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*Phytotechnologies to promote sustainable
land use and improve food safety*



PHYTOTECHNOLOGIES LESSONS FROM PILOT AND FIELD SCALE

Book of Abstracts



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Key note lectures

Phytoremediation of metal contaminated soils: data from field experiments

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Abstract

An overview will be given of existing information concerning the use of plants for the remediation of metal contaminated soils. Both, site decontamination (phytoextraction) and stabilization techniques (phytostabilisation) will be discussed, but focus will be on phytoextraction.

Recent studies investigated the feasibility of phytoextraction and confirmed that both biomass production and metal concentration factor (metal (hyper) accumulation) determine the efficiency of the remediation process. Several studies claiming to have demonstrated a high potential of different plant species for remediation of contaminated sites in fact were using plants showing a bioconcentration factor of lower than 0.5. A simple calculation using realistic biomass production levels leads to the conclusion that remediation of a moderately contaminated soil should take at least more than 100 years. In (hyper) accumulator plants, the bioconcentration factor is usually higher than 1 and in some cases even up to 100. For easy harvesting, the root to shoot transport should be efficient, resulting in a shoot to root ratio of metal concentration higher than 1. Few studies report on the use of natural metal hyperaccumulators under field conditions. The metal concentration factor is not only depending on plant but also soil factors (soil type, pH, organic matter content, ...). Metal availability in soils plays an important role in the efficiency of the remediation process. The use of amendments for mobilization (f.i. chelating or acidifying agents) of metals in the soil can sometimes improve the metal accumulation by plants. Chemically assisted phytoextraction is based on the use of non-accumulator plants with metal accumulation levels far below those of hyperaccumulators, but with high biomass potential. Restrictions apply, however, to both the use of complexing agents and artificial soil acidification. It was found that EDTA and EDTA-heavy metal complexes are toxic for some plants and that high dose of EDTA inhibited f.i. the development of arbuscular mycorrhiza. Furthermore, EDTA is poorly photo-, chemo- and biodegradable. *In situ* application of both poorly degradable but also easily degradable chelating agents can cause groundwater pollution by uncontrolled metal dissolution and leaching. Since in many cases metal uptake by plants is limited by low metal solubility, it is necessary that the efforts for selection of appropriate rhizosphere manipulation be continued. There is a need to find cheaper, environmentally benign chemical compounds with chelating properties as well as to better understand the role of rhizospheric bacteria in metal solubility, plant uptake and tolerance. Another possibility that should be considered is the use of Plant Growth Promoting Bacteria that stimulate root formation by plants and also produce siderophores. These siderophores can interact with heavy metals, in certain cases reducing their toxicity and increasing their bioavailability and uptake by plants. Endophytic bacteria can be engineered for increased heavy metal sequestration. The (combined) activities of these bacterial strains could enhance heavy metal uptake and translocation by the host plants. Bacterial siderophores can be considered as natural chelators and the bacterial production of which is in tight equilibrium with plant activity, thus improving heavy metal uptake and translocation as part of the phytoextraction process. Besides of the more biological and technical aspects, the economical impacts of changed land-use, eventual valorisation of biomass and cost-benefit aspects of phytoremediation will be briefly addressed. An opinion exists that phytoextraction will get only economically feasible if, in addition to metal removal, plants produce biomass with an added economical value (see abstract and presentation of T. Thewys). An increasing number of reports are confirming the rationale of this option.

Conclusions

It is clear that, in spite of the a growing public and commercial interest and success, more fundamental research still is needed to better exploit the metabolic diversity of the plants themselves (knowledge of the molecular mechanisms of (hyper)accumulation of metals), but also to better understand the complex interactions between metals, soil, plant roots and micro-organisms (bacteria and mycorrhiza) in the rhizosphere. Improvement also could be achieved by optimization of agronomic practices further, more demonstration experiments are needed to measure the underlying economics, for public acceptance and last but not least to convince policy makers.



Phytotechnologies in developing countries

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Keywords: Phytotechnologies, wastewater treatment, constructed wetlands, water reuse, effluents

Abstract

The term Phytotechnology describes solutions involving plants to address environmental challenges. An environmentally related application is the use of constructed wetlands for wastewater treatment (minimizing environmental impact) and reuse of process water (protection of groundwater). In developing countries provides the reduction of discharge of untreated and insufficient treated wastewater a basis for minimizing the environmental impact. In this countries above all not available capital resources for water treatment leads to a lack of water treatment plants and adequate maintenance. The water reuse is also very important in developing countries.

The use of constructed wetlands for wastewater treatment is an economic facility for an appropriated water treatment. Constructed wetlands concern mechanical and biological treatment steps. They can be integrated in existing, traditional treatment plants to improve the effluent quality. The main advantages of this technology are the simple construction, long lifecycle and low maintenance. On the base of the knowledge of local conditions, the application of constructed wetlands can be considered. By using of natural processes to treat wastewater, the environmental quality can be effectively improved. BioPlanta has planned und built constructed wetlands in Latin America for the treatment and reuse of municipal wastewater for irrigation of green areas in Peru and tannery effluents in Mexico. For the treatment of process water from a copper mine in Chile BioPlanta is planning a pilot scale wetland system.

- 1) In Peru the lack of water forced the local farmers to use untreated wastewater, taken from the main sewage collector, for irrigation of vegetable cultures. BioPlanta planned and installed four constructed wetlands for wastewater hygienization in the region of Callao in recent years. Small area constructed wetlands on each of the small agricultural plots treat the needed amount of water for irrigation of the surrounding fields. The systems reduce the contamination of the water with germs by up to 6 orders of magnitude. Operated properly, the standards of the Peruvian Water Act (Class III and IV) are met.
- 2) The leather processing industry is very important for Mexico. In the tanning process large amounts of highly contaminated wastewater are produced. After a physical-chemical treatment, effluents do not meet the required regulatory limits, and it's difficult to reuse the water as process water. A new concept for the water management in the Teneria Europea in León has been established, integrating the existing physicochemical treatment systems and a new constructed wetland. For the supply of reusable process water a modular wetland system was planned. The plant were built and put into operation in 2005. The constructed wetland shows excellent reduction in COD and BOD (up to 99%).
- 3) By mining of copper and molybdenum polluted process water occurs in a mine in Chile. At present the untreated process water flows to a water reservoir and for leaching in green spaces. For the reduction of water pollution before leaching a constructed wetland will be planned and installed in pilot scale in 2006.

The application and use of Phytotechnologies range from effects on the environment and human health to impacts on social and economic conditions.

To reduce environmental impact is important to use sustainable solutions which take into account ecosystem needs. Technology decisions should consider overall life-cycle costs, benefits and risks, the mix of human and capital resources required, and the conditions where environmentally sound technologies such as Phytotechnologies may be applied. Governments should support the consistent and fair application of environmental regulations within a framework that support the development of innovative, ecosystem -based solutions, such as those which can be achieved through the appropriate use of Phytotechnologies.

Phytoremediation: socio-economic aspects

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Keywords: phytoremediation, economics, renewable energy, agricultural income

Abstract

The replacement of a traditional crop in favour of a phytoremediating culture, is not a neutral operation. Apart from physical constraints - like not fitting in the crop rotation - the social acceptability of introducing a phytoremediating crop depends on the condition that the present value of the gross labor income (the total revenue diminished by the non-labor variable costs) earned on the area to be cleaned up and calculated over a sufficient long period, is at least not decreased. Phytoextraction will be implemented only if it is a financially attractive long-term option. From this perspective, when a farmer considers to introduce (if any) phytoremediating crop, his decision making can be assisted by the device called 'cost-benefit analysis' in which the timely evolution of costs and benefits of phytoremediation can be taken up. The outcome is then summarized by the net present value of the result. Assuming a predefined time period for the study, a cost-benefit approach could distinguish the following items (Vassilev et.al., 2004, p.28):

1. The cost of the phytoremediation action, capital and operational costs will be strongly connected with the pollutant removal performance of the remediation crop, the soil conditions, the difference between the initial and the target level of pollution, etc. All these items will also determine the length of the remediation period.
2. The loss of the income that the soil is still generating even in its polluted situation.
3. The eventual income through the valorisation of the biomass. This can be considered as 'recovering' some of the costs of phytoremediation.
4. The regained income of the soil after reclamation, determined by its functional use for which the reclamation target is decisive.

The case study considered applies to a large area cross bordering the eastern part of Flanders and the Netherlands in which diffuse heavy metal pollution forms a heritage from the historical zinc smelters in the region. The surface of the area is so vast that traditional techniques are far too costly to be considered. In this case a cost-benefit analysis is useful in deciding which phytoremediation crops are most fitted from the point of view of their impact on agricultural income. For the larger part the actual income comes from dairy cattle rearing. The cattle feeding (roughage), mostly maize, forms the most important land use. In switching this land use towards phytoremediation accumulator crops the income of the land use would change. As a reference for this change we use the present value of the actual labor income of the average farm (36 ha) earned by cultivating roughage (grassland and maize). Two alternative phytoremediation crops are considered: (i) rape (brassica nappus), and (ii) willow (salix spp.) in 'short rotation forestry'. The choice for rape is motivated by the possibility of using its biomass as a source of renewable energy like bio-diesel. With the purpose to create the value added as much as possible on the farm itself, we opt for the production of pure plant oil (PPO) by the farmer to be used as fuel (with a price of € 0,50/lit.) for his own tractor(s) equipped for such an application. In the phytoremediation scenario, the yearly cultivation scheme then looks as follows: 8 ha of rape (in 4 year rotation); 4 ha of willow (whole period, with harvest every 4th year); 24 ha of roughage (to continue the cattle rearing). Remark that the lost produce of the surface of roughage substituted by rape and willow (12 ha), is compensated by external buying, so that the cattle population can stay the same. The reclamation activity aims at removing on average 1,9 kg Cd/ha (depth of 30 cm). The net present value (NPV) over a period of 40 years of the gross labor income resulting from the mentioned phytoremediation cultivation scheme is 4,5% higher than the NPV of continuing the actual land occupation, that is 36 ha only for roughage - as if no soil reclamation would have been necessary. If the farmer would sell the PPO as a fuel for cars to a distribution network (at a price of € 0,65/lit.), the NPV then would be 11,7% higher. Increasing the ratio of willow versus rape from 4 ha/8 ha to 8 ha/12 ha (and thus also increasing the phytoremediation surface) remarkably shortens the calculated remediation period from 38 to 21 years (willow has a relative higher uptake performance), but the NPV is now only 2% (instead of 4,5%) higher than the reference (the biomass of willow has less profitable applications than the biomass of rape).

Reference

Vassilev, A., Schwitzguébel, J-P, Thewys, T., van der Lelie, D., Vangronsveld, J. (2004), *The use of plants for remediation of metal contaminated soils*, TheScientificWorldJOURNAL, vol. 4, p.9-34.



How the French government manage the « faint person in charge » of the Métaleurop Nord site? Integrative approach around a smelting area

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Abstract

Located in the North part of France (\approx 12 kms from Douai), the smelting plant « Métaleurop Nord » is being dismantled. Created in 1894, the main production of the plant was nonferrous metals. For about one century, the plant released huge amounts of lead, cadmium and zinc. Consequently, soils and the environment were greatly contaminated. Thanks to the French administration, numerous metal concentration measurements in the soil were performed that permitted to draw the isoconcentration curves. The results of Cd and Pb concentrations showed that something had to be done.

On January 2003, Métaleurop S.A. decided to cut all links with Métaleurop Nord. On March 2003, Métaleurop Nord was finished off leading to a serious socio-economic crisis (at least 830 dismissals). In this context, the French government allowed the ADEME (Agence pour l'Environnement et la Maîtrise de l'Energie) to act for preventing health risks. The allocated budget is 1.488.000 euros.

Thus, the tasks of ADEME are:

- to end the diagnosis and the detailed risk assessment of the outside parts of the plant
- to clean school playground in order to prevent metal contamination
- to take charge of polluted scraped soils and demolition materials in case of private new building, to look for perennial management solutions
- to manage and finance a device for preventing health risk linked to agricultural products and concerning farms impacted by soil pollution, to think about new activities
- to buy wood and maintain agricultural soils in the zone where the soil concentration of Pb is higher than 250 mg kg⁻¹.

The talk will focus on:

- the presentation of the smelting plant site (interactive visit)
- the quick presentation of the French administrative process to manage sites called « faint person in charge of the site » illustrated by the case of Métaleurop Nord
- the detail of the actions carried out by the ADEME in the case of the Métaleurop Nord zone.



The Use of Constructed Wetlands for Re-habilitating Wastewater Treatment in Tsunami-affected Areas of Thailand

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Keywords: constructed wetland; reuse; plant; phytotechnology; wastewater

Abstract

The tsunami of December 2004 destroyed infrastructure in many coastal areas in South-East Asia. In January 2005 the Danish Government gave a tsunami relief grant to Thailand to re-establish the wastewater management services in some of the areas affected by the tsunami through the development of appropriate wastewater collection and treatment systems. Constructed wetlands are in this context being selected as an appropriate on-site wastewater treatment technology. The paper will describe the systems which have been built at the following three locations:

Koh Phi Phi Don. The new constructed wetland treats the wastewater from the main business and hotel area on the island thereby reducing the amount of pollutants such as organic matter and nutrients at the beaches of Koh Phi Phi. The project comprises the establishment of a wastewater collection system for the main business and hotel area of Koh Phi Phi, a pumping station and a pressure pipe system to the treatment facility, a two-stage constructed wetland system, and the establishment of a system for reuse of treated wastewater, as freshwater is a scarce resource at the island. A Solar system is installed to power the pumps, and puls-loading of the vertical flow constructed wetland is secured by the use of siphons.

