

Nagata, T., M. Kiyono, et al. (2006). Engineering expression of bacterial polyphosphate kinase in tobacco for mercury remediation. *Applied Microbiology and Biotechnology* 72(4): 777-782. To develop the potential of plants to sequester and accumulate mercurials from the contaminated sites, we engineered a tobacco (*Nicotiana tabacum*) plant to express a bacterial *ppk* gene, encoding polyphosphate kinase (PPK), under control of a plant promoter. The designated plant expression plasmid pPKT116 that contains the entire coding region of *ppk* was used for *Agrobacterium*-mediated gene transfer into tobacco plants. A large number of independent transgenic tobacco plants were obtained, in some of which the *ppk* gene was stably integrated in the plant genome and substantially translated to the expected PPK protein in the transgenic tobacco. The presence of Hg²⁺ did not cause considerable morphological abnormalities in the transgenic tobacco, which grew, flowered, and set seed similarly to the wild-type tobacco on the medium containing normally toxic levels of Hg²⁺. The *ppk*-transgenic tobacco showed more resistance to Hg²⁺ and accumulated more mercury than its wild-type progenitors. These results suggest that *ppk*-specified polyphosphate has abilities to reduce mercury toxicity, probably via chelation mechanism, and also to accumulate mercury in the transgenic tobacco. Based on the results obtained in the present study, the expression of *ppk* gene in transgenic tobacco plants might provide a means for phytoremediation of mercury pollution.

Nagpal, V. and K. M. Paknikar (2006). Integrated biological approach for the enhanced degradation of lindane. *Indian Journal of Biotechnology* 5(3): 400-406. Lindane is a persistent organic pollutant (POP) and its removal from various environmental compartments is a global priority. In the present study, three lindane degrading bacterial cultures, viz. *Pseudoarthrobacter* sp., *Pseudomonas* sp. and *Klebsiella* sp., were selected from among ten isolates obtained from lindane-exposed soils by enrichment culture technique. The cultures exhibited a maximum lindane degradation efficiency of similar to 50%, having little significance in bioremediation. However, in the presence of protozoa (ciliates) obtained from the same enrichment culture, lindane degradation efficiency of bacterial cultures increased to the tune of 90-92% with concomitant formation of 1,2,4-triclorobenzene, a key degradation product. Lindane degradation by the bacterial cultures was also found to get enhanced by 10-15% in the presence of root exudates of lindane-tolerant plants, viz. corn, chili and coriander, even though these plants were unable to uptake any lindane and thus were unsuitable for phytoremediation per se. These data highlight the need for an intensive investigation on (a) bacteria-protozoa interactions for bioremediation of POPs and (b) use of POP-degrading bioinoculants in the rhizospheric soil of resistant plants as a possible alternative, in the absence of a viable conventional remedial option.

Nair, D.R. (1991). Atrazine fate modeling and mineralization studies in soil-plant systems. Ph.D. Thesis, University of Iowa, Iowa City, IA.

Nair, D.R., and J.L. Schnoor (1990). Modeling of alachlor and atrazine at a small plot in Amana, Iowa. Pp. 89-112. In L.E. Erickson, D.L. Tillison, S.C. Grant, and J.P. McDonald (eds.), *Proceedings of the 8th Annual Conference on Hazardous Waste Research*, May 25-26, 1993, Manhattan, KS.

Nair, D.R., and J.L. Schnoor (1992). Effect of two electron acceptors on atrazine mineralization rates in soil. *Environ. Sci. Technol.* 26(1):2298-2300.

Nair, D.R., and J.L. Schnoor (1994). Effect of soil conditions on model parameters and atrazine mineralization rates. *Water Res.*

Nair, D.R., J.G. Burken, L.A. Licht, and J.L. Schnoor (1993). Mineralization and uptake of triazine pesticide in soil-plant systems. *J. Environ. Eng.* 119:842-854.

