater and the increase of purification efficiency have become the central questions in Hungary. Presently based on the results of the water chemical analysis, the hydrobiological and microbiological investigations show that the properly functioning the wastewater treatment systems, including the constructed wetlands, have water quality conditions, which are very similar to that of the natural waters.

The present study offers a brief survey of the Hungarian constructed wetland types that have been established for wastewater treatment in the last thirty years, and gives a detailed analysis of the structure and functioning of those reed ponds that have been constructed for the post-treatment of petrochemical waste waters.

Today, the application of natural treatment processes, being harmless to the environment, is in great demand, and other requirements include low operation costs and satisfactory purification efficiency. The Hungarian constructed wetlands have three major types: free water surface system, subsurface flow system, and artificial floating meadow system. From the 1970s, sewage treatment systems have been completed with ponds of emergent and/or submerged macrophyte vegetation that operate as free water surface systems, and have a considerable role in the wastewater treatment of petrochemical industry.

In this paper the operation of petrochemical wastewater treatment systems with activated sludge and post-treatment constructed wetland-systems is demonstrated. Furthermore the optimisation of systems, the energy saving of their function and the enforcement of environmental protection are also discussed. Comparative analysis of some procedures applied during the residual waste sludge treatment in petrochemical plants is also presented.

In the wastewater treatment system of Nyirbogdány, the average COD removal efficiency is around 60%, while the reed-submerged weeds pond performs an efficiency of 25%. In the reed pond of the TIFO post-treatment pond system, the total phosphorus has shown an average of 40% removal for several years, while the nitrogen removal efficiency has not gone beyond 35%. For the enhancement of the bio-filter function in the constructed wetlands, the introduction of a reed-periphyton - growth constructed wetlands (RPGCW), suggestion has been made.
Simulation of Subsurface Flow Constructed Wetlands

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Constructed wetlands for wastewater treatment have been used in Europe and North America for more than 20 years. Today vertical subsurface flow constructed wetlands with intermittent feeding are used widely due to their good efficiency regarding the removal of ammonia nitrogen. Although there are many experiences of constructing and operating constructed wetlands their design is still based mostly on 'rules of thumb', providing a specific area requirement per person. The main task of the development of a simulation model for subsurface flow constructed wetlands is to provide a better understanding of the processes within the black box 'constructed wetland'. Once the simulation model provides reliable results the used design criteria can be checked and finally the design of constructed wetlands can be optimised.

The main task of the development of a simulation model for subsurface flow constructed wetlands is to provide a better understanding of the processes within the black box 'constructed wetland'. Once the simulation model provides reliable results the used design criteria can be checked and finally the design of constructed wetlands can be optimised.

The hydraulic behaviour of constructed wetlands can be simulated successfully using available simulation programs for unsaturated flow and transport. When using single-component transport models it is also possible to simulate tracer experiments. Tracer experiments are used widely to determine the hydraulic retention time of constructed wetlands. For two-dimensional simulations of the hydraulic behaviour the simulation program HYDRUS-2D was used.

The multi-component reactive transport model CW2D (Constructed Wetlands 2 Dimensional) was developed to model transport and reactions of the main constituents of wastewater in subsurface flow constructed wetlands. CW2D is able to describe the transformation and elimination processes of organic matter, nitrogen, and phosphorus. The mathematical structure of CW2D is based on the matrix notation as used for the 'Activated Sludge Models'. The multi-component reactive transport model CW2D is implemented into the source code of the simulation program HYDRUS-2D.

A sensitivity analysis shows the most influential parameters of CW2D. The parameters of the model that describe the properties of the unsaturated zone turn out to be the most influential parameters. Besides hydraulic parameters the oxygen re-aeration rate, and yield coefficients and lysis rates of the micro-organisms are the most important parameters. The result of the sensitivity analysis also supports the statement that good results with CW2D can only be achieved when a calibration of the flow model is possible.

Simulation results are presented for an indoor pilot-scale constructed wetlands for wastewater treatment. A lot of data are available to describe the hydraulic behaviour of the pilot-scale constructed wetland. Therefore a good calibration of the flow model is possible and the results of the reactive transport simulations with CW2D fit the measured data.