Patong: The wastewater management system in Patong consists of a horizontal flow constructed wetland systems and associated river bank improvements. The new constructed wetland will treats the water in two small rivers/drains-Khlong Pak Bang and Khlong Pak Lak during low tide thereby reducing the amount of pollutants such as organic matter and nutrients in the river water and at the outlet to Patong Beach.

Baan Pru Teau: The constructed wetland treats the wastewater from 80 households which are being constructed with the generous contribution of the Thai Red Cross. The project is under the auspices of Her Royal Highness Princess Maha Chakri Sirindhorn. The subsurface flow constructed wetland consists of five units and is fed by a pumping station with an average flow rate of approximately 40 m³ per day.

The constructed wetland technology is considered an appropriate technology for Thailand, and the constructed wetland systems established will serve as demonstration facilities for Thailand.



Oral Presentations

Phytoextraction of metals: Field-testing

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Keywords: soil pollution, heavy metals, phytoremediation, pilot scale

Abstract

Phytoextraction, an *in situ* remediation technique that uses hiperaccumulator plants to extract contaminants from soils and accumulate them in the harvestable parts of the plant, which can then be removed from site, is a relatively new environmentally sustainable technology, low-input approach for remediation of contaminated soils.

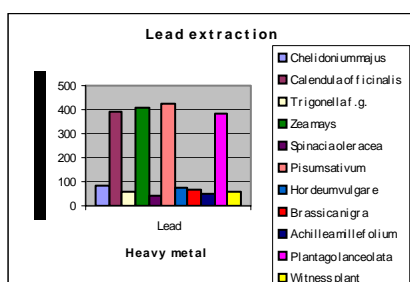
In this investigation, two field trials were undertaken to evaluate the ability of eleven plant species (*Calendula officinalis*, *Trigonella foenum graecum*, *Lactuca sativa*, *Spinacia oleracea*, *Pisum sativum*, *Hordeum vulgare*, *Chelidonium majus*, *Achillea millefolium*, *Plantago lanceolata*, *Zea Mays* and *Brassica nigra*) to extract heavy metals (Cu, Pb, Zn, Cd, Ni, Mn, Cr, Co) from contaminated soils around a major Romanian power plant: Deva power plant.

We have collected and analysed (by means of atomic spectrometry) soil and plant samples from each plot before and after the experiment.

In the paper, we have presented the encouraging results of heavy metal accumulation in plants and the percent of heavy metal extracted from soil compared to total soil pollutant content.

The feasibility of phytoextraction was reviewed for the two field trials undertaken in this project. The literature would indicate that incineration and pyrolysis appear to be the most promising techniques for post harvest disposal or possible recovery of metal from biomass grown on contaminated soils. However, both these techniques have to be fully tested on a commercial scale. The estimated costs of disposal of plant material containing “hazardous” concentrations of heavy metals/metalloids to landfill sites are around 200 euros per tonne.

Along with the other costs associated with phytoremediation such as biomass production and pre-treatment of biomass, phytoremediation remains cheaper than many other remediation technologies currently available, but may take more time.



Lead extraction



Calendula officinalis



Achillea millefolium

Heavy metal accumulation in *Solanaceae* plants grown at highly contaminated area

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Keywords: heavy metals, *Solanaceae* plants, phytoextraction, microbial activity, field experiment

Abstract

In previous studies we showed that the contamination of the soils in general (Shilev et al., 2003; Shilev et al., 2006) and particularly, in south of the city of Plovdiv (South Bulgaria), had been strongly influenced by the Non-Ferrous-Metal Smelter, NFMS (Shilev & Naydenov, 2005). In this study, crops of the family *Solanaceae* - tomato (*Lycopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annum* L.), were grown in this industrially polluted region. The experimental plots were situated at two different distances (0.5 and 15 km) from the source of pollution. We investigated the level of soil heavy metal contamination as well as the concentration into the plant shoots. The plant heavy metals content (roots, stems, leaves and fruit-sets) were determined after the method of the wet mineralization (Lozano-Rodríguez et al., 1995). The quantitative measurements were carried out by atomic absorption spectroscopy (AAS).

We found that the level of soil contamination depends of the distance from the source (NFMS) and the winds, and is very high, reaching levels of 630 mg Pb kg⁻¹, 13.2 mg Cd kg⁻¹, 60.1 mg Cu kg⁻¹, 974 mg Zn kg⁻¹, in the studied field area, which is between 3 and 8 folds up to the Maximum Permissible Concentrations (MPC) according the Bulgarian Law.

There were observed significant differences in the accumulated values of metals in the plant tissues. The highest values of Pb were found in the leaves of eggplant, while the Pb content in the leaves of pepper and tomato was much lower (4 and 2 times). The lowest concentration of this metal was observed into the stems, particularly of tomato. In surprise, the concentrations of lead in fruit-sets were found to be significant - 81.82 mg kg⁻¹ for tomato, 115.38 mg kg⁻¹ for pepper and 42.1 mg kg⁻¹ for the eggplant. Meanwhile, cadmium was the unique metal for which some tissue concentrations were found to be higher than the soil ones. The highest concentration of Cd was observed in the roots of tomato, while the lowest one was found into the leaves of pepper.

A strongly exhibited tendency towards decrease of the contents of heavy metals in these crops was observed as the distance from the NFMS increases. Generally, crops from the family of *Solanaceae* are not suitable cultures for growth in industrially polluted regions – they remove considerable quantities of heavy metals from the soil with their roots, leaves and fruit-sets, and they are potential danger for the end consumer in the food chain also because of high quantities used for food. On the other hand, the soil microbial activity was strongly influenced by the contamination. Decreasing the contamination, the microorganisms' activity goes up (at 0.5 km from the NFMS), while near to the factory was observed that the MPN and microbial respiration CO₂ had reduced levels.

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Feasibility of phytoextraction using sunflower mutants with enhanced metal uptake characteristics

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Keywords: phytoextraction, sunflower mutants, metal accumulation, extraction

Abstract

Classical mutagenesis has been successfully used for induction of metal tolerance and to increase metal shoot concentration in some plants (Delhaize, 1996; Nawrot et al., 2001). Classical mutation techniques were also assumed to be appropriate to improve the efficiency of metal accumulation of high yielding crops (Herzig et al., 1997).

The main goal of the present study was the assessment of new sunflower mutants, obtained by means of chemical mutagenesis, for metal accumulation and extraction characteristics under real field conditions. In addition, classical fertilisers were used to enhance both metal accumulation and extraction efficiency of sunflower mutants.

Sunflower mutants of M₂ generation were tested on the sewage sludge contaminated field in Rafz (2004) and M₃ sunflower mutants were tested on two metal contaminated fields in Switzerland; on the Rafz field and on the Zn contaminated field in Bettwiesen (2005).

The sunflower mutants of the second generation showed a 2-3 times higher metal shoot accumulation than the control plants and, even more, the metal extraction of the best sunflower mutants was increased 7.5 times for Cd, 9.2 times for Zn and 8.2 times for Pb, as compared to the control plants. By sunflower mutagenesis we obtained mutants with an enhanced metal accumulation/extraction (for phytoremediation use) as well as mutants with a lower metal accumulation (for improved food quality).

Theoretical calculations of phytoextraction potential of sunflowers point out that the best M₂ sunflower mutant 14/185/04 can produce up to 26 t dry matter yield per ha and remove 13.3 kg Zn and 31.2 g Cd per ha and year at the metal contaminated site in Rafz (total metal concentration: Cd 0.9 mg/kg, Zn 813 mg/kg, Pb 492 mg/kg, for a soil pH of 5.8). From a practical point of view the metal removal of the best sunflower mutants looks very promising for boosting future phytoextraction research and application.

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Prosopis juliflora plays a significant role in reclamation of mine soils, flyash land fills, perturbed ecosystems and adsorbs toxic metals - evidence from lan and field

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Keywords: *Prosopis juliflora* (Velvet mesquite), heavy metals, absorption/adsorption, fly ash, mine reclamation, lan, field.

Abstract

Prosopis juliflora DC (Velvet Mesquite), an evergreen, fast growing, drought resistant, widely distributed ptreatophyte not only in India but also in other arid and semi-arid tropical countries. Metal adsorption using pods (Figure 1) and its field potential for reclamation of mine soils, flyash land fills and turbed ecosystems are highlighted in this presentation. (Figure 2 and 3). *P.juliflora* seedlings cultivated in hydroponics are able to bioaccumulate Ni, Cd and Cr. It is helpful for reclamation of mica, copper, tungston, marble, dolomite mine tailings. Arbuscular mycorrhizal inocula have been isolated from its rhizosphere (low cost agrotechnology) were found to accelerate the growth of other agroforestry and social forestry legumes in perturbed ecosystems (Kailappan 2000, Rai et al 2004 and Senthilkumar 2005).

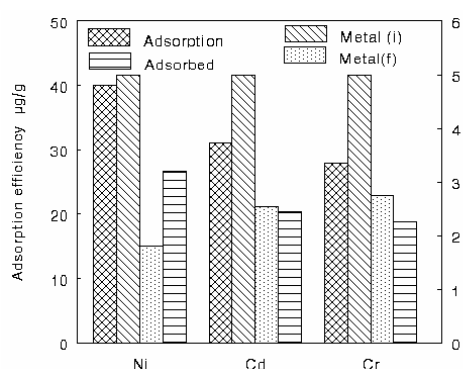


Figure 1. Comparison of biosorption of Ni, Cd and Cr using pod powder of *P. juliflora* after 24 hrs.



Figure 2. *P. juliflora* growing on mine tailings accumulate large concentrations variety of heavy metals

Figure 3. *P.juliflora* providing shelter to migratory birds, contributes environmental protection and conseration.

Its bioresource potential and contribution to sustainable development are presented with lan and field data.

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Field research of sites polluted with pesticides

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Keywords: obsolete pesticides, soil, phytoremediation, herbicides, POPs

Abstract

The obsolete pesticides's storehouses (their quantity is over 5000 in Ukraine) causes big problems. Around those storehouses sites polluted with different pesticides and their metabolites where formed. There are substantial human health threats from pesticide residues in surface water, in groundwater as a result of the vertical and horizontal migration, and suspended in air. Our task was to make a representative sampling of such soils, select the control parameters of such soils, evaluate the level of the soil pollution and propose a method of their remediation. For this purpose, we developed a scheme of soils selection. Using this method, soil samples were taken from six distances from a warehouse - 1, 5, 10, 15, 25, 50 meters in four directions - northern, southern, western, and eastern, and from six soil depths of 0-10, 10-20, 20-40, 40-60, 60-80, and 80-100 cm of soil. Pesticides were quantified by gas chromatography according to accepted engineering specifications and state standards of Ukraine. We determined the concentration of persistent organochlorine pesticides, which are the major pollutants of these sites.

Table 1. Pesticides concentration in the soils of storehouses sites (ppb).

Region	Sum of DDT	Sum of HCH	PCB	Year of research
Biosphere preserve Askania-Nova	1696.5	616.5	692.2	2002
Kobelyaky, Poltava region	417510.9	691.3		2005
Makarov, Kiev region	2632.5	182352.2	-	2005

Pollution with organochlorine pesticides is directly proportional to storehouse's lifetime. Pollution with PCB is also observed for storehouses, which are situated close to parks and gardens. PCB was in the "Garden's carbolineum" mixture, which was used as tree insecticide.

Besides high concentrations of organochlorine pesticides, plots near some warehouses are polluted with persistent herbicides. Pollution with persistent herbicides provides a bad treat on the plants that are grown on these sites. That's why determination of concentration of their residues in the soil is required for making a decision about a possibility of their remediation. We have conducted pot experiments using soil that was sampled near the pesticides storehouse. We used *Cucurbita pepo* (pumpkin) as a test-plant. Their lifetime is proportional to the concentration of herbicides in the soil.

Table 2 Concentration of persistent herbicides in soil and plants, ppm

Herbicides	Plants	Soil	Toxicity level
Atrazine	0.21	0.32	0.10
Simazine	0.35	2.51	0.10

Cucurbita pepo plants can be used in the technologies of phytoremediation of non-polluted with persistent herbicides storehouses' plots. Soil enzymes are sensitive indicators of soil ecological stress or restoration. For evaluation of biological quality we have researched the activity of Peroxidase and Phenol Oxidase ferments of polluted with pesticides soddy podzolic and "chernozem" soils. Activities of Peroxidase and Phenol Oxidase in the polluted and non-polluted soil were compared.

Table 3 Activity of soil enzymes

Type of soil	Location	Soil enzyme's activity, mg purpureogalin in 1 g of soil		Factor of accumulation of humus
		Phenol Oxidase	Peroxidase	
"Chernozem" soil	Lea soil	0.44	0.22	2.04
	Polluted soil	0.18	0.13	1.3
Soddy podzolic soil	Lea soil	0.18	0.15	1.2
	Polluted soil	0.06	0.08	0.77

Pesticide pollution has bad influence on the biological properties of soil. The activity of enzyme's processes in polluted soil is decreasing, which slows down the pesticides decomposition.