Nakajima, N., Y. Ohshima, et al. (2002). Processing of bisphenol A by plant tissues: Glucosylation by cultured BY-2 cells and Glucosylation/translocation by plants of *Nicotiana tabacum*. *Plant and Cell Physiology* 43(9): 1036-1042. Bisphenol A (BPA, 4,4'-isopropylidenediphenol), an endocrine disrupter with estrogenic properties, was supplied to tobacco BY-2 cells in suspension culture and the chemical nature of its metabolites was investigated. The concentration of BPA in the culture medium decreased rapidly

and became undetectable at 2.5 h after the application. Four metabolites of BPA were observed in a methanol extract of the cells when the culture was supplemented with [C-14]BPA. The most abundant metabolite was determined to be 4,4'-isopropylidenediphenol-O-beta-(D)-glucopyranoside (BPAG) by mass spectrometry, nuclear magnetic resonance spectroscopy and by hydrolysis with beta-glucosidase. This identification was confirmed by synthesis. When [C-14]BPA was administered to tobacco seedlings from their roots, radioactivity was incorporated in BPAG and three unidentified metabolites. These metabolites were accumulated in the leaves after 4 h exposure, indicating that tobacco seedlings absorbed BPA through their root systems, metabolized to its beta-glucoside and translocated the metabolites to their leaves.

Nakajima, N., Y. Oshima, et al. (2004). Glycosylation of bisphenol A by tobacco BY-2 cells. *Phytochemistry (Amsterdam)* 65(10): 1383-1387. Tobacco BY-2 cells in suspension culture absorbed and transformed bisphenol A dissolved in the culture medium. Major products were bisphenol A mono-O-beta-D-gentiobioside and the trisaccharide bisphenol A mono-O-P-D-glucopyranosyl-(1 fwardw 4)-(beta-D-glucopyranosyl-(1 fwardw 6)) P-D-glucopyranoside. Also produced were the mono- and di- O-beta-D-glucopyranosides. As glycosides of bisphenol A lack the estrogenic activity of the parent compound, these findings enhance the possibilities of phytoremediation of natural waters contaminated by bisphenol A. Copyright 2004 Elsevier Ltd. All rights reserved.

Nakamura, T., T. Motoyama, et al. (2004). Biotransformation of pentachlorophenol by Chinese chive and a recombinant derivative of its rhizosphere-competent microorganism, *Pseudomonas gladioli* M-2196. *Soil Biology & Biochemistry* 36(5): 787-795. The use of plants or microorganisms to detoxify contaminated soil or groundwater is a potentially cost-effective alternative to traditional remediation technologies. This study investigated the effects of a rhizosphere microbe on the biotransformation of pentachlorophenol (PCP). Chinese chive (*Allium tuberosum* Rottler) and its rhizosphere-competent bacterium, *Pseudomonas gladioli* M-2196, were used as a plant-bacterium pair. The genes encoding PCP-degrading enzymes from *Sphingobium chlorophenolicum* ATCC39723 were introduced into the chromosome of *P. gladioli* M-2196. The resultant transformants were able to degrade PCP almost completely in liquid medium within 4 d in culture. PCP degradation experiments showed that the amount of PCP in soil (3.3 mug g⁻¹) planted with the *P. gladioli* transformant (T-9) and Chinese chive decreased by 40% as compared with untreated soil (control) by day 28. Strain T-9, which was used in the PCP degradation experiments, retained the ability to colonize the Chinese chive rhizosphere after 28 d. Tetrachlorocatechol (TCC) was detected as a metabolite of PCP in Chinese chive extract. The amount of PCP in soil treated only with Chinese chive decreased by 30% as compared with the control, but the total amount of PCP plus TCC detected in the plant was less than 10% of the amount of PCP removed from soil. This might be due to the enhancement of a soil microflora population capable of degrading PCP by root exudates from Chinese chive. Therefore, Chinese chive itself, in addition to the rhizosphere-competent bacterium, seemed to play an important role in reducing the PCP level in the soil. (C) 2004 Elsevier Ltd. All rights reserved.