The strong relationship between the hydraulic behaviour and the removal efficiency of constructed wetlands gives the need for a model that considers the processes, which have a big influence on the hydraulic properties. Therefore main mechanisms of pore clogging, settling of suspended matter and growth of micro-organisms, will be included into an improved model for constructed wetlands.

In general simulation results with CW2D show a good agreement with measured data when the hydraulic behaviour of the constructed wetland can be modelled successfully. The multi-component reactive transport model CW2D proofs to be a promising tool for a better understanding of the transformation processes inside the black box 'constructed wetland'.
Abstract

Landfill leachates treatment and the elimination of the pollution they generate is one of the major problems the society is facing at the present time in term of environmental protection. This work, achieved in the frame of a E.C. funded LIFE project (CEE- LIFE 97 ENV/B/000403), presents the results of three years of research and adjustment of a purification technique called Epuvalisation and its adaptation to landfill leachates treatment. This purification technique, based on the principle of the hydroponics, has already shown its efficiency as a tertiary treatment for common (urban) wastewater in the removal of nitrogen forms, phosphorus, COD and pathogens. During these three years of experimentation, and in spite of the fact that the leachates’ quality has changed a lot from one year to the other, it has been possible to adapt the technique, by the use of other plants’ species and other configurations and to have an idea of its efficiency in this particular case. If Epuvalisation has not shown any real efficiency in reducing the leachates' pollutant concentration to the point they could be rejected in the environment, it has, however, shown real capacities to "consume" a quite big amount of the effluent, what could be considered as a reduction of the means needed to treat the rest below in the treatment line.
Session 4  Pollutants in wastewater: pre-treatment and behaviour during utilisation

Treatment of wastewaters of agricultural origin in a constructed wetland

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The application of phytoremediation techniques in the treatment of polluted waters (rhizofiltration or rhizosphere bioremediation) seems particularly promising in all those situations in which isolated settlements have difficulties in connecting or in utilizing conventional water treatment plants (large scale depuration). This is the case, for instance, of agro-industrial plants like milk and cheese production farms.

A constructed wetland consisting in two horizontal sub-surface flow beds to treat dairy parlour wastewaters and domestic sewage was built in 1999 in a mountain agricultural settlement, at 600 m above sea level, in Northern Italy. The plant was originally designed to treat wastewaters, which are more polluted than domestic sewage, from a dairy farm with an 80-cow herd: 3000 mg/l COD, with a 40% BOD, for a total of 25 liters wastewater produced by each animal each day.

Each bed was 12.5 m long by 6 m wide and 1.1 m deep with a base slope of 1.5%. They were lined with PVC and filled with gravel each to a depth of 0.9 m. Each bed was equipped with three vertical perforated tubes to control and sample water flow, at different positions of the bed’s length. The beds were planted with Phragmites australis in April 2000 on a total surface of 150 m².

Pre-treatment of wastewaters was done in a septic tank followed by a plastic filter, both mainly used to store up suspended solids before they reach the reed beds.

Wastewater inflows and outflows were sampled throughout the first year of the trial, from April 2000 to March 2001 (20 times).

Wastewater inflows were sampled at three different times during the sampling day:
1) when no operation occurred in the milking parlour;
2) when milking occurred;
3) when parlour washings occurred;

Wastewater outflows were always sampled:
A) at the pre-treatment outflow;
B) at the first reed bed outflow;
C) at the second reed bed outflow;
and sometimes along the crossing of the reed beds in vertical control tubes.

Analyses were done for pH, Total Suspended Solids (TSS), COD, BOD₅, total nitrogen (TKN), NH₄-N, NO₃-N, P and heavy metals (copper and zinc).

The reeds were sampled at the end of the vegetative period (October 2001) to determine above- and below-ground growth, chlorophyll and heavy metal content and to verify the occurrence of mycorrhizae in roots. Heavy metal distribution was also verified in plant tissues by means of Scanning Electron Microscopy and microanalysis (SEM/EDX).