Phytoremediation of heavy metals in muds of sewage treatment plants of industrial waters

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Keywords: Boron; *Nicotiana glauca*; phytochelatin synthase; phytoremediation, mercury, muds, sludge.

Abstract

Up to the date the conventional treatments of the muds do not allow the elimination of the heavy present metals in the same ones. Three lines of *Nicotiana glauca* incorporating *TaPCS1* gene have been used to analyze the capacity of these GMOs to remediate heavy metals from muds proceeding of sewage treatment plants of industrial waters. The muds were mixed with sand and plants were cultivated during three months in greenhouse under controlled conditions. The concentration of heavy metals was determinate by ICP masses, showing the presence of B, Ni, Cu, Zn, Cd, Hg and Pb. The amount of these heavy metals accumulated in the plants was determinate in three different tissues of the plant: roots, shoots and leaves. This determination was done at the very beginning of the experiment and after three months of growth of the plants.

This work complements other recently published by Martínez et al, (2005) in that the capacity of these plants is evaluated concerning its capability to phytoremediate contaminated soils. Coherently to the above mentioned experience on soils it is found a capacity of accumulation of heavy metals increased by the overexpression of the gene *TaPCS1* with regard to the wild species of *Nicotiana glauca*. The maximum values of accumulation were observed for Zn like in the experiment performed previously in contaminated soils. In contrast to the experiment carried out in contaminated soils, the muds contain mercury that is accumulated in the tissues of the GMOs. The information obtained only can be interpreted of coherent form if part of the mercury is volatilized once it is accumulated in the tested plants.



Advances in phytoremediation and restoration of plant communities for wood preservative treatment sites

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Keywords: aided phytostabilisation, CCA, phytoextraction, *Populus nigra*, plant community, field trial.

Abstract

Various chemicals such as Creosote, Zinc sulphate, Copper sulphate, and CCA-type C (Copper oxide 11.1% w/w, Chromium trioxide 30% w/w, Arsenic pentoxide 19.9% w/w) have been successively used in soluble formulations as wood preservatives against insect and fungal attack at treatment sites since the 19th century. For a long-time, there were no regulations in place concerning the treatment or disposal of waste repositories and dust. Consequently such wood preservative treatment sites were entry points for chemicals such as PAHs, Cu, Cr and As into surrounding environments by ways of leaks of the treating fluid, lixiviates from treated timber, and natural agencies (wind erosion and water runoff). Nowadays, large derelict areas at these sites frequently displayed either barren soils or dwarf vegetation patches. Increasing concern for environmental quality and redevelopment of derelict treatment areas led to more closely scrutinise for soil impacts and soil remediation. Advances in phytoremediation technologies, Pro's and Con's, and options for restoring plant communities are reviewed in this communication. When Cu is a key contaminant, special emphasis is given to chemical stabilisation (Kumpiene et al., 2005), and aided phytostabilisation (Mench et al., 2005). Stabilisation with iron oxides is an option to render As less leachable (Kumpiene et al. 2005). Dark acid oxalate extraction dissolves the amorphous Al- and Fe-oxides and hydroxides and mobilises the adsorbed inorganic As species (Bhattacharya et al., 2002), and thus may enhance As phytoextraction. However oxalate also acts as a ligand for the cationic metals, releasing them from exchangeable sites. Chinese brake fern (*Pteris vittata* L.), an arsenic (As) hyperaccumulator, can take up As from the iron-bound fraction in CCA soil and is an option to phytoextract As in such contaminated soils. Phosphate and compost additions can enhance As phytoextraction by this fern (Cao et al., 2003), but high Cu content limits its development. Soil amendments and plant species are currently investigated to refine aided phytostabilisation and phytoextraction.

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Phytotechnologies evaluation with multicriteria analysis

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Keywords: phytotechnologies, multicriteria analysis, wetland

Abstract

Nowadays phytoremediation is a well-known technique that presents some advantages: sustainability, environmentally friendly, low cost technology. In order to investigate the economical benefits of a wetland system we wanted to understand its feasibility starting from comparing the most common wastewater treatments in terms of cost-benefits analysis. Phytotechnologies are low-cost and environmentally friendly technologies, so that their efficiency is getting higher thanks to the several efforts and studies by a lot of scientific teams in Europe. They target extraction, degradation or fixation of the pollutants of contaminated soils and water around formers, mines, slag dumps from coal-fuelled power plants and are expected to play a major role especially in the restoration of former industrial areas. Thanks to their wide employment possibility and efficiency in the land use management a single-criterion, approach (such as cost-benefit analysis) was not considered enough, especially where significant environmental and social impacts cannot be assigned monetary values. For this purpose a Multi-Criteria Analysis (MCA) was developed in order to allow decision makers to include a full range of social, environmental, technical, economic and financial criteria. In a situation where multiple criteria are involved confusion can arise if a logical, well-structured decision-making process is not followed. For each waste water treatment technique the MCA defines criteria (in order to identify the studied ratings), attributes (to measure the studied ratings) and rules. The second step aims to compare the different techniques based on each criterion in order to find the priorities among the different alternatives, and then every criterion has to be weighted. In a Multi-Criteria Evaluation (MCE), an attempt is made to combine a set of criteria to achieve a single composite basis for a decision according to a specific objective (Eastman et al., 1995). Decisions about the allocation of land typically involve the evaluation of multiple criteria according to several, often conflicting-objectives. The advantage of MCE is that it provides a flexible way of dealing with qualitative multi-dimensional environmental effects of decisions (Munda, 1995). Although a variety of techniques exists for the development of weights for the criteria, one of the most promising would be developed by Saaty (1977): it is a comparison method where the decision-maker is asked to give the relative importance to the criteria by comparing them two by two. Then the priorities between the criteria is calculated according to the following alternative but equivalent procedures:

- 1) Calculating the priorities using an approximation method starting from the Saaty's matrix of pairwise comparison between the criteria;
- 2) Calculating the priorities using the exact method based on the idea of consistency. If the matrix is consistent all its powers give the same priority or dominance pattern.

When the matrix is consistent, the normalized sum of each row tells us how much each element dominates the others in relative terms. The sum of the entries in each column tells us how much each element is dominated by other elements.

To derive the priorities from the matrix we add the numbers in each row and divide each of the results by their total sum to obtain the normalized scores. The operation is repeated until results are stabilized.

This methodology is actually tested on a case history in order to verify its applicability.

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Persistence of smelter wasteland phytoremediation using biosolids - 10 years story

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Keywords: Smelter waste, metal, biosolid, field, grasses

Abstract

One of the aims of the EU Soil Thematic Strategy is to create framework for identifying and managing risk associated with historical contamination sites. Metal mining and smelting wasteland sites are serious sources of heavy metal pollution. The sites are usually barren due to metal toxicity and harsh physical conditions which causes metal spread in local environment through wind erosion and runoff. Metal waste sites located in Silesia region in southern Poland are known to contain almost 90 million tons of waste (Stuczynski et al., 2002). Due to low organic matter such smelter wastes are infertile and have low holding capacity. High metal contents along with acidic conditions of many types of smelter wastes causes metals to be very mobile and toxic to plants. Reclamation of such environments is usually a substantial challenge and involves a number of technical and cost difficulties. Application of biosolids followed by introduction of properly selected grasses to revegetate such extreme environments has proved its potential effectiveness (Brown et al, 1998; Li et al., 2000). The reclamation potential of biosolid is related to its organic matter and nutrient content which contribute to fertility and binding capacity of the system (Basta et al. 2005).

The goal of our studies was to assess to what extent biosolid treatment along with selected grass mixture would ensure persistent functioning of revegetated metal waste ecosystem as measured by such biological activities as microbial activity or plants yield. Experiments were located at a brownfield resulted from the shutdown of a smelting plant in Piekary Slaskie. This site contained waste from two different smelting processes - Welz and Doerschel. The reclamation work which took place in 1994/1995 resulted in vegetation of 3 ha pilot areas. The Welz waste site was reclaimed by application of municipal sewage sludge at the rates ranging from 150-300 tons per hectare (dry matter basis) combined with the incorporation of lime as an oxide and carbonate form at the rate of 1.5 tons and 30 tons, respectively. Due to higher salinity and metal toxicity Doerschel material required a different approach and involved use of a 30 cm by-product lime cap followed by incorporation of sewage sludge at a rate of 300 t ha⁻¹ (dry matter basis). The treatments afforded successful establishment of a grass ecosystem on both experimental fields.

The most crucial question is how persistent and sustainable are such constructed ecosystems. Different chemical and biological parameters were used for monitoring of revegetated site including metals extractability, plant biomass and microbial activity (enzyme activities, respiration). Samples were collected in a regular grid over 10 years period. Analysis of samples collected after reclamation showed strong reduction of metals solubility as a result of treatments used. Metals solubility generally remained at the same level non-toxic to plants. However spatial variability of that parameter was significant. Plant biomass and microbial activity generally were not correlated to soluble metal contents. Correlations to organic matter content reflected a role of even biosolid distribution over the reclamation area.

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Study of mercury uptake by common vetch at different scales

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Keywords: mercury; common vetch; pot experiments, lysimeter; greenhouse, pilot, field; alternative land use

Abstract

Objectives:

This work studies the mercury root uptake by *Vicia sativa* (common vetch) and the distribution of this metal through the plant. The final aim is to evaluate the potential use of common vetch as forage plant in a mercury contaminated area. Furthermore, its contribution to Hg-phytoremediation is discussed. To reach these goals, an integrated approach is being developed. This one includes three study levels: studies in plant pots under greenhouse conditions, under close to real conditions lysimeter experiments and near future application in field plots under real conditions.

Methods:

The experiments of the three study levels have been carried out using a soil from the same plot of mercury mining district of Almadén (Spain). Plant pots used in experiments under greenhouse are filled with a mixture of soil, perlite and sand on equal proportions, this mix has a mercury concentration of $6.74 \pm 0.63 \text{ mg kg}^{-1}$ on average. Lysimeter which is an unalterable soil monolith of 1 m^3 has a mercury concentration of $22.83 \pm 1.80 \text{ mg kg}^{-1}$ on average, similar value than in field plot. In all experiments the percentage of mercury easily available for plants in soils is very small ($< 0.1\%$).

Four samplings are performed along plant development. The samples collected are separated into several parts. Hg concentration is determined using an Advanced Mercury Analyser (AMA-254)

Results:

In experiments under greenhouse and in lysimeter, general trends are observed. Concentration of mercury in root is higher than in shoot in all stages of development. The highest mercury content in aerial part of the plant is in leaves while the lowest mercury content is located in seeds and pods. Other aspect to remark is that the values of mercury content in plants grown under greenhouse are up to 10 fold higher than plants grown in lysimeter. This fact could be due to more favourable conditions in greenhouse, specially because of better isolation and better fertilization, so, plants are more developed (up to 3.8 times more) and also, the concentration of mercury is higher.

Discussion:

Common vetch has different uses. The seeds can use as food for birds and other animals or for industry uses, also, it is cultivated as fodder for livestock. Regarding forage use, European legislation proposes a mercury limit of 0.1 mg kg^{-1} in raw material for animal nutrition. The values obtained in leaves are between 0.5 to 1.8 mg kg^{-1} that are higher than the limit established by the proposal. Consequently, it would be necessary to beware of using the aerial part of this crop for fodder. However, their seeds could be used as food for birds and other animals because mercury concentration is around 0.02 mg kg^{-1} that is lower than 0.10 mg kg^{-1} . Although the future experiments in field will ratify this results.

Regarding phytoextraction capacity, the results show that this culture could decrease very slowly, but progressively the soil mercury concentration.

In summary, in this study, a possible economical and environmental double profit due to the cultivation of this plant is discussed.

Behaviour of Antimony in soil, transfer from soil into plant, risk management strategy

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Abstract

Antimony contamination of soils in ground waters below shooting ranges is a recently discovered environmental problem of considerable concern in Switzerland (Wersin et al. 2002) because of the toxicity of this element. Many Sb compounds are known to be toxic, some are even considered to be carcinogenous. A potentially important Sb exposure pathway of humans and animals to antimony in areas with contaminated soils is through the food chain (Ainsworth et al. 1990b, Hammel et al. 2000).

Predominant anthropogenic sources:

- Fossil fuel combustion
- Non-ferrous metal refining
- Waste incineration
- Transports
- Shooting-ranges (local contamination)

Current world production of Sb is about 140'000 tons per year (Nriagu and Pacyna, 1988).

Predominant natural sources:

- Soil dust (95% of natural background deposition)
- Volcano

Sb in the earth's crust is about 0.3 ppm but background concentration in soil may amount to 2 ppm (W. Shotyk, 2005).

- Antimony is present in 3 oxidation states in soil:
 - Sb(0) = metallic
 - Sb(III) and Sb(V) = oxidised
- Sb(V) is the predominant redox species in soil (> 90%).
- Sb(III) and Sb(V) are strongly sorbed to Fe, Al and Mn (hydro)oxides.
 - Hydrous Fe oxides showed the highest sorption capacities.
- The major part is sorbed to the soil matrix as up to 3 % is soluble.