Namvargolian, R., J.W. Warner, T.W. Gates, P. Miller, and G. Comes (1993). Fouling of groundwater recharge facilities. Pp. 283-297. 6th Biennial Symposium on Artificial Recharge of Groundwater, May 19-21, 1993, Scottsdale, AZ. USDA Water Conserv Lab/et al.

Nanda Kumar, P.B.A., S. Dushenkov, D.E. Salt, and I. Raskin (1994). Crop *Brassicas* and phytoremediation - a novel environmental technology. *Cruciferae Newsletter*. 16:18-19.

Nandakumar, R., L. Chen, et al. (2004). Factors affecting the *Agrobacterium*-mediated transient transformation of the wetland monocot, *Typha latifolia*. *Plant Cell Tissue and Organ Culture* 79(1): 31-38. An *Agrobacterium*-mediated transformation system, using transient transformation assays, was used to evaluate conditions influencing transformation for the wetland monocot *Typha latifolia*. These studies were aimed at the long-term objective of evaluating candidate genes for phytoremediation. The binary plasmid vector pCAMBIA1301/EHA105, containing the beta-glucuronidase coding sequence, was used in combination with factors known to affect transformation. These included callus age at the time of cocultivation with *Agrobacterium tumefaciens*, type and concentration of auxin for explant growth, light or dark culture environment, the presence or absence of acetosyringone (AS), explant type, explant

wounding and the number of days used for cocultivation. The number of days needed for the first detection of transient expression of the beta-glucuronidase gene was also examined. Three days of *Agrobacterium* cocultivation of 50-day-old seedling-derived calluses, grown on 20.7 μM (5 mg l⁻¹) picloram supplemented medium, in the dark, resulted in higher levels of transient beta-glucuronidase expression than were seen in calluses cultured on 4.5 or 22.6 μM (1 or 5 mg l⁻¹) 2,4-dichlorophenoxyacetic acid containing media. The addition of 100 μM acetosyringone significantly enhanced transient beta-glucuronidase activity. Wounding of explants, by cutting into two or three pieces, 3 days before cocultivation, increased expression of beta-glucuronidase only in calluses cultured under light conditions. Transient beta-glucuronidase expression was observed as early as 24 h after cocultivation and increased as the days post cultivation increased. The developed transient system will be used for stable transformation of *Typha* species.

Nandakumar, R., L. Chen, et al. (2005). *Agrobacterium*-mediated transformation of the wetland monocot *Typha latifolia* L. (Broadleaf cattail). *Plant Cell Reports* 23(10-11): 744-750. An *Agrobacterium*-mediated model transformation system was standardized for the wetland monocot *Typha latifolia* L. to achieve the long-term objective of introducing candidate genes for phytoremediation. Two binary plasmid vectors, pCAMBIA1301/EHA105 and pTOK233/LBA4404, both containing the gus (beta-glucuronidase) and hptII (hygromycin phosphotransferase II) genes, were used for transformation. Fifty-day-old 5 mg/l picloram-derived calli were cocultivated and selected on medium containing 20 mg/l or 40 mg/l hygromycin. Resistant calli were regenerated on medium supplemented with 5 mg/l 6-benzylaminopurine, with or without 20 mg/l or 40 mg/l hygromycin and with or without charcoal (10 g/l). Transient GUS activity in explants ranged between 28% and 36%. Hygromycin-resistant calli, selected after 3 months, showed stable GUS expression. A total of 46 plants were regenerated and established in the greenhouse; 13 showed stable GUS expression. Cocultivation of dark culture-derived calli, directly selected on regeneration medium containing 20 mg/l hygromycin and rooted on medium with 20 mg/l hygromycin was the best protocol. The addition of charcoal did not have any effect on regeneration. PCR and Southern analyses of transgenic calli and transgenic plants confirmed the presence of the introduced genes. In conclusion, *T. latifolia* could be genetically transformed by *Agrobacterium tumefaciens*.