Measured mean flow rates were 6m³/day. The reed’s growth (root and shoot biomass, leaf number, plant height) in the first bed was significantly higher than in the second, whereas no difference could be evidenced in the frequency of occurrence of mycorrhizae. Significant
differences could also be found for the zinc content in reed stems and roots. SEM/EDX analyses evidenced the precipitation of iron on the outside surface of the root periderm, reported to be acting as a barrier towards uptake of other metals. The performance of the process was checked during the winter time to ascertain the feasibility of the treatment during the period in which plant activities are reduced.

The main results obtained from the whole system are shown in the following table. Bacteria transform input nitrogen in NH$_4$-N, then a nitrification process occurs in the presence of oxygen thanks to other bacteria strains. Probably the concentration in NH$_4$-N of the output water slightly increases because there is a general lack of oxygen that slows the nitrification reactions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INPUT (mg/l)</th>
<th>OUTPUT</th>
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<tbody>
<tr>
<td>TSS</td>
<td>760</td>
<td>70</td>
</tr>
<tr>
<td>COD</td>
<td>1380</td>
<td>105</td>
</tr>
<tr>
<td>BOD5</td>
<td>505</td>
<td>32</td>
</tr>
<tr>
<td>TKN</td>
<td>57.3</td>
<td>30.5</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>INPUT (mg/l)</th>
<th>OUTPUT</th>
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<tbody>
<tr>
<td>NH$_4$-N</td>
<td>21.8</td>
<td>23.3</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>8.5</td>
<td>5.6</td>
</tr>
<tr>
<td>P</td>
<td>14.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Organic N</td>
<td>35.5</td>
<td>7.2</td>
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According to the Italian legislation, wastewaters from dairy parlour are included in the regional law: “Legge della Regione Emilia Romagna 29 Gennaio 1983, n.7” and must conform to specific parameter values in order to be delivered to surface waters, as shown in the following table.

<table>
<thead>
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<th>Parameter</th>
<th>Limits</th>
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<tr>
<td>pH</td>
<td>5.5 – 9.5</td>
</tr>
<tr>
<td>TSS (mg/l)</td>
<td>80</td>
</tr>
<tr>
<td>BOD5 (mgO$_2$/l)</td>
<td>80</td>
</tr>
<tr>
<td>COD (mgO$_2$/l)</td>
<td>160</td>
</tr>
<tr>
<td>P (mg/l)</td>
<td>15</td>
</tr>
<tr>
<td>NH$_4$-N (mg/l)</td>
<td>25</td>
</tr>
<tr>
<td>NO$_3$-N (mg/l)</td>
<td>20</td>
</tr>
</tbody>
</table>

All parameters in the final outflow were therefore well below the acceptable values for discharge into water courses during the whole year of operation, showing that reed beds are able to reduce pollutant levels of wastewaters.
MUNICIPAL WASTEWATER DISINFECTION USING A CONSTRUCTED WETLAND

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Human pathogens are typically present in municipal wastewater, and their control is one of the fundamental reasons for wastewater treatment. Investigations have shown that constructed wetlands provide a low-cost, environmentally acceptable and high efficient method of wastewater treatment and pathogen removal.

The present study was carried out in Greece, in order to investigate the influence of climate, on the ability of a constructed wetland to disinfect municipal wastewater. The objectives of the project, were to: a) observe the seasonal changes in the reduction of coliform populations in a constructed wetland, b) study the effect of solar radiation and temperature on the ability of the wetland to reduce the concentration of total coliforms (TC), and c) evaluate whether the ability of the wetland to remove Salmonella spp. could be predicted by the concentration of total coliforms in the effluents of the wetland. For that reason, a prototype constructed wetland has been built in 1996 near Gallikos River in Thessaloniki, Greece, and operated since April 1997. The wetland is used for the secondary treatment of 100 m³/day of primary treated municipal wastewater, and consists of four parallel surface flow beds (planted with Typha latifolia), one facultative stabilization pond, and two subsurface flow beds (planted with Phragmites communis).

The constructed wetland effectively reduced total coliforms during spring, autumn, and summer, while the analysis of variance showed a significant difference between the inflow and outflow of the system. The percent reduction of coliforms was significantly lower during winter compared to all the other seasons.