2000 shooting ranges in Switzerland

Lead alloy used in making ammunition contains between 2 to 5 % antimony. 5.6 mm Gw Pat 90 bullet and GP 11 bullet used in Switzerland contain 95% Pb, 2% Sb, 3% Cu and 0.5% Ni (A. Johnson, 2005). Shooting ranges in Switzerland lead to 10 to 25 tons Sb entering the soil annually. 5 main pathways are concern by soil antimony contamination:

- Crop
- Animal products and human
- Water for drinking/other use
- Surface water
- Groundwater

Until now, little is known about the risk management of oxy anions. In presentation following aspects will be given more stress- antimony transfer to crop and pasture plants and to the use of plants and soil and plant technologies to avoid contamination of plant, ground and surface water.

A Review of the Constructed Wetland Technologies in Mediterranean Countries

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Keywords: mediterranean countries, wastewater treatment and reuse, extensive treatment systems, constructed wetlands (CWs), manual of practice.

Abstract

In most of the southern and eastern Mediterranean countries, the application of the conventional wastewater treatment systems is limited because of their high cost and technological complexity. As a result, the Mediterranean basin receives tremendous amounts of highly polluted wastewater on a daily basis. Moreover, as complete sewerage in the Mediterranean region seems still unlikely in the near future, the urgent need for decentralized systems that will serve the communities with a PE below 2000, has been reported almost for each country in the Mediterranean basin. Sustainable treatment technologies like “constructed wetlands” (CWs) are found to be within the economical and technological capabilities of most of the Mediterranean countries.

In the framework of the Med-Reunet Support Programme-II (EU project INCO-CT-2003-502453), a report called “Manual of Practice on Constructed Wetlands for Wastewater Treatment and Reuse in Mediterranean Countries” including both theoretical and practical aspects of the subject has been prepared (Korkusuz, 2005). This manual aims to provide a broad outline of the CW technology to the engineers, researchers and stakeholders, who are interested in designing, constructing, operating and using CWs as one of the decentralized treatment system options for wastewater treatment and reuse, especially for small agglomerations in rural areas of several Mediterranean countries. In this manual, some of the CW case studies conducted in the Mediterranean countries (Portugal, Spain, France, Italy, Slovenia, Croatia, Greece, Turkey, Israel, Palestine, Egypt, Morocco) have been summarized in details.

As a result of reviewing CW studies published in scientific journals and proceedings (number of reviewed studies: 656), it has been found out that CWs have been used mostly to treat the municipal and domestic wastewater, whereas they have been used rarely for leachate treatment and sludge dewatering, both worldwide and in Mediterranean countries. Around 18% of the CW studies done worldwide were conducted in Mediterranean countries (number of reviewed studies: 118). Almost half of the CW studies conducted in Mediterranean countries were to treat the domestic wastewater. France, Italy and Spain were found to have leading CW studies in Mediterranean countries, which might be due to the stringent legislation on wastewater treatment and reuse of the EC. Even though the number of the CW studies carried out in Portugal, Slovenia, Croatia, Greece, Turkey, Israel, Palestine, Egypt, and Morocco has been found to be lower, the number of CW studies is expected to increase rapidly in the following years (Korkusuz, 2005).

According to the reviewed literature, the majority of the CW systems build in the Mediterranean countries are subsurface flow (SSF) CW system. In Portugal and Italy, horizontal subsurface flow (HSSF) systems are the most preferred systems, whereas in France, the most favourite systems are vertical flow (VF). Free water surface (FWS) wetland studies are performed in Greece, Israel, Palestine and Egypt. Use of the Hybrid systems, like combinations of the VF+HSSF or FWS+SSF CW systems, seems to be gaining popularity in the recent years. Moreover, addition of the different types of CWS to the treatment line of the conventional treatment plants becomes a current issue (Korkusuz, 2005).

Acknowledgements

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Constructed wetland pilot units for wastewater treatment in the tannery industry

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Keywords: constructed wetlands, pilot scale units, tannery industry, *Phragmites australis*, *Typha latifolia*

Abstract

Constructed wetlands can be used for primary and secondary treatment of domestic wastewaters and for the treatment of a variety of other water sources including stormwaters, landfill leachate, industrial and agricultural wastewaters and acid mine drainage.

For the treatment of tannery wastewater no detailed studies using constructed wetlands are known in the literature. Frequent problems occur in this industry concerning the effluent with high organic loadings. In these situations, a constructed wetland can be potentially used to support the existing wastewater treatment.

The present study refers to the applicability of subsurface horizontal flow constructed wetlands for the treatment of tannery wastewater in two series of pilot units planted with *Typha latifolia* and *Phragmites australis*.

The units were subject to different hydraulic loadings, 180 mm/d and 60 mm/d, and the organic matter removal efficiency was evaluated. The average COD for the inflow of the units was 1579 mg/l for an hydraulic loading of 180mm/d (1421 KgCOD/had) and 1297 mg/l for an hydraulic loading of 60mm/d (385 KgCOD/had). Maximum removal efficiencies, in terms of COD, for hydraulic loading of 180 and 60 mm/d in *Typha* unit were 63% and 92%, respectively. For *Phragmites*, the maximum values were 64% and 92%.

The decrease in COD, BOD₅ and TSS at the outflow of the units was found to increase with their influent values in a linear correlation. This behavior corresponds to a first order kinetics, showing that the pollutant removal rate was proportional to the influent strength. There was no significant difference between the two systems in series, indicating that the type of plant did not influence the treatment performance.

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Removal of chlorinated benzenes from contaminated groundwater in a pilot scale constructed wetland

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Keywords: chlorinated benzenes, removal, pilot scale constructed wetlands

Abstract

Constructed wetlands are applied for treatment of water containing chlorinated organics. While fundamental and applied aspects are already studied for chlorinated aliphatic compounds, little is known about the fate of chlorinated aromatic hydrocarbons in such systems.

In a pilot scale horizontal subsurface flow constructed wetland (area of 1 m x 4.5 m and depth of 0.5 m, planted with *Phragmites australis*) removal of chlorinated benzenes from an originally anaerobic contaminated groundwater was investigated. The wetland was operated in Bitterfeld (Germany) under long term field conditions during different seasons in comparison to an unplanted reference system.

Implementing a linear flow velocity of about 0.5 m/d in both (planted and unplanted) wetlands, the inflow concentrations amounted to 15-22 mg/l monochlorobenzene (MCB), about 0.3 mg/l 1,4-dichlorobenzene (1,4-DCB), and 0.04-0.2 mg/l 1,2-dichlorobenzene (1,2-DCB). After a flow distance of 4 m, in the planted wetland the contamination of MCB was reduced by 60-90%, 1,4-DCB by 60-80%, and 1,2-DCB by about 10%. In the unplanted control wetland, the removal was considerably lower, about 10-40% for MCB and 1,4-DCB and only about 0-10% for 1,2-DCB. Surprisingly, there were no clear differences of the elimination in winter time in comparison to the summer season in both systems. In contrast to the unplanted control, in the planted wetland decreasing concentration gradients of MCB and 1,4-DCB could be estimated depending on depth. At a depth of 30 cm below the bed surface, the removal of MCB was up to 5 times higher than at a depth of 50 cm, for example.

The results show significantly higher removal efficiency in the planted system than in the reference system independent of the season. The low removal efficiency of 1,2-DCB in comparison to MCB and 1,4-DCB is a strong indication of microbial removal processes. Furthermore, isotope ratio measurements show an increase of the ¹²C/¹³C ratio of the MCB in the pore water depending on the flow distance which also proved the microbial degradation of the compound.

Influence of Abiotic Factors in the Kinetics of a Vertical Flow Constructed Wetland by a Mechanistic Model

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Keywords: constructed wetlands (CW), vertical flow, modelling, abiotic factors, Acid Orange 7 (AO7).

Abstract

Constructed wetlands (CW) are treatment systems that mimic natural wetlands by the use of processes involving wetland vegetation, soils and their associated microbial populations to improve water quality (Davies et al, 2005). The complexity of CW, which can be regarded as eco-reactors, makes them difficult to model. Several mathematical models of CW have been proposed most based on retention time distributions (RTD) and first order degradation kinetics (Rousseau et al., 2004). Another approach that has been used involves mechanistic models such as continuous stirred tank reactors in series and plug flow reactors with dispersion involving finite or infinite number of stages (Werner and Kadlec, 2000).

To evaluate the influence of abiotic factors, like Temperature (T) and Evapotranspiration Rate (ETR), the first part of experimental data took place during spring/summer time. A Vertical Flow CW (VFCW) at pilot scale that was being used in the treatment of an azo dye was modelled using a mechanistic approach. Acid orange 7 (AO7) was the selected azo dye to pursue these studies due to its characteristic orange colour, which is easily followed. The VFCW was pulsed with an inlet concentration of 700mg/l, 13 minutes every 3 hours at 1l/min, giving a hydraulic load of 108 l m⁻² day⁻¹. The model was conceived by analogy with the hydrodynamics of pulsed systems and the RTD of a continuous system. Using a mechanistic approach, we selected the simplest possible combination of ideal reactors that best reproduce the experimental results (2 continuous stirred tank reactors in series, the first of variable and the second of fixed volume, and 1 in parallel, accounting for dead volumes). The outflow was calculated using a simplification of Carmen-Kozeny relation, and it was considered that the degradation reaction rate was irreversible and of first order. The outlet AO7 concentration data were fitted using MSEXcel, by means of Solver, non-linear optimisation of the least squares sum function. Based on the model results we obtained the optimal operational conditions that give the best removal efficiencies.

As the selected inlet AO7 concentration is high and simulates an accidental discharge, we used the model to predict how many VFCW beds would be necessary to fulfil the quality of the effluent for environmental discharge. It was concluded that, for the same operation conditions, 3 VFCW in series with identical characteristics would be necessary. These results were validated, afterwards, experimentally.

We aim to study the influence of the two abiotic factors with new experimental data at much lower T and ETR, and this will confirm the model adequacy.

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Bacterial communities in subsurface flow constructed wetlands

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Keywords: constructed wetlands, bacterial diversity, municipal wastewater, pilot scale, *Miscanthus gigantea*

Abstract

Basic research is necessary to understand the decomposition processes in constructed wetlands (CWs) more in detail. The waste water purification within CWs is a result of the interactions between plants, soil and microorganisms. Although it is well known that degradation processes are mainly caused by bacteria only little information is available regarding the bacterial diversity of the filter material of constructed wetlands.

The research work was carried out at 8 indoor subsurface vertical flow small scale constructed wetlands (SSCWs) and at one outdoor CW with similar design and operation. The filter body of the SSCWs consists of a sandy substrate with a grain size of 0.06-4 mm and has a depth of 50 cm. 6 SSCWs have been planted with *Miscanthus gigantea* whereas the other 2 are unplanted. The outdoor CW located in Lower Austria (Ernstshofen) has 3 beds with 20 m² each and they are planted with *Phragmites australis*. All wetlands are fed with mechanically pre-treated municipal wastewater.

During the first phase of the project the distribution of the microbial biomass over the depth of the filter substrate was analysed by different methods, e.g. SIR, ATP, fumigation extraction, microscopic direct counts, PLFA (Tietz et al., 2005). In the second phase of the project the focus was on the bacterial diversity inside the filter substrate. Therefore molecular fingerprint techniques like DGGE and TRFLP have been applied.

During a stay at the Centre for Environmental Research (UFZ) in Leipzig (Germany), funded by a STSM scholarship, a general inventory of the bacteria was done by the construction of a 16S rRNA clone library to characterize the diversity of active bacteria. The isolated RNA from rhizosphere and bulk soil samples from the SSCWs and from the outdoor CW was transformed into DNA by reverse transcriptase (RT-PCR). After a PCR targeting the 16S rDNA the PCR products were cloned into the pGEM vector by using the Easy Vector System II kit (Promega). After a screening of the clone library via restriction analysis (ADRA) the most dominant species have been sequenced. With these sequences we constructed a phylogenetic tree of the bacterial community.

The main part of the identified species belonged to the group of proteobacteria (alpha-, beta-, gamma-, and delta-). The species composition of the bacterial community also provides information about the physiological potential of the biocoenosis. For example we found typical nitrifying bacteria and also species of the family *Planctomycetales*, which presumes the occurrence of Anammox-species. The CW system, which is a hybrid between soil and sediment, contained typical soil and rhizosphere bacteria, typical wastewater bacteria and also human intestinal bacteria. From these results we can conclude that a CW system is very unique and has a highly diverse bacterial community.

Acknowledgements

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Producers and Users of Wastewater - The Meeting Point

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Abstract

Optimally, regulations should be the meeting point between wastewater producers, and potential users: agriculture, rivers and other natural water bodies, and ground water.

As a ground rule, the regulations should mandate the producer to manufacture effluents that fit the needs of existing users in the region.

This meeting point is not without problems. Different interests, different jurisdictions, and different sources of authority are involved.

Formerly, the Ministry of Health alone set regulations for wastewater quality. Today, the Ministry of the Environment also has a say as does the Ministry of Agriculture, which sets parameters for irrigation with treated wastewater. The Ministry of Infrastructure sets regulations for emitting treated water back to the environment.

Needless to say, the regulatory bodies do not have a common ground. There is no agreement as to who should take responsibility and pay for differences in costs incurred, when user and producer demands and regulations differ from one another.