Narayanan, M., L. Erickson and L. Davis (1999). Simple Plant-Based Design Strategies for Volatile Organic Pollutants. *Environmental Progress* 18(4): 231-242.

Narayanan, M., L.C. Davis, and L.E. Erickson (1995). Fate of volatile chlorinated organic compounds in a laboratory chamber with alfalfa plants. *Environ. Sci. Technol.* 29:2437-2444.

Narayanan, M., N. K. Russell, L. C. Davis and L. E. Erickson (1999). Fate and Transport of Trichloroethylene in a Chamber with Alfalfa Plants. *International Journal of Phytoremediation* 1(4).

Narayanan, M., N.K. Russell, L.C. Davis, and L.E. Erickson (1996). Fate and transport of trichloroethylene in a chamber with alfalfa plants: Experimental and modeling studies. In *Proceedings of the 1996 HSRC/WERC Joint Conference*, Albuquerque, NM. Great Plains/Rocky Mountain Hazardous Substance Research Center.

Nardi, S., F. Reniero, and G. Concheri (1997). Soil organic matter mobilization by root exudates of three maize hybrids. *Chemosphere.* 35:10, 2237-2244.

Nascimento, C. W. A. d. (2006). Organic acids effects on desorption of heavy metals from a contaminated soil. *Scientia Agricola.* C. W. A. d. Nascimento. 63: 276. Phytoremediation of heavy metals is a biotechnology that extracts metals from soils and transfer them to plant. As hyperaccumulator species have demonstrated low potential for commercial phytoextraction, synthetic chelates have been successfully used to induce accumulation of metals by high-biomass plants. However, they pose serious environmental drawbacks regarding excessive amount of metals solubilized. In search for synthetic chelate-alternatives, this paper evaluate the performance of DTPA, EDTA, citric acid, oxalic acid, vanillic acid, and gallic acid in desorbing Cd, Pb, Zn, Cu, and Ni from soil. DTPA and EDTA were highly effective

in desorbing Cd, Pb, Zn, Cu, and Ni from soil. However, the excessively high concentration of metals brought in solution by such chelates limits their application in the field. Citric and oxalic acids desorbed substantial quantities of Zn, Cu, and Ni if applied at 10 or 20 mmol kg⁻¹. At the 20 mmol kg⁻¹ dose, vanillic and gallic acids solubilized significant amounts of Zn, Ni, and Cd from soil. None of the tested low molecular weight organic acids substantially increased the Pb concentration in soil solution.

Nascimento, C. W. A. d. and B. S. Xing (2006). Phytoextraction: a review on enhanced metal availability and plant accumulation. *Scientia Agricola*. 63: 299. Phytoextraction has emerged as a novel approach to clean up metal-polluted soils in which plants are used to transfer toxic metals from soils to shoots. This review provides a synthesis of current knowledge on phytoextraction of metals from soils and their accumulation in plants. The objective is to integrate soil-related (root exudates and chemical amendments) and biological advances to suggest research needs and future directions. As far as can be deduced from the literature, it will be some time before phytoextraction may be established as a commercial technology. For chemically-assisted phytoextraction, research has not shown easily biodegradable compounds to overcome the risks associated with the use of EDTA for poorly available metals in soils. On the other hand, significant progress has been made on the physiological and molecular aspects regarding tolerance and phytoaccumulation of metals in plants. A multidisciplinary approach is warranted to make phytoextraction a feasible commercial technology to remediate metal-polluted soils.