A regression model of percent coliform reduction as dependent variable and temperature, solar radiation and their product as independent variables indicated a good fit ($r^2 = 0.89$). Especially, solar radiation and temperature had a positive effect on percent reduction of coliforms, with temperature having less impact. Temperature x solar radiation had negative effect, indicating that solar radiation has a direct lethal effect on coliforms, but its severity regulated by temperature.

Finally, the probability of Salmonella spp. appearance in the outflow of the system was positively related to $\log_{10}$ coliform levels, indicating that Salmonella spp. appearance may be predicted by the concentration of total coliforms.
Phytostabilisation of heavy metals in mine tailings by wetland plants
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When mine tailings containing pyrite react with water and atmospheric oxygen acid mine drainage, with elevated metal concentrations, is created. If an organic layer with plants is formed on top of water-saturated mine tailings it will probably consume oxygen, due to the processes of microbes and plant roots, which prevents the weathering of pyrite. However, plant roots might not just consume oxygen, but also release it, and thus may increase the leakage of heavy metals. Plants can take up such elements by the roots and either accumulate them there or translocate them to the shoots. To establish wetland plants for phytostabilisation it is important to know the action of roots in the tailing and weather they accumulate certain elements in roots. Furthermore, if metals are accumulated in the shoot, it could be an ecological problem for grazing animals. To establish vegetation some kind of amendment should be added, since mine tailings are low in nutrients.

The aim of this study was to find out the impacts of growth of Eriophorum angustifolium on the content of Cd, Cu, Pb, Zn and As in the drainage water from mine tailings, as well as to compare the element uptake and translocation properties of E. angustifolium with other wetland plant species.

Seeds of Eriophorum angustifolium and mine tailings were collected from a mine site in Kristineberg, northern Sweden. One of the tailings was lime treated (pH 10.9) at the impoundment for metal precipitation, while the other was unlimed (pH 5.0). Two thirds of one litre plastic pots were filled with tailings and on top, 2-3 cm of sewage sludge (plant nutrient source) were added, except in control pots. The pots had six holes (ø=1mm) in the bottom, and they were placed in an outer pot in order to collect the drainage water. Germinated seeds from Eriophorum angustifolium were added to half of the pots with sewage sludge. The pots were placed in a greenhouse and water covered. After about 7 months, samples were taken from the drainage water and analysed for pH and Cd, Zn, Cu, Pb and As. In addition, root and shoot samples of E. angustifolium, Carex rostrata, Phragmites australis and Salix ssp. growing on a waste-water treated tailing impoundment at the Boliden mine site, northern Sweden, were collected. The samples were wet digested and analysed for the mentioned elements.

On the one hand, the presence of plants increased the pH and reduced the metal concentrations of the drainage water from pots with unlimed tailings, but did not affect the As concentration. On the other hand, the metal concentrations and the pH of the drainage water from pots with lime treated tailings were not affected by the presence of plants. The addition of sewage sludge increased the As concentration in the drainage water from these pots, while this increase was depressed in the presence of plants. The comparison of the element uptake and translocation of E. angustifolium with other wetland species show that E. angustifolium had significantly higher concentrations of Cu, Pb and As in the shoot tissue compared to the other species. In addition, there were also tendencies that the root concentrations of all measured elements were higher in E. angustifolium compared to the other species.
It is likely that *E. angustifolium* is able to work well as a phytostabiliser in unlimed tailings since it increases pH and have a high accumulation of metals in the roots. The high shoot accumulation of Cu, Pb and As of 23, 38 and 8 µg g⁻¹, respectively, could be compared to the concentrations of a “normal” plant of 4-15, 0.1-10 and 0.1-5 µg g⁻¹ (Pais and Jones Jr., 1997). It is doubtful if *E. angustifolium* cause metal dispersion through grazing animals, since even though the plant tissue concentrations are higher compared to the other wetland species grow in the mine tailings, they are not so far from the concentrations of a “normal” plant.

Reference
BASIC REQUIREMENTS FOR RADIOPHYTOREMEDICATION AND RADIOPHYTOMONITORING

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Phytoremediation is a relatively young technology to reduce some pollutants in the environment. It is used, especially, for heavy metals till now. During the last years, different analyses have been described in the literature using phytoremediation also for radionuclides (radiophytoremediation) (e.g.1,2,3). However, no systematic work has been done to analyze different conditions and relevant consequences concerning large-scale application of this approach.