In an ideal world, the regulations would be enforced. But when the wastewater producer does not meet the regulations, the recipient farmer resultantly irrigates with water that not only fails to meet the parameters set by the Ministry of Agriculture, but also fails to meet the initial parameters of BOD and TSS set for the producer.

The farmer can then seek another profession, irrigate with water he knows will harm his land and crops, or treat the water by himself. Such a case will be presented.

Another clash of interests occurs in the case of the Yarkon River, from which natural sources are harnessed for drinking water supply. The river gets its water from two activated sludge factories, the first producing tertiary effluents and the second secondary effluents, with a plan for improvement from secondary to tertiary treatment.

The terms tertiary and secondary relate to a very small range of parameters (neither of which are met), while other parameters essential to maintain life in the river are not covered by regulations. The effluent is very unstable, with peaks of BOD, E. Coli, mineral oil, aluminum and probably many other unmeasured parameters. Furthermore, high hormone content is wreaking havoc and causing sex changes in fish.

We will present two projects built to provide a solution and serve as a buffer before emitting water from an activated sludge plant to the river. In these cases the principle of "polluter pays" does not work. It is up to the user to improve water quality for use.

Naturally there is a need to enforce existing laws and regulations, but there is also a need for regulators to look more closely at the needs of the user and develop a comprehensive set of abiding norms for safe and enduring use.

Can polluters be made to pay for emitting water of inferior quality? Should they pay? Can the producers of wastewater refuse to supply "tough" users? Will too high standards eliminate potential sources of water for the rivers?

All these questions must be answered to ensure the development and ultimate implementation of an effective research and regulation process and set of guidelines that all parties can abide by.



Cologne Scheme for Eco-Management of Recreational Lakes

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Keywords: Aquatic sports facilities, Cologne scheme, eco-management, Lake Fuehlingen, lake restoration

Abstract

Numerous groundwater-fed gravel-pits are located along the Rhine River. They are attractive landscape components and valuable aquatic ecosystems. Distances among these lakes and to the river are near enough for aquatic organisms to move easily from one aquatic habitat to the neighbored one or being transported by vectors. In this way, an extended net-shaped aquatic ecosystem is created in the river valley for free exchange of genetic resources contributing significantly to ecologically important bio-diversity in that industrialized region.

As highly attractive places for any kind of recreation and aquatic sports, lakes are hot spots in tourism. Especially in that densely populated area, they are endangered from mechanical destruction of lakesides and from water pollution. Sophisticated eco-management is necessary to protect these aquatic ecosystems from degradation or extinction by non-sustainable anthropogenic over-use.

Today, ecological and landscape aspects are not enough in nature conservation. In many cases financial resources were provided for setting up conservation projects. But, funds are not considered for maintenance or groups of conservationists do not exist long enough to serve for these projects over longer periods. A few years later, the protected areas are degrading and enormous financial resources are going lost finally by such non-sustainable eco-management. Instead of simple conservation activities, nature conservation should focus on wise-use of sites worthy of protection by including economic instruments.

Restoration of Lake Fuehlingen at Cologne is a proper example following that philosophy. Lake Fuehlingen is located in a water protection area in the Northern outskirts of Cologne. Some 25 years ago, an old gravel-pit was developed into an attractive aquatic sports facilities and Leisure Park of some 75 ha water surface area. The internationally standardized regatta course is accompanied by seven sub-lakes of 2.5-12.6 ha surface area and up to 16 m in depth. All sub-lakes are connected with the regatta course outlining a well structured lake system without any surface inflow and out-let. During the last 2-3 decades, the site has been developed into a most valuable aquatic ecosystem.

In having been used for international sports events, open air festivals and any kind of recreational activities by up to some 30,000 visitors at sunny weekends, water quality of Lake Fuehlingen degraded in the mid 90th of the last century. Based mainly on eco-compensation funds and budget resources of the site managing authority, some projects have been set up by research institutions from natural, technical, social, and juridical sciences in cooperation with landscape engineers and the regional drinking-water supplier for developing and adjusting techniques of lake restoration and conservation which can be applied without any restrictions of public access for generating continuously operating income from the site. The resulting 'Cologne scheme for eco-management of recreational lakes' is a set of instruments for conserving recreational lakes under anthropogenic stress and improving their ecological quality. Promoted by the 'Friends of Lake Fuehlingen at Cologne', this Cologne scheme is supplemented continuously by further suitable methods to stabilize ecological and commercial conditions of Lake Fuehlingen also in future.

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Poster Presentations

An investigation of olive tree stress as caused by excessive application of municipal sewage sludge

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Keywords: sewage sludge, olive tree, heavy metals fractionation, soil remediation

Abstract

Excessive amounts of municipal sewage sludge (300 Mg ha⁻¹) were applied in a 5 ha olive orchard in 1999. Seven years after application, the appearance of stress symptoms persisted in the leaves of approximately half of the trees. Prior to consideration of remediation measures, a preliminary study was undertaken in order to assess the causes of the stress. Soil quality parameters and leaf nutrient status was measured in randomly selected positions within the sludge-amended field. These were compared to an adjacent area of unamended and naturally growing trees.

Sludge application resulted in a significant increase of soil pH and a multi-fold increase in soil nutrients (electrical conductivity, N, P, K, Mg) and heavy metals (Cd, Zn, Cu, Pb, Mn, Ni) in comparison to the unamended control. In particular, the acidic soil pH increased by 1.8 units, soil EC increased six-fold and nitrate-N nine-fold in the top 20 cm. Therefore, mineralization of sludge and associated nitrification processes appear to be responsible for the rise in soil EC. Maximum concentrations of heavy metals were detected in the top 10 cm of sludge-amended soil and rapidly declined thereafter with depth. In the case of Cd, Zn and Cu, their concentration in the top 10 cm approached the maximum recommended concentrations by EU in soils treated with sewage sludge. Fractionation of Ni and Mn revealed that in the top 10 cm of sludge amended soil about 1% was found in the exchangeable fraction, 15-17% in the organic matter associated fraction, 7-8% in the carbonates fraction and the rest 62-72%, in the residual fraction.

Olive trees responded to sludge application by an increase in N uptake. This increase was caused by the uptake of sludge-derived N as evidence by the highly enriched ¹⁵N signal of the leaves ($\delta^{15}\text{N}=7.55$) relative to that naturally growing trees ($\delta^{15}\text{N}=2.26$). A comparison of leaf $\delta^{13}\text{C}$, as an indicator of WUE, showed that sludge amended trees suffered from water stress in the summer but not in the winter. It is probable that due to the surface application of sludge, trees developed a shallow rooting system causing water shortage during periods of drought in the summer. No differences have so far been detected in leaf elements and their isotopic composition between stressed and unstressed trees in the sludge amended field.



Changes in the rhizosphere of *Lupinus albus* L. affecting metal availability in a contaminated calcareous soil

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Keywords: heavy metals, *Lupinus albus*, microbial biomass, rhizosphere

Abstract

The conditions of the rhizosphere are different from those of the bulk soil, and the complexity of the soil-plant relationship can lead to changes in the rhizosphere affecting metal speciation and therefore availability to plants. Accumulator plant species may facilitate metal solubility in the rhizosphere, while excluders should immobilise them to reduce plant absorption. In this work, the availability of metals in the rhizosphere of the metal-tolerant species *Lupinus albus* L. has been studied and related to the chemical characteristics of the rhizosphere in comparison with the bulk soil.

A rhizotron was designed to allow easy sampling of the rhizosphere. It consists of two compartments separated by a nylon mesh; the upper part holds the soil where plant and roots are growing, while the inferior part is filled with the bulk soil. A 2mm-thick layer of soil was placed between the two parts when a complete development of a root system filled the bottom of the upper part, this was considered the rhizosphere. After two months of plant growth, the rhizosphere soil was added, and two weeks later, the rhizosphere soil and the bulk soil from the inferior part were sampled. Aerial parts of the plants and roots were cut, the fresh and dry weight determined before ground for metal and nutrient analysis. Roots were washed two times with distilled water, a third time for 7 minutes under sonication to remove soil particles, and then with 0.1 mM SrCl₂ for 30 sec to remove metals from the root apoplast.

Bulk soil and rhizosphere were analysed for pH, redox potential, soluble organic-C, microbial biomass and heavy metal fractionation by a sequential extraction procedure. Compared with the bulk soil, only small increases were detected for pH, Eh and microbial biomass in the rhizosphere, but the initial results showed that soluble metal concentrations decreased in the rhizosphere of *Lupinus albus*.



Influence of effluent irrigation on soil microbiological parameters under willow coppice

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Keywords: microbial activity, microbial biomass, vegetation filter, willow coppice

Abstract

The effect of establishment of short-rotation willow coppice and effluent irrigation on microbial parameters in soil of previous arable land was evaluated using soil biochemical tests. Analyses were conducted on soil samples in October of 2003 after willows were planted and repeated in October 2005. Part of the willow coppice (9.5 ha) was irrigated during two vegetation seasons with pre-treated domestic wastewater. A significant increase occurred in soil microbial biomass, respiration and nitrogen mineralisation activity during study period both in irrigated and non-treated parts of willow coppice. Wastewater irrigation increased soil potassium concentration and enhanced activity of alkaline phosphatase. Plant growth and irrigation did not affect potential nitrification and acidic phosphatase activity as well as microbial community metabolic activity measured with Biolog EcoPlates. Response of soil microbiological parameters to irrigation was dependent on soil type. Comparison of soil profiles indicated that temporal changes in soil microbiological parameters were allocated to upper 10 cm. From our results, we can conclude that willow coppice affects positively soil biological activity and irrigation of pre-treated wastewater has weak effect on soil chemical and biochemical properties.

Evaluation of the impact of sunflower on PAH degradation in soils amended with compost and sewage-sludge

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Keywords: PAH, rhizosphere degradation, greenhouse study

Abstract

Soils contaminated with PAHs are difficult to bioremediate because PAHs are hydrophobic compounds, poorly soluble in soil solution and strongly sorbed to the soil matrix. However some higher plant may directly (e.g. root adsorption) or indirectly (e.g. increase of microbial population) enhance PAH degradation in the soil environment. From another hand, use of sewage-sludge or compost is a cost-effective and practised widely method that improves properties of the soil by supplementing organic matter and nutrients as well as by introducing new microbial species - including those that may degrade organic contaminants.

The aim of the study was to evaluate the influence of the joint effect of both those factors (phytostimulation and organic amendment) on dissipation of PAHs in highly contaminated soils.

Two soil materials collected from industrial areas were used in the greenhouse pot study (two replications each): CS1; $C_{org}=3.54\%$; $pH_{KCl}=6.8$; $\Sigma 13PAH=14.2$ mg/kg and CS2; $C_{org}=5.93\%$; $pH_{KCl}=6.0$; $\Sigma 13PAH = 20.6$ mg/kg. Potentially bioavailable PAH fraction (as related to the total PAH content) was 13% and 3% for soil CS1 and CS2, respectively. Each soil was amended separately and univocally with peat-earth compost and sewage sludge at the dose of 100g/kg of the soil (300t/ha). Sunflower (*Helianthus annuus* L.) which can be used for industry purposes, creating abundant root system was cultivated for one vegetation period - 90 days.

The effectiveness of phytoremediation was assessed on the basis of the determination of PAH residue in planted and unplanted enriched and non-enriched soils after vegetation period. Bioavailable fraction of PAH using TENAX-TA method was determined to predict the effectiveness of microbial degradation. Content of 13 PAHs was determined using GC/MS system.

None of applied combinations gave a statistically significant enhancement of PAH dissipation after one vegetation season although small effects of sunflower cultivation were observed in both soils. Obtained results suggested that promising effect of phytostimulation may be observed after rather long period of time.

The studies were performed within the activity of the EU Centre of Excellence PROLAND

The influence of annual plants cultivation on PAH degradation in contaminated soils - as affected by the root system abundance

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Keywords: polycyclic aromatic hydrocarbons, annual plants, phytostimulation, greenhouse study

Abstract

Polycyclic aromatic hydrocarbons are group of common soil pollutants. One of the methods that can be applied for cleaning-up PAH contaminated soils is phytostimulation. The efficiency of this process depends on specific interactions between plants and microorganisms associated with their roots - in relation to soil property and the nature of compounds to be degraded. The rhizosphere effect can be affected by the abundance of the root system. Plants with highly developed roots provide more exudates and can more effectively “distribute” microorganisms throughout the soil that improves their contact with degraded contaminant. The aim of the study was to evaluate the effectiveness of phytostimulation as a tool for cleaning up soils contaminated with polycyclic aromatic hydrocarbons as affected by the abundance of root system.

Two different soil materials were used in the pot experiment: HLS - heavy loamy sand ($C_{org} = 3.54\%$, $pH_{KCl} = 6.8$, $\Sigma 13PAH = 12,9$ mg/kg) and LL - light loam ($C_{org} = 5.93\%$, $pH_{KCl} = 6.0$, $\Sigma 13PAH = 22,1$ mg/kg). Soil samples were collected from industrial areas from Silesia district, Poland. Eight annual plants: maize, sunflower, soybean, and millet (rich root system), white lupine, oat and garden pea (moderately rich root system) and horse bean (weak root system) - were cultivated for one vegetation season. Unplanted soils were applied as controls. Content of PAH 13 compounds was determined by the GC/MS technique after 0, 30, 60 and 90 days in all experimental samples (2 soils*8 plants*2 replicates).