Nascimento, C. W. A. d., D. Amarasiriwardena, et al. (2006). Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. *Environmental Pollution* 140: 114. Chemically assisted phytoremediation has been developing to induce accumulation of metals by high biomass plants. Synthetic chelates have shown high effectiveness to reach such a goal, but they pose serious drawbacks in field application due to the excessive amount of metals solubilized. We compared the performance of synthetic chelates with naturally occurring low molecular weight organic acids (LMWOA) in enhancing phytoextraction of metals by Indian mustard (*Brassica juncea*) from multi-metal contaminated soils. Gallic and citric acids were able to induce removal of Cd, Zn, Cu, and Ni from soil without increasing the leaching risk. Net removal of these metals caused by LMWOA can be as much as synthetic chelates. A major reason for this is the lower phytotoxicity of LMWOA. Furthermore, supplying appropriate mineral nutrients increased biomass and metal removal.

Natarajan, K. and S. Malathy (2005). The role of ectomycorrhizal fungi in bioremediation of heavy metals. *Biodiversity of fungi: their role in human life*. K. Natarajan. Enfield, Science Publishers, Inc.: 251. Heavy metal accumulation in soils, besides fluctuating edaphic factors and soil pH, is an important factor in the deterioration of biodiversity at those sites and has become a global problem, particularly the loss of vegetation leading to barren wasteland. An attempt to revegetate these lands would involve either phytoremediation, wherein plants like *Thlaspi caerulescens* that have a remarkable ability to concentrate elements and compounds from the environment and metabolize various molecules in their tissues are employed, or bioremediation through bioaccumulation by the use of microorganisms that show a high level of tolerance to heavy metals. The effect of both plants and their associated microbes is essentially phytodegradation. Although both these methods are cost-effective and feasible, the former is limited by the fact that very few plants are naturally metal consuming; but in the latter, there are no such limitations. The availability of metal-tolerant fungi found in close association with plants is fairly high. The occurrence of a group of fungi called mycorrhizal fungi that help in enhancing the growth of the plants to which they are associated is quite common and a lot of research has been focused on utilizing this group of organisms. Since mycorrhizal fungi are the only ones among soil microorganisms to provide a direct link between the soil and plant roots, they can be of great importance in ameliorating heavy metal availability and toxicity to plants. Field studies on adverse sites show that reclamation and reforestation of such sites can be expedited by using seedlings tailored with ectomycorrhiza formed by fungi capable of growing under adverse conditions; this implies that the planting of seedlings with root systems, physiologically and ecologically adapted to the adversities of the planting site, can be an important biological tool in land reclamation.

Navari-Izzo, F. and M. F. Quartacci (2001). Phytoremediation of Metals - Tolerance Mechanisms against Oxidative Stress. *Minerva Biotechnologica* 13(2): 73-83.

Navaza, A. P., M. Montes-Bayon, et al. (2006). Study of phytochelatins and other related thiols as complexing biomolecules of As and Cd in wild type and genetically modified *Brassica juncea* plantst. *Journal of Mass Spectrometry* 41(3): 323-331. The accumulation of As and Cd in *Brassica juncea* plants and the formation of complexes of these elements with bioligands such as glutathione and/or phytochelatins (PCs) is studied. The genetic manipulation of these plants to induce higher As and Cd accumulation has been achieved by overexpressing the genes encoding for gamma-glutamyl cysteine synthetase (gamma-ECS) and glutathione synthetase (GS). These two enzymes are responsible for glutathione (GSH) formation in plants, which is the first step in the production of PCs. The biomass produced in both the wild type and the genetically modified plants, has been evaluated. Additionally, the total Cd and As concentration accumulated in the plant tissues was measured by inductively coupled plasma mass spectrometry (ICP-MS) after extraction. Speciation studies on the extracts were conducted using size exclusion liquid chromatography (SEC) coupled online with ICP-MS to monitor As, Cd and S. For further purification of the As fractions, reversed phase high performance liquid chromatography (RP-HPLC) was used. Structural elucidation of the PCs and other thiols, as well as their complexes with As and Cd, was performed by electrospray-quadrupole-time-of-flight (ESI-Q-TOF). In both the Cd and As exposed plants it was possible to observe the presence of oxidized PC2 ([M + H]⁺, m/z 538), GS-PC2(-Glu) ([M + H]⁺, m/z 716) as well as reduced GSH ([M + H]⁺, m/z 308) and oxidized glutathione (GSSG) ([M + H]⁺, m/z 613). However, only the GS plants exhibited the presence of As(GS)₃ complex ([M + H]⁺, m/z 994) that was further confirmed by MS/MS. This species is reported for the first time in *B. juncea* plant tissues. Copyright (c) 2006 John Wiley & Sons, Ltd.