Low levels of radionuclides can enter into soils and/or waters by different human activities and nuclear accidents. As it was proved after the Chernobyl accident, the highest long – term environmental effect, existing till now, has been the distribution of $^{137}$Cs, almost within the whole Europe.

In our experiments we test the utilization of radiophytoremediation approach for accumulation of $^{137}$Cs, $^{125}$I and $^{63}$Ni using three plant species (Phragmites australis L., Heliantus annuus L., Populus simonii L.) in hydroponic conditions. We measure the decreasing of amount of radionuclides in liquid media, as well as their distribution in plant tissues using autoradiography.

Generally, localisation of the nuclides was found mostly in the areas of the cell expansion and active metabolism and thus in the parts of the increased requirement of water and nutrients i.e., in the node, leaf tips and young leaves and in the young shoot meristems. These laboratory results were partially confirmed in preliminary field studies.

On the basis of above mentioned results it was possible to elucidate serious differences in plant system requirements for “normal” metals phytoremediation and radiophytoremediation, both from practical as well as theoretical point of view. This requirements will be discussed in our contribution.

The utilization of selected plant species for radiophytomonitoring in surroundings of new nuclear power plant in South Bohemia will be presented too.

References

2) Entry JA, Watrud LS.: Potential remediation of Cs-137 and Sr-90 contaminated soil by accumulation in Alamo switchgrass. WATER AIR SOIL POLL 104: (3-4) 339-352 JUN 1998


Acknowledgement
This work was supported by Grant Agency AVCR project No. A6055902, S4055014, COST project No. 837.10, and Z4 055 905 research project.
Passive Biological Treatment of Waters Contaminated by Uranium Mining

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Treatment of radioactively contaminated and metal-laden mine waters and of seepage from tailings ponds and waste rock piles is among the key issues facing WISMUT GmbH in their task to remediate the legacy of uranium mining and processing in the Free States of Saxony and Thuringia, Federal Republic of Germany.

Generally, contaminant loads of feed waters will diminish over time.

At a certain level of costs for the removal of one contaminant unit, continued operation of conventional water treatment plants can hardly be justified any longer. As treatment is still required for water protection, there is urgent need for the development and implementation of more cost efficient technologies.

BioPlanta GmbH and WISMUT GmbH have studied the suitability of helophyte species for contaminant removal from mine waters.

In a first step, original waters were used for an In-vitro bioassay. The test results allowed to determine the effects of biotic and abiotic factors of influence on helophytes' tolerance range, growth, and uptake capability of radionuclides and metals. Test series were carried out using Phragmites australis, Carex disticha, Typha latifolia, and Juncus effusus. Relevant contaminant components of the mine waters under investigation included uranium, iron, arsenic, manganese, nickel, and copper.

Investigations led to a number of recommendations concerning plant selection for specific water treatment needs.

Investigations on constructed wetlands in technical scale for the treatment of flood water from the Pöhlä-Tellerhäuser mine were done in a second step. Relevant constituents of the neutral flood water include radium, iron, and arsenic.

BioPlanta further pursue a technical wetland plant for remediation of drainage waters contaminated with explosives. The remediation performance achievable for the plant-microorganism-based process is been tested. The total nitroaromaten contamination is about 1,200 – 1,500 µg/L. The results demonstrate the fundamental efficacy of the passiv biological process.
The higher biomass of crop plants and their subsequent higher root development converts them in a plausible alternative to (hyper)accumulators as pollutant extractors from waters or liquid media (Salt et al. 1998). *Lupinus* species are considered as acidophilous, with strong root system and high capacity of solubilization and uptake of elements from soils (Belteky et al. 1983; LópezBellido and Fuentes 1986). Our aim was, to study the Cd uptake by *Lupinus albus* L. (two cvs. Multolupa and Marta) grown hydroponically in a glasshouse.