During one vegetation season the observed efficiency of phytoremediation of “industrially” contaminated soils was very low comparing to unplanted control. The highest influence of plant cultivation on PAH dissipation from soils was noticed for some 3- and 4- ringed compounds. The efficiency of phytostimulation - as evaluated in relation to unplanted control soil - was not related to the abundance of root system nor the soil property. It suggests that for obtaining higher effects, much longer times - with allowances for PAH transport processes - has to be applied in further studies.

The studies were performed within the activity of the EU Centre of Excellence PROLAND

Assessment of Cu stabilisation in a contaminated soil from a wood preservative treatment site using the PlantTox test

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Keywords: aided phytostabilisation, *phaseolus vulgaris* L., oxidative stress, organic matter, soil amendment

Abstract

Various chemicals such as Creosote, Zinc sulphate, Copper sulphate, and CCA-type C (Copper oxide 11.1% w/w, Chromium trioxide 30% w/w, Arsenic pentoxide 19.9% w/w) have been successively used in soluble formulations as wood preservatives against insect and fungal attack at a treatment site since the 19th century. Wood preservative treatments were entry points for chemicals such as PAHs, Cu, Cr and As into the soil by ways of leaks of the treating fluid, lixiviates from treated timber, wind erosion and water run-off. Topsoil ecotoxicity was assessed using terrestrial bioindicators (Mench et al, 2005). According to soil analysis and bioassays, Cu in top soils ranged from 65 to 2600 mg kg⁻¹ and was identified as main metal contaminant limiting plant growth. Stabilisation of Cu is a relevant soil remediation option (Kumpiene et al, 2005), especially in the purpose to further use phytostabilisation and to restore plant community with the highest biodiversity. Therefore, various soil amendments (i.e. composted sewage sludge, composted poultry manure, mushroom bedstraw, activated carbon, zerovalent iron grit, Thomas basic slags, triple superphosphate, ammonium phosphate, silicates, and Ca oxide) were either singly or in combination applied into a high Cu contaminated soil. Their effectiveness at reducing root exposure to Cu and promoting plant growth was assessed using the PlantTox test with *Phaseolus vulgaris* (Ruttens et al, 2006). Dwarf beans were grown in potted soils placed in a climatic chamber. Biometrical and biochemical plant parameters were monitored after a 2-week growth period. *Rhizobium* nodules were count on bean roots. Metal contents in primary leaves and roots were determined. The effectiveness of soil amendments for enhancing plant growth and *Rhizobium* symbiosis and for reducing Cu concentrations in plant parts will be discussed.

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Plant communities at a wood preservative treatment site, and Cu tolerance patterns of plant species

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Keywords: *Sporobolus tenassissimus*, *Agrostis capillaris*, *Populus nigra*, *Populus deltoides*, *Carex* sp.

Abstract

Creosote, Copper sulphate, and CCA-type C (Copper oxide 11.1% w/w, Chromium trioxide 30% w/w, Arsenic pentoxide 19.9% w/w) have been successively used in soluble formulations as wood preservatives against insect and fungal attack at a treatment site (Mench et al., 2005). Various ways, i.e. leaks of the treating fluid, lixiviates from treated timber, waste repositories, and natural agencies (wind erosion and water run-off) have consequently disseminated chemicals which have contaminated soils to various extends. Copper content in topsoils ranged from 65 to 2600 mg kg⁻¹. Large derelict areas frequently displayed either barren soils or dwarf vegetation patches, whereas other experienced natural attenuation and reforestation based on *Populus* and *Salix* sp. Firstly, plant species richness in quadrates at the treatment site is presented and related to soil contamination. 94 different plant species have been identified. *Hedera helix* and *Genista* sp were dominant at low topsoil contamination. *Agrostis capillaris* and *Rumex acetosela* were the most frequent plant species at high topsoil contamination. Secondly, Shannon diversity index in quadrates was calculated, and it ranged from 0 to 1.2. Finally, Cu tolerance patterns of several plant species are reported. Poplar (*P. nigra*, *P. deltoides*) cuttings collected on site were cultivated in either nutrient solution with increasing Cu concentrations or contaminated soils. Maximum root growth vs. Cu concentration (0 - 40 µM) in solution was monitored for Poplar sp. (Schat and ten Bookum, 1992). *Sporobolus tenassissimus*, *Carex* sp and *Agrostis capillaris* from both this wood preservative treatment site and surrounding uncontaminated sites were grown on a soil series with increasing contaminant contents, obtained by dilution of the contaminated soil with a control soil. Potted soils were placed in a greenhouse. Biometrical and biochemical parameters of plant species, metal uptake and distribution in plant species are presented. Potential of plant species for either phytostabilisation or phytoextraction is discussed regarding plant tolerance to Cu and Cu accumulation in plant parts.

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Zinc accumulation and histolocalisation in *Solanum nigrum* grown in contaminated soil: effect of arbuscular mycorrhizal fungi

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Keywords: *Solanum nigrum*, arbuscular mycorrhizal fungi, Zn, phytoremediation, autometallography

Abstract

Zn tissue accumulation in *Solanum nigrum* grown in a non-contaminated and a naturally contaminated Zn matrix and the effect of inoculation with different arbuscular mycorrhizal fungi (AMF) on metal uptake were assessed. The Zn level in the soil used in this study - 426 mg Zn/ kg dry soil - is higher than what is set in the Portuguese legislation (EC Directive 86/278/CEE from June 12th). *S. nigrum* plants grown in the contaminated soil accumulated up to 1622 mg kg⁻¹ of Zn in the roots, 411 mg kg⁻¹ in the stems and 253 mg kg⁻¹ in the leaves, with no visual toxicity signs. The levels registered for the roots are considered as phytotoxic for plants - 500 to 1500 mg kg⁻¹, according to Chaney (1989) - but all the tissues showed Zn levels above what is considered as normal levels in plant tissues - 10 to 100 mg kg⁻¹, according to Frisberg et al. (1996), independently of the inoculation with AMF. The presence of AMF did not influence the biomass of *S. nigrum* individuals, but both *Glomus claroideum* and *Glomus intraradices* enhanced the uptake and accumulation of Zn by *S. nigrum*. The inoculation with *G. claroideum* induced an increase of 58, 44 and 120% in the Zn accumulation levels for the roots, stems and leaves, respectively, when comparing to the non-inoculated plants. When inoculated with *G. intraradices*, the increase in the accumulation levels was of 54, 39 and 122% for roots, stems and leaves, respectively.

The knowledge of the metal distribution in cell compartments is important for understanding the tolerance mechanisms in plant species. In the present study, autometallography was performed on root samples of all treatments as a way of determining the location of the accumulated Zn in root tissues of *S. nigrum*. Electron-dense deposits of the metal were found in the appoplast (cell walls and intercellular spaces) and inside the cell, in the vacuoles. According to Memon et al. (2001), Zn location at the cell wall level is one of the resistance mechanisms of tolerant plants, as it allows avoiding the Zn in the most sensitive areas, where the metabolic activities take place. Compartmentalisation of the metal in the vacuole also avoids these sensitive areas to be affected. The accumulation of Zn in the cell walls and intercellular spaces was observed in plants for all the AMF treatments, as for the non-inoculated control.

These findings indicate that *S. nigrum* inoculated with selected heavy metal tolerant AMF presents extracting and accumulating capacities, constituting a potentially suitable remediation method for Zn polluted soils.

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Phytorestoration of highly alkaline industrial sediments: effect of native and non-native arbuscular mycorrhizal fungi

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Keywords: arbuscular mycorrhizal fungi; plant; stress; phytorestoration; sediments

Abstract

The presence of arbuscular mycorrhizas may reduce plant stresses caused by adverse soil conditions. In a previous field survey *Conyza bilbaoana* was found to be the dominant mycorrhizal herbaceous plant species in a highly alkaline sediment resulting from the disposal of waste originated at an acetylene and polyvinylchloride factory. The arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* BEG163 and *Glomus mosseae* BEG198 have been isolated from the sediment. To test the symbiotic effectiveness of these two fungi, seedlings of *C. bilbaoana* were inoculated with either native *G. intraradices* BEG163 or *G. mosseae* BEG198 and non-native *G. intraradices* BEG75 or *G. mosseae* BEG25 isolates in sterile and non-sterile sediment collected from the study site. All four isolates were able to colonise *C. bilbaoana*. However, AMF native to the target sediments were generally more effective than the non-native fungi in promoting plant establishment and growth under highly alkaline conditions. The non-native *G. intraradices* was, however, more effective than the non-native *G. mosseae*. The results of this study suggest the use of adapted AMF as inoculants for phytorestoration of alkaline anthropogenic-stressed sediments.



Environmental impact and soil remediation in contaminated areas of Madrid

EIADES

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Keywords: heavy metals, organic contaminants, bio-(phyto)remediation, electro-kinetic techniques, ecotoxicological tests, pilot and field scale.

Abstract

Europe soil degradation due to contamination represents an increasing hazard that requires the development of improved methods for soil remediation. In Spain, the new legal frames, mainly at regional level, provides for assessments of the environmental impact of a number of activities and projects, including those of regeneration of the contaminated sites. In this sense, a Research Consortium *EIADES* has been constituted recently in the Region of Madrid.

The Program *EIADES* is implemented by groups from eight research organisms including, three National and one Regional Institutes, and four Universities. A methodology to assess the environmental impact of industrial activities on soils and of the remediation projects themselves according to the risk analysis techniques applying last generation scientific development (PRA Probabilistic Risk Assessment, Biotech-DTA, biotechnology-based -Direct- Toxicity Assessment) in relation to characteristics of the Region of Madrid will be developed.

The application of different remediation strategies such as electrokinetic, bioremediation and phytoremediation, will be considered in relation to the size and type of contaminant, heavy metals or organic compounds, characteristics of the ecosystem and the future use of the soil. For this purpose, a selection of adequate microorganisms and plants; and, on the other hand, of representative placements will be carried out. With the aim to evaluate the efficiency of the remediation processes, an ecotoxicological analysis system will be developed to quantify the ecosystem remediation in terms of cost/benefit. The present communication aims to display the first approach to the investigation proposed by *EIADES* Program.

Cadmium and Zinc compartmentation in biota of a naturally-remediated woodland: Seasonal changes

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Keywords: cadmium, zinc, woody plants, compartmentation, natural attenuation.

Abstract

Seasonal changes in cadmium and zinc compartmentation have been followed in tissues of mature *Salix caprea* trees, naturally established on metal-contaminated canal sediment at a site in Staffordshire, UK (maximum pseudototal soil metal contents were 376 mg kg⁻¹ Cd, pH 5.53, and 787 mg kg⁻¹ Zn, pH between 4.6 - 6.7). Above ground tissues (twigs and leaves) were collected at regular intervals from a representative cohort of trees over the course of a single growing season and analysed for elemental content. The sampled trees all grew in soil with a range of Cd and Zn contents that significantly exceed UK statutory guidelines for any form of plant-based land use. The highest metal concentrations in leaves and twigs were found at the end of the growing season. Maximum values in leaves were 121 mg kg⁻¹ for Cd and 640 mg kg⁻¹ for Zn; in twigs maximum values were 152 mg kg⁻¹ for Cd and 306 mg kg⁻¹ for Zn.

Elemental distribution in current year stems and foliage was investigated using SXRF techniques. These revealed that Cd and Zn were localised in bark of current years' twigs and that Zn was closely associated with vein tissues in the mature leaves. This information, coupled with the seasonal measurements has been used to carefully assess the risk posed to primary consumers from element accumulations in current year stems and foliage as well as considering the potential for rapid element recycling via decomposition processes. Lignified tissues are frequently ignored by primary consumers, which tend to flourish during the earlier part of the growing season. As it is a British native species, *S. caprea* has a wide diversity of potential primary consumers, in turn attracting an equally wide range of secondary consumers. The absence of large numbers of one or two preferential consumers reduces the potential for large-scale food chain transfer of both Cd and Zn. The close association of both elements with lignified tissues would indicate that there would be a reduced rate of release during decomposition, as this type of tissue is the most recalcitrant component of plant litter.

Qualitative investigation of the soil fauna at the site showed this to be varied, with all major groups (decomposers, herbivores, predators) represented. The main groups of soil invertebrates present were Collembola, mites, Dipteran larvae, Coleopteran larvae and spiders. *Salix caprea* is only one component of the woodland; the other dominant species, *Salix viminalis* shows a much lower accumulation of both Cd and Zn in current years stems and foliage. These inter-specific differences in metal accumulation also contribute to the stability of this woodland ecosystem. It is concluded that the woodland that has developed naturally on this extremely polluted substrate is clearly sustainable, possesses a fully functional soil faunal community and presents a significantly reduced environmental risk compared to the unvegetated contaminated sediment.

Use of special substrates to optimize nutrient removal in vertical flow constructed wetlands

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Keywords: optimization, subsurface flow constructed wetlands, wastewater treatment, special substrates, nutrient removal, simulation tool, Turkey, Austria

Abstract

To reduce the large surface area requirement (3-10 m²/person) of vertical flow (VF) constructed wetlands (CWs) in Europe, several wetland researchers have recently focused on the optimization of CW design and on the use of special substrates in CWs for enhanced nutrient removal. The project ONUREM (*Optimization of Nutrient Removal in Constructed Wetlands Using Special Substrates and Numerical Simulation*), which is funded by the E.C. Marie Curie Intra European Individual Fellowship Programme, was also developed to optimize the specific surface area requirement of VFCWs.