Naylor, S., J. Brisson, et al. (2003). Treatment of freshwater fish farm effluent using constructed wetlands: The role of plants and substrate. *Water Science and Technology* 48(5): 215-222. Freshwater fish farm effluents have low nutrient concentrations but high flow rates, resulting in a pollutant load, especially phosphorus (P), causing eutrophication. The feasibility was tested of a treatment combining, within a single constructed wetland, the contribution of macrophytes for reducing organic matter and nitrogen (N), with the high efficiency of steel slag and limestone for P removal. Twenty subsurface flow (SSF) basins of 280 L with different combinations of plants (*Phragmites communis* or *Typha latifolia*) and substrates (steel slag, limestone, gravel, peat) were fed with a reconstituted fish farm effluent in a greenhouse experiment. Pollutant removal was generally very good under all treatments. N and organic matter removal were correlated with plant biomass while P removal was better in substrates with steel slag and limestone. However, the high pH of the P-adsorbing substrate was detrimental to plant growth so that no combination of plants and substrates could maximise in one step the simultaneous removal of all evaluated pollutants. Therefore, the use of two sequential units is recommended, a first one consisting of a macrophyte planted basin using a neutral substrate to remove organic matter and N, followed by a second unplanted basin containing only a P-adsorbing substrate.

Nazarov, A. V. and S. A. Ilarionov (2005). Potential of the use of microbial-plant interaction for bioremediation. *Biotechnologiya*(5): 54-62. The basic mechanisms of phytoremediation are reviewed. The data on a role of microbial-plant systems in remediation are summarized. The influence of microorganisms on the increase of plant resistance to environmental contamination is analyzed.

Nedelkoska, T. and P. Doran (2000). Characteristics of Heavy Metal Uptake by Plant Species with Potential for Phytoremediation and Phytomining. *Miner. Eng.* 13(5): 549-561.

Nedelkoska, T. and P. Doran (2000). Hyperaccumulation of Cadmium by Hairy Roots of *Thlaspi caerulescens*. *Biotechnol. Bioeng.* 67(5): 607-615.

Nedelkoska, T. V. and P. M. Doran (2001). Hyperaccumulation of Nickel by Hairy Roots of *Alyssum* Species: Comparison with Whole Regenerated Plants. *Biotechnology Progress* 17(4): 752-759.

Nedunuri, K., R. Govindaraju, M. Banks, A. Schwab and Z. Chens (2000). Evaluation of Phytoremediation for Field-Scale Degradation of Total Petroleum Hydrocarbons. *J. Environ. Eng.-ASCE* 126(6): 483-490.

Negri, M. C., E. G. Gatliff, et al. (2003). Root development and rooting at depths. *Phytoremediation: transformation and control of contaminants*. M. C. Negri. Hoboken, Wiley-Interscience: 233. Phytoremediation can often be extended to contamination at depths below the normal rooting zone of 1 m. Root growth at the desired depth is dependent on the spatial availability of water, nutrients, and oxygen and is limited by the mechanical impedance of the soil overburden. Natural rooting at depths as deep as 50 m occurs when plants that are genetically predisposed to root deeply (phreatophytes) do not find sufficient water and nutrient resources in the surface soil to satisfy growth needs. Thus, semiarid climates with porous soil may be the best for phytoremediation of deeper contamination with minimal manipulation or engineering of root growth. In temperate regions, however, where water is easily accessible in surface soil horizons, rooting at depth may never occur spontaneously and increasingly complex rooting manipulation or engineering techniques need to be utilized to achieve root development in the target zone. Case studies conducted at Argonne site in Illinois, Aberdeen Ground site in Maryland and Staten Island in New York (USA) establish that engineered deep rooting is feasible to at least 12 m.