Two experiments were carried out with both lupin cvs. Seedlings were transplanted to pots filled with vermiculite or perlite in a glasshouse (T 10-26 °C; HR 50-70%). Nutrient solution (pH 6) was: Macroelements (mM): 2,75 K₂SO₄; 0,48 KH₂PO₄; 1,05 MgSO₄; 0,60 CaSO₄. Microelements (μM): 36 FeEDDHA; 32,4 MnSO₄.H₂O; 92,5 H₃BO₃; 1,5 ZnSO₄.7H₂O; 1,3 CuSO₄.5H₂O; 1,2 (NH₄)₂MoO₄.24H₂O; 0,001 NiCl₂.6H₂O and 0,001 CoCl₂.7H₂O. Cd treatments were 9 and 18 μM. Plants were inoculated with *Bradyrhizobium* sp (*Lupinus*), strain ISLU-16. Plants not inoculated were supplied with 1 mM KNO₃ and 0,5 mM Ca(NO₃)₂. Plants were sampled after three or five weeks of the beginning of treatments.

Total content of mineral elements were determined in plants, remaining nutrient solutions and also the elements retained by the substrate.

Plant biomass of treated plants was no altered in the same way of Costa and Morel (1993) reporting no effect in biomass production of lupinus treated with 10 μM Cd. Cd level in roots is higher than in leaves and stems, but considering total distribution of Cd after 5 weeks of treatment, 46% of Cd was found in the aerial part and 54% in root. On the other hand, Cd bound to cell wall of leaves account by a 40% of total leaf Cd. This percentage is double than the found in pea plant (Hernández et al. 1998).

Plant exported Cd reflects closely the treatments, but several difficulties appeared when tried to fit these data with those of Cd that disappeared from the solution; the discordance was solved considering the Cd retained by the substrates. As Cd was retained by vermiculite, Mn was desorpted.

After these first results, we can consider lupin plant as a good candidate for phytoextraction or removal of Cd from polluted media, and probably for other elements as As, Pb or Zn.

Acknowledgments: Finantial support was given by DGESI, PB98-0114-CO2-02. Seeds and bacterial strains were a kind gift from Dra. Fernández-Pascual (CSIC) and from Ing. Gil Aragón (Serv. Investig. Desarrollo Tecnológico, Junta de Extremadura) Technical assistance of Ana I. Gamo is acknowledged.

Phytoremediation is an experimental technology for remediation of contaminated soils. One problem, presently limiting its applicability for certain organic pollutants, is insufficient degradation of the pollutants by the plants. As a result, the original compound or its metabolites build up in the plants (poisoning the plants) or are volatilized through the leaves (causing a new environmental problem). This project aims to develop and test specific endophytic (plant-internal) bacteria capable of degrading key pollutants. These new bacteria, when inoculated to the inside of plants, will degrade the pollutants as they are being transported from the roots to the leaves. This will reduce phytotoxicity and volatilization of the pollutants. As a result, phytoremediation technology will be applicable to more toxic compounds or higher pollutant concentrations, and will maintain its social and regulatory appeal.

Objectives:

This project intends to solve the question: “Can endophytic bacteria be utilized to improve phytoremediation of organic pollutants?” We hypothesize that endophytic bacteria can be made to degrade toxic organic compounds within the plant vascular system. Thereby they will assist the plants in metabolising soil contaminants. As a result, even when the plants are taking up pollutants at a high rate, the concentration of toxic compounds in the plant sap will be kept low. This will reduce contaminant toxicity for, and volatilization from, the plants.

Description of the work:

This project will

- isolate and identify endophytic bacteria from selected phytoremediation plants,
- characterize these bacteria and determine their colonisation properties using molecular ecological techniques,
- construct endophytic bacterial strains for degradation of organic pollutants by natural gene transfer and strain engineering,
- re-inoculate modified endophytic bacteria into their host plant and study re-colonisation of the plant vascular system and expression of catabolic properties,
• mathematically model the effect of inoculated modified endophytic bacteria on phytoremediation efficiency in laboratory systems, achieving contaminant mass balances, 
• assess the risk of modified endophytic bacteria for humans, animals, plants and environment, and 
• demonstrate improved phytoremediation at pilot and field scale.