ONUREM is aimed to quantify the effect of different natural and artificial substrates, which are commercially available in Turkey and Austria, on the removal performance of nutrients in lab-scale VF CWs treating municipal wastewater. Moreover, this research is aimed to develop a catalogue of the physico-chemical and hydraulic characteristics, and transport parameters of the special substrates. The substrate catalogue will contain the substrate parameters in the way so that they can be used in the simulation tool CW2D (Constructed Wetlands 2-Dimensional) developed by Langergraber (2001). The multi-component reactive transport module CW2D is able to describe the transformation and biochemical elimination processes of organic matter, N&P compounds of wastewater. Integration of the results of this study is expected to improve the reliability of the CW2D and to foster its use as a practical design tool for optimal sizing of VF CWs.

Materials and Methods

Quantification of the effect of different substrates on treatment performances

A literature survey has been conducted to determine the candidate natural and artificial substrates originated from Turkey (sand, zeolite, pumice, perlite and blast furnace granulated slag) and Austria (sand, zeolite, Ferrosorp and crushed-concrete). Batch-scale sorption experiments will be performed both with real wastewater and model N&P solutions to determine Langmuir and Freundlich isotherms of selected substrates. Suitable substrates will be further studied in lab-scale VFCWs as filter media and the treatment performances of these filters will be monitored. The experimental results will be evaluated to develop a database of substrates that can be used for VFCWs. The database will contain all relevant parameters describing the hydraulic behaviour and the nutrient removal capacity.

Investigation of the predominant removal mechanisms in special substrates

Chemical extraction experiments will be performed for substrate samples taken from the lab-scale VF CWs to understand the predominant nutrient removal mechanisms. Mass balance calculations will be conducted for nutrients using the related data of the monitored parameters, hydraulic loading rate, and chemical extraction results.

Inclusion of the results in the simulation tool CW2D

Since the experience of Langergraber (2003) showed that the characterization of the substrate regarding the hydraulic properties is a cornerstone in the successful application of CW2D, the database will provide input parameters for CW2D.

Validation of the results of the modified design tool with the results of the experimental wetlands

A simulation study will be performed using the results of the analysis of the substrates to optimize VF CWs. The simulated results will be validated with the measured data obtained from the lab-scale VF CWs. Finally, the results will be summarized and a design tool for VF CWs will be developed.

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Phytoextraction with rapeseed and tobacco: a Tool for Sustainable Management of Cd-Contaminated Soils

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Keywords: phytoextraction, *Brassica napus* L., *Nicotiana tabacum* L., cadmium, hydroponics and field exposure.

Abstract

Phytoextraction is a promising tool to extract metals from contaminated soils and species like *Brassica napus* and *Nicotiana tabacum* seemed to be suitable candidate species for this purpose (Salt et al., 1998). The selected plants will then be implemented into current agricultural practice and used for non-food purposes (biomass energy and/or bio-diesel).

To select accessions with the ability to accumulate cadmium, hydroponically grown 21 day old seedlings of 77 *B. napus* L. accessions were exposed to 0.2 μM CdSO₄ for an additional 10 days. The effects of Cd on several parameters were quantified i.e.; shoot Cd concentration ([Cd]_{shoot}), total amount of Cd in shoots (Total Cd) and the shoot to root Cd concentration ratio (S/R ratio). Though generally natural variation was low for [Cd]_{shoot}, Total Cd and S/R ratio, a number of accessions could be selected. Our results indicated that Total Cd and S/R ratio are independent parameters for Cd accumulation and translocation. The selected varieties were then tested in field experiments on two locations nearby metal smelters. The two locations differed in extractable soil Cd, Zn, Ca concentration and pH levels. On both locations *B. napus* accessions showed significant differences in [Cd]_{shoot} and Total Cd. Furthermore we found significant correlations between Cd and Zn accumulation in shoots. There were site-specific effects with respect to Cd accumulation in the *B. napus* accessions, however two accessions seem to perform equally well on both sites. The results of the field experiment suggest that certain *B. napus* L. accessions are suitable for phytoextraction of moderately heavy metal contaminated soils.

To select for higher Cd accumulation properties we crossed a few promising rapeseed winter varieties and screened 8 F₂ families on Cd accumulation and S/R ratio after exposure to 0.2 μM CdSO₄ in hydroponics for 10 days. However, this selection procedure was not successful because no segregation was found among the families.

Therefore, we decided to concentrate on a molecular approach i.e. to combine the characteristics of high biomass production with specific genes, responsible for extremely high uptake of metals in hyper-accumulators (Assuncao et al., 2003). We are now in the process to establish transgenic tobacco plants overexpressing some of these specific genes.

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Vertical flow surface constructed wetland as a treatment to remove pollutants from the pig slurry liquid phase: study case in La Aljorra, SE Spain

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Keywords: *Phragmites australis*, pig slurry, ecological treatment, constructed wetlands, pollutant.

Abstract

Spain produces about 3 millions tones of pork meat, and is the second larger pig producer in Europe. As a consequence, a large amount of pig slurry is generated. Pig slurry constitutes a very serious environmental issue mainly due to the change of pig production to an intensive, confinement type system without soil contact.

Nowadays, there are different available technologies to remove pollutants from waste water. Constructed wetlands represent a low -cost and ecological treatment. In this study, constructed wetlands are used to treat pig slurry. Wetlands are a suitable system to reduce pollution from agrarian effluent and, as in this case, by pig slurries. There are different types of constructed wetlands. For this research, a vertical flow surface constructed wetland was used.

Experimental wetlands were constructed at a pig farm located in La Aljorra, Cartagena (Murcia, SE Spain). The farm has a phase separator for the processing of the pig slurry by bioaeration process: the solid material is dried-out and used directly for fertilization, while the liquid is disposed into a waterproof cement pond.

The liquid, with a high nutrient load, and high levels of soluble salts, heavy metals, etc may not be used directly for irrigation. In order to solve to this problem, a set of 5 plastic containers (1 m³) filled with sand and gravel layers, (with 6 and 24 mm size gravels, and 2 mm sand) was designed. The different thicknesses for each layer are: 50 cm of sand, 10 cm of thin gravel and 15 cm of coarse gravel. Suspended solids are filtered by this system.

Biological processing in constructed wetlands is an important part of the system and essential for the improvement of water quality. Plants can remove different contaminants including, organic matter, nitrogen, phosphorus and heavy metals (Haberl, Perfler, 1991). The plant species selected for this experiment is *Phragmites australis*. *Phragmites* is well adapted to the semiarid climate of the area and grows locally. For the test, influent and effluent are periodically chemically and microbiologically analyzed.

The goal is to treat effluent sufficiently for use as irrigation water.

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Influence of soil treatment with zerovalent Fe grit on growth and As accumulation in Chinese Brake fern (*Pteris vittata*)

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Keywords: CCA, stabilization, plant mortality

Abstract

Zerovalent iron grit (Fe^0) has been shown to immobilize As in contaminated soil by providing effective surfaces for sorption of As anions (Mench et al., 2000, Kumpiene et al., 2006). To demonstrate the usefulness and compliance of this *in situ* soil remediation method, long-term reduction of As bioavailability should be achieved. Chinese Brake fern (*Pteris vittata*), that can hyperaccumulate As in its fronds at levels 3-6 times greater than soil As, has a potential to remediate As-contaminated soils (Ma et al., 2001). It can also be used to study the Fe^0 -treatment efficiency in CCA-contaminated soils. This study aimed at assessing changes in growth and As accumulation in *Pteris vittata* caused by soil amendment with Fe^0 . Three field topsoils containing different CCA concentrations (Table 1) were treated with 1% Fe^0 and planted with fern seedlings.

Table 1 Contaminant contents, pH and OM content of the studied soils.

	Soil		
	A	B	C
pH	5.6	7	6.1
OM (%)	1.9	2.7	1.7
As (mg/kg)	4673	26.3	14.4
Cu (mg/kg)	1379	2600	536
Cr (mg/kg)	2181	31	22
Zn (mg/kg)	562	58	21

The fern mortality in the treated soils was compared to that in the untreated soils. The survival rates of ferns in untreated soils decreased in the following order (A>B>C) (Fig. 1), which corresponded to the total As content in soil.

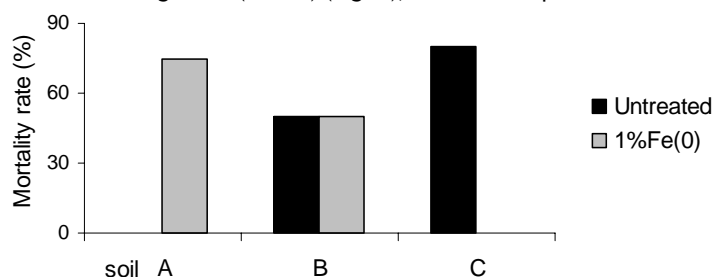


Figure 1. Mortality rate of *Pteris vittata* in untreated and 1% Fe^0 -treated soils.

Fern mortality had an opposite pattern in soils A (high contamination with both Cu and As - higher survival in untreated soil) and C (medium Cu contamination, low As - higher survival in Fe^0 -treated soil). Soil B that contained the highest Cu concentration and more As than in soil C had 50% survival in both untreated and 1% Fe^0 -treated soils.

The contaminant concentrations in soil pore water and fronds will be analyzed to determine the translocation and accumulation of As in ferns and to identify possible reasons of fern mortality.

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Laboratory scale model experiments on improving nitrogen removal in subsurface constructed wetlands by enhancing the anaerobic ammonia oxidation

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Keywords: laboratory scale wetland, model experiments, anaerobic ammonia oxidation

Abstract

Subsurface constructed wetlands are used for nitrogen removal, but their performance is limited. In case of domestic sewage the removal rate fluctuates between 0.2 and 1.0 g N/m²d⁻¹. The main removal mechanisms have been associated to microbial nitrification and denitrification processes. The limiting factor for this relatively low removal rate could be associated to the low oxygen transfer that the plants can do, and low content of organic carbon. In the last years new nitrogen transformation processes have been studied and partial nitrification combined with anaerobic ammonia oxidation (Anammox) have been identified as a new process strategy for nitrogen removal in wastewater treatment. Due to the presence of aerobic and anaerobic micro-zones in subsurface flow constructed wetlands there can be assumed that the Anammox process could play an important role in the nitrogen removal in such systems.

In order to evaluate the role and effects of Anammox process lab scale model experiments were done with planted fixed bed reactors. The experiments lasted for two years. In the first year a reactor (planted with *Juncus effusus*) was fed with synthetic wastewater containing 150 mg l⁻¹ NH₄⁺ and 60 mg l⁻¹ NO₂⁻.

At these running conditions the nitrogen removal rate was in the same range as already reported by different authors. The pore water in the rooted gravel bed and the effluent of the model system contained a mixture of nitrite, nitrate and ammonia, however plants were affected by high ammonia and nitrite concentrations.

In the beginning of the second year Anammox biomass was added, without changing the other operation conditions in the reactor. As the result of this inoculation, the nitrogen removal rate increased between 4 and 5 times and also the toxicity effects to the plants disappeared. The pore water of the rooted gravel bed and the effluent of the model systems at present contains mainly ammonia and low concentration of nitrate.

By applying nitrogen isotope tracer tests with ¹⁵N-NH₄⁺ and ¹⁴N-NO₂⁻ ²⁹N₂ was formed which proves the anaerobic oxidation of ammonia (Anammox process).

The results from these model experiments show that in principle subsurface flow wetlands can function also as a "Anammox bioreactor". Further investigations have to show the role of plant roots in such systems.

For the complete process design for the treatment of waters with high ammonia load especially simple technical solutions for realising the first step, the partial nitrification, have still to be developed.

Effect of organic matter, plants and type of flow on chromium and zinc removal in constructed wetlands

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Keywords: small scale constructed wetlands, chromium and zinc removal

Abstract

Constructed wetlands have been used for the treatment of wastewater containing heavy metals. However, aspects like the role of plants, flow (subsurface or surface) and the impact of organic matter are not clear. In order to clarify such questions, an experimental design and research with three factors and two levels for each factor were carried out. The evaluated factors and levels were: type of flow (subsurface and surface); presence of plants (planted with *Typha latifolia* and unplanted), and presence of organic matter (with and without). In total, eight different experimental units or treatments were operated during a period of one year. The units were fed with synthetic wastewater containing chromium(VI) (1.5 mg.l^{-1}), zinc (1.5 mg.l^{-1}) and macro and micro nutrients. Additional organic matter (COD: 250 mg.l^{-1}) was added to those units defined with this factor. After statistical analysis (ANOVA, multifactorial) subsurface constructed wetlands showed, with significant difference, a higher removal rate of chromium in comparison with surface systems ($97 \text{ mg.m}^{-2}.\text{d}^{-1}$ and $60 \text{ mg.m}^{-2}.\text{d}^{-1}$, respectively). Planted systems removed, with significant difference, more chromium in comparison with unplanted systems ($85 \text{ mg.m}^{-2}.\text{d}^{-1}$ and $76 \text{ mg.m}^{-2}.\text{d}^{-1}$, respectively) and the presence of organic matter increased the removal rate regarding the units without it ($88 \text{ mg.m}^{-2}.\text{d}^{-1}$ and $69 \text{ mg.m}^{-2}.\text{d}^{-1}$, respectively). In a similar way, subsurface systems removed more zinc than surface systems ($88 \text{ mg.m}^{-2}.\text{d}^{-1}$ and $57 \text{ mg.m}^{-2}.\text{d}^{-1}$, respectively) and planted systems removed more zinc regarding unplanted systems ($79 \text{ mg.m}^{-2}.\text{d}^{-1}$ and $68 \text{ mg.m}^{-2}.\text{d}^{-1}$, respectively). No significant differences were found for zinc, if organic matter is added.