Negri, M. C., R. R. Hinchman, et al. (2003). Salt tolerant plants to concentrate saline waste streams. *Phytoremediation: transformation and control of contaminants*. M. C. Negri. Hoboken, Wiley-Interscience: 753. Produced water is a salty brine generated during the production of oil and gas. Brine disposal is typically achieved by reinjection in deep wells, and the cost is highly dependent on volume. We investigated, evaluated, and developed the principal components of a cost-effective, engineered bioreactor system that will, through natural plant processes of transpiration and salt tolerance, reduce the volume of produced water. After a screening of plant effectiveness at the greenhouse scale, the system was tested at the pilot scale at a gas and oil field in Oklahoma, USA. Saltwater cordgrass (*Spartina alterniflora*) and great bulrush (*Scirpus validus*) were chosen to treat the waste water with chloride levels between 30000 and 60000 milligrams per litre in a two-compartment bioreactor. These tests confirmed greenhouse data and demonstrated volume reductions of 75% in less than 5 days. Compared to evaporation from an open surface, plants achieved 30% more volume reduction in the same time. Cost savings between 36 and 70% are feasible under full field conditions.

Negri, M.C. and Hinchman, R.R. (1996). Plants That Remove Contaminants From the Environment *Laboratory Medicine*. 27, 1, 36-40.

Negri, M.C., R.R. Hinchman, and E.G. Gatliff (1996). Phytoremediation: Using green plants to clean up contaminated soil, groundwater, and wastewater. *International Phytoremediation Conference*, May 8-10, 1996, Arlington, VA. International Business Communications, Southborough, MA.

Nehnevajova, E., R. Herzig, et al. (2005). Screening of sunflower cultivars for metal phytoextraction in a contaminated field prior to mutagenesis. *International Journal of Phytoremediation* 7(4): 337-349. Sunflower can be used for the remediation of metal-contaminated soils. Its high biomass production makes this plant species interesting for phytoextraction and using sunflower oil for a technical purpose may improve the economic balance of phytoremediation. The aim of the present field study was to screen 15 commercial cultivars of *Helianthus annuus* L, grown on metal-contaminated soil, to find out the variety with the highest metal extraction, which can be further improved by mutation or in vitro breeding procedures. Two different fertilizers (ammonium sulphate and ammonium nitrate) were also used to enhance the bioavailability of metals in soil. Highly significant differences were observed within tested varieties for metal accumulation and extraction efficiency. Furthermore, ammonium nitrate increased cadmium extraction, whereas ammonium sulphate enhanced zinc and lead uptake in most tested cultivars. In this field-based sunflower screening, we found enhanced cumulative Cd, Zn, and Pb extraction efficiency by a factor 4.4 for Salut cultivar. We therefore emphasize that prior to any classical breeding or genetic engineering enhancing metal uptake potential, a careful screening of various genotypes should be done to select the cultivar with the naturally highest metal uptake and to start the genetic improvement with the best available plant material.

Neidorf, R. (1996). Phytoremediation: phytechs hungry plants take up toxic metals. *EI Digest Industrial and hazardous Waste management*. April 1996.

Neitch, C. T., J. T. Morris and D. A. Vrblosky (1999). Biophysical Mechanisms of Trichloroethene Uptake and Loss in Baldcypress Growing in Shallow Contaminated Groundwater. *Environ. Sci. Technol.* 33(17): 2899-2904.

Nelancon, P., G. Harvey, C. Giammona