**Deliverables:**
The feasibility of the concept of endophytic bacterial degraders will be demonstrated. In addition, the project will generate an endophyte inoculation technique, useful modified bacterial strains for phytoremediation, knowledge on the risk associated with their field use, and protocols for field application of the new technology. As a result, phytoremediation will become applicable to soils otherwise too toxic for plants to grow in, and contaminant volatilisation will be avoided.

_Acknowledgement_
This work is supported by the European Commission as project number QLK3-2000-00164.
Physiological and structural characteristics and *in vitro* cultivation of some willows and poplars

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Several species and clones of willow and poplar were cultivated in both hydroponics and *in vitro*. For hydroponics stem segments ca 20 cm long were used and hydroponic solution contained $10^{-5}$ M Cd(NO$_3$)$_2$. After three weeks of cultivation adventitious roots and new shoots were formed. The content of cadmium was determined in individual parts of plants. Majority of Cd remained in roots, the values of Cd ranged between 4100 and 5000 µg.g$^{-1}$ DW. The values in stem segments were 40 – 160 µg.g$^{-1}$ DW and leaves contained only 3 – 29 µg.g$^{-1}$ DW. The main barrier for cadmium uptake seems to be the root, which might be connected with early development of endodermis and/or exodermis – main apoplastic barriers of the root.

*In vitro* cultures were used to test the sensitivity of cells and to select cell clones tolerant to high concentrations of cadmium and capable to accumulate this toxic metal. Cultures of *Salix viminalis*, *Salix alba*, *Salix sp.* clone 171, *Populus x euroamericana* Gigant, *Populus x euroamericana* Robusta, and *Populus alba pyramidalis* were initiated. From all species organ (shoot and root) cultures and callus cultures were induced. High multiplication of shoot cultures was achieved especially from *Salix viminalis*. The long-term callus culture of *Populus alba pyramidalis* showed high tolerance to cadmium in the medium and ability to accumulate exceptionally high amounts of this metal. The highest content of cadmium after 35 days of cultivation on the medium with $10^{-3}$ M of CdCl$_2$ was 8600 µg.g$^{-1}$ DW. The viability of this culture on medium supplemented with cadmium was high even after 10 subcultures (each subculture was 35 days long). Structurally the callus became more compact lacking intercellular spaces and gradually the intensity of originally green pigmentation decreased.
Effects on *Phragmites* plants of toxic concentration of Cd $^{+2}$ in the root environment
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Many constructed wetlands use *Phragmites australis* to biofilter waste waters where many different contaminants including heavy metals could be found. Soluble Cd$^{+2}$ can be one of the most concentrated metal ion in waters particularly if they receive agricultural run off. It is known that this metal is very toxic to *Phragmites* but scant information is available on the mechanisms impaired and damages inferred to plants. Here we report a preliminary investigation on the interaction of Cd$^{+2}$ with the root surface, on the partitioning of the metal ion between roots and leaves and on its effects on primary leaf processes. We analysed the redox status (NAD/NADH, NADP/NADPH, GSH/GSSG, ascorbate) and the antioxidant activities (GPX, GR, GST, SOD APX and Cat) in roots exposed to 50 and 100 ppm Cd$^{+2}$. Similar biochemical analysis and a detailed characterisation of photosynthetic gas exchange and of photochemical activities were also done in leaves. We found that roots kept most of the absorbed Cd$^{+2}$ and that this induced a there significant oxidative stress. The higher increase of glutathione peroxidase, relatively to the activity of other enzymes, seems to indicate that this stress was particularly severe in membranes. The low amount of Cd$^{+2}$ transported to leaves was, however, enough to inhibit the photosynthetic gas exchange almost proportionally to the concentration around the root environment. This inhibition was not reversed by exposing leaves to a very high CO$_2$ concentration to overcome possible stomatal limitation brought about by root damages. On the contrary, the photochemical efficiency was not affected by any Cd$^{+2}$ concentration despite a strong decrease of leaf chlorophyll content. The short term imposition of a further stress such as low temperature and strong light evidenced a much higher susceptibility of Cd$^{+2}$ leaves with respect to control leaves indicating a lower energy dissipation capacity in the chloroplasts. The implications of these results for the selection of *Phragmites* genotypes to include in constructed wetlands for the biofiltration of waters with high concentration of Cd$^{+2}$ are discussed.