Soil analysis showed that metal accumulation and retention took place in the first part of the wetland and decreased along it. Through multiple sequential extraction procedure, different metal fractions susceptible for remobilization were determined.

Simulation experiments on post-treatment of anaerobic effluents in laboratory scale wetlands

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Keywords: post-treatment, anaerobic effluents, sulphur compounds, laboratory scale wetlands

Abstract

Anaerobic treatment technology has found remarkable application in the treatment of wastewaters especially in developing countries. However, it is recognized that a sulphate-rich wastewater can cause some problems resulting from hydrogen sulphide formation. To tackle the "H₂S problem" the anaerobic digestion step can be linked with further treatment in a constructed wetland. Questions left open are the possible limiting loading rates, the transformation processes of the sulphur compounds etc.

This study shows simulation results of two planted (with *Juncus effusus*) and one unplanted (control) model wetlands in laboratory scale which were run with a synthetic wastewater containing varying sulphide (1.5 and 2.5 mg/l) concentrations. The hydraulic retention time (HRT) was 5 and 2.5 days.

As the HRT was 5 days, removal did not show significant differences (22.2, 23.1 and 24.8 mg S²⁻/m²d) for the unplanted and two planted systems respectively. When the HRT was decreased by a half the S²⁻ removal in the two planted systems was more than twice as high (25.5, 51.3 and 56.9 mg S²⁻/m²d). At the highest S²⁻ loading rate, the planted systems showed even a higher removal rate (76.1 to 85.9 mg S²⁻/m²d). Applying different analytical methods, the sulphur species (S⁰, S²⁻, SO₃²⁻, S₂O₃²⁻, SO₄²⁻) were characterized within the model wetlands and in their effluents depending on different loading conditions.

These results underline the role of wetland plants in the removal/transformation process for S²⁻ probably mainly by their oxygen input into the rooted soil systems by which S²⁻ gets oxidised.

Removal of arsenic and dynamics of sulphur compounds in a laboratory-scale constructed wetland

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Keywords: laboratory-scale constructed wetlands, arsenic, sulphate, elemental sulphur

Abstract

A laboratory reactor planted with *Juncus effusus* treating an artificial wastewater that contains sulphate (150 mg l⁻¹) and arsenic (0.3 mg l⁻¹) was used to investigate the removal of arsenic (As(III) and As(V)) and the dynamics of sulphur compounds. Permanent circulation of process water enabled to evaluate the micro-gradient processes for an operation period of 225 days.

Sulphate reduction started immediately and was found highly efficient at approximately 84% during the whole experimental period. Concentrations of sulphide and elemental sulphur in the process water increased permanently up to 34-47 mg l⁻¹ (S⁻²) and 8-21 mg l⁻¹ (S⁰) at the end of the experimental period. Finally, nearly all reduced sulphate-sulphur was found to be transformed to S⁻² and S⁰.

In a first part (0-90 d) of the total operation period, the As(V) inflow concentration of 0.2 mg l⁻¹ was reduced continuously at a range of 50-70%. Starting the operation, As(III) was completely removed, but the outflow concentration linearly increased up to the value of the inflow (0.1 mg l⁻¹) at the end of this first operation part. In all, 65% of the added arsenic was removed. During the second part of the whole operation period (90-176 d) without arsenic in the added wastewater, As(V) was found to be remobilized. During the third operation part (176-225 d) in presence of high concentrations of S⁻² and S⁰, nearly 92% of the added arsenic (0.1 mg l⁻¹ As(III) and 0.2 mg l⁻¹ As(V)) was removed. During the whole operation period, COD was removed highly efficiently in a range of 86-97%. Stable ammonia removal of approximately 50% could only be estimated during the first operation part. For the remaining operation period, the ammonia concentrations in the outflow increased to the value of the inflow at the end of the experiment. The results suggest high removal efficiencies for As in the root environment in presence of sulphur compounds but its partially unstable immobilization in case of sulphate depletion.

Dynamics of redox processes in a laboratory-scale constructed wetland

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Keywords: redox processes, laboratory-scale constructed wetlands, *Juncus effusus*

Abstract

A laboratory reactor planted with *Juncus effusus* treating an artificial wastewater was used to investigate the short-term and long-term variations and interactions in the redox conditions as well as the removal efficiency of C and the N turnover. The permanent circulation of the process water enabled the micro-gradient processes to be evaluated for an operating period of 20 months.

Steady-state conditions were achieved throughout the operating period with high mean removal efficiencies of 92.7% TOC, 82.0% ammonia and 97.6% nitrate. Daily variations in the redox state of the rhizosphere of a few hundred mV were observed, ranging from about -200 to oxidized conditions of about +200 mV and driven by daylight. Variations in pH associated with changes in light and redox were linked to the dynamics of the fates of organic and inorganic carbon species.

The ammonia removal processes were found to be firmly established, including for moderately reduced redox conditions with high efficiencies for $E_h > -50\text{mV}$. The enrichment of ammonia (up to 13 mg l^{-1}) closely linked to the light, particularly during summertime, indicates the existence of hitherto unconsidered additional N turnover pathways in the rhizoplane involving N_2 produced by microbes or released by plants.

C turnover was strongly related to the seasonal variation in illumination with minimum efficiencies during the dark season. In addition it was characterized by oscillation with periods of approximately one month.

The relationships found are dominant for biofilms on the rhizoplane and decisive for the removal efficiency of especially simple constructed and natural wetlands. The results highlight the importance of helophytes and their physiological specifics for removal processes.

Removal of sulphate, organic carbon and ammonia in a laboratory-scale constructed wetland

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Keywords: sulphate, ammonia, removal, laboratory-scale constructed wetlands

Abstract

Sulphate is a usual constituent of domestic wastewater. In case sulphate is reduced, mainly by microbial processes, the resulting reduced sulphur compounds are known to be potent inhibitors of plant growth and certain microbial activities. However, knowledge about sulphate reduction and the effect on the removal of C and N in constructed wetlands is still limited. Investigations in planted laboratory-scale reactors as model systems for constructed wetlands were performed to evaluate the interrelation of organic carbon and ammonia nitrogen removal with the sulphate reduction using artificial domestic wastewater.

Organic carbon removal was found to be only slightly affected and remained at high levels of efficiency (75 to 90%). Only at sulphate reduction intensities above 75 mg l⁻¹ (50% removal) decrease in organic carbon removal of up to 20 % was observed. A highly contrary behavior of ammonia removal was found in general, which decreased exponentially from 75 to 35% related to a linear increase in sulphate reduction up to 75 mg l⁻¹ (50% removal). Since sulphate removal is considered to be dependent on the load of electron donors, the organic carbon load of the system was varied. Variation of the load changed the intensities of sulphate reduction immediately, but did not influence the organic carbon removal effectiveness. Doubling the organic carbon concentration of 200 mg l⁻¹ BOD₅ for domestic wastewater usually led to sulphate reduction of up to 150 mg l⁻¹ (100% removal). The findings show that, particularly in constructed wetland systems, the sulphur cycle in the rhizosphere is highly important for the performance of wastewater treatment and may initiate a reconsideration of the amount of sulphate present in tap water systems.

Phytoremediation of PAHs from the air

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Keywords: air pollution; urban area, waxes, *Canna x generalis*

Abstract

Polluted sites of anthropogenic origins, due to industrialization and intensive transportation, might create serious hazard to environment and human health. Urban areas, with often-elevated level of pollutants, are especially dangerous sites because of permanent exposition of citizens to harmful substances. Phytoremediation seems to be promising technology for urban areas. In fact, for the phytoremediation of the pollutants from the air in practice there is no other alternative technology. Polycyclic aromatic hydrocarbons (PAHs) are major constituent of air pollutants in places with heavy traffic. As very hydrophobic substances they are very easy captured by lipophilic waxes that covers the aerial parts of plant mostly leaves and stems. Almost half of PAHs, emitted from the transport vehicles, are found in waxes covering plant organs (1); glossier leaves trap better vapor 2-3 rings PAHs and leaves with trichomes and hairs keep particles of 4 and more rings, which tend to be solids (2). In our Laboratory several ornamental plant species were evaluated for amount of waxes and PAHs including *Canna x generalis*, known from previous studies as good phytoremediant of heavy metals. In this work 3 *C. generalis* cvs were included: Delibab, Halina and Hercules, all having leaves and stems well covered with waxes. Plants were growing in 3 distances from roadways edge (1-3, 50 and 100 m) and during vegetation season they were harvested 4 times for waxes determination. In the end amount of PAHs in plant material was also determined. Waxes were washed twice with chlorophorm (40 and 20 s), which after deprived from impurities was evaporated. The remained wax was weighted and calculated per square cm². PAHs were extracted from dried leaves by dichloromethane and analyzed with GC/MS technique.

The amount of wax deposited on leaves changed during vegetation and in the end of growing season varied depending on distance from the roadway from 15,6 to 25,4 $\mu\text{g} \times \text{cm}^{-2}$ and on cultivar from 14,9 to 25,6 $\mu\text{g} \times \text{cm}^{-2}$. These differences are more evident when plant leaf area was taken into account and ranged from 100.2 mg plant⁻¹ (Halina) to 322.5 mg plant⁻¹ (Herkules). Seventeen PAHs compounds were identified, including 8 carcinogenic ones: benzo(a)anthracene, chrysene, benzo(b)fluorantene, benzo(k)fluorantene, benzo(a)pirene, dibenzo(a,h)anthracene, benz(g,h,i)perylene and indeno(1,2,3-cd)pirene. Leaves, in average for studied cvs. contained 325, 235 and 127 $\mu\text{g} \times \text{kg}^{-1}$ in the distances of 1-3 m, 50m and 100 m from the roadways edges. Respective values for carcinogenic PAHs where as follow: 79, 47 and 18 $\mu\text{g} \times \text{kg}^{-1}$. PAHs concentrations negatively correlated (highly) with the distance from roadways but no differences were recorded between cultivars in regard to amount of captured of these pollutants.

These results show that vegetation surrounding roadways, with high transportation emitting pollutants, plays role of the green safety belt for cultural environment.

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Phytotechnology for the treatment of dairy effluent

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Keywords: farm effluents; short rotation forestry; willow; Salix; lysimeter

Abstract

The rapid growth of the dairy industry is having a significant detrimental impact on groundwater, streams, and lakes on a worldwide scale. Legislation of developed countries is beginning to require landowners to treat wastewaters to avoid environmental degradation and contamination of soil and water resources likely to be caused by their disposal. Farm effluents are often treated biologically using storage-ponds systems and spreading on pasture and cropland. A potential low-cost solutions to dispose of dairy shed effluents are short rotation forestry (SRF) plantations. In SRF, fast growing species, such as *Salix* sp., may be used to remove or degrade contaminants while providing secondary products such as feed supplement to stock and bio-fuel. However, these green approaches need to be carefully managed to avoid excessive leaching of nitrogen and metals: deep-rooting, phreatophyte plants reduce leaching by evapotranspiration and rapid nutrient-uptake in the summer months, some leaching and soil accumulation of contaminants may occur, especially if contaminants are rendered unavailable for plant uptake by complexation with the effluent-borne organic matter. Therefore a greenhouse experiment with lysimeters (containing 80 kg of sandy soil), planted with willows stalks fertilised with dairy shed effluent was set up to monitor in a controlled condition how a SRF systems evolves in time. The experiment lasted three months during which the lysimeters, planted with male specimens of *Salix kanayunagy*, were subjected to three levels of fertilisation (high, medium, low). Lysimeter leachates were collected each week; after three months, samples of soil at different depths and plants organs were collected. All the solid samples were digested with concentrated Nitric acid using a Velp Scientifica (Italy) Digestor. Macro and micro nutrients, and heavy metals content was measured with analytical chemistry techniques as ICP-OES, the content of N was measured through the Kjeldahl method, total N was assessed as well. The lysimeters leachates were analysed for all metals and N to monitor the changes in elements content in time, according to the treatment intensity. High concentrations of N were found in soils and in leachates at the end of the experiment. Metals accumulated in soil, but did not increase in leachates. Plant roots had a higher content of nutrients and metals than shoots and leaves for all the treatments. Plants growth and health during the experiment were strictly related to the treatment intensity: plants treated with the highest fertiliser concentration grew healthier and produced significantly more biomass than those treated with low doses of fertiliser. The total amount of leachates used for the highest treatment added up to 200 litres plus 300 litres supply of water for irrigation. The total N applied to the soil was of 300mg/L per day, as in areal on-site situation. This experiment revealed that almost 80% of N was used by plants to grow, providing a valuable above ground biomass, liable of further exploitation as biofuel or fodder.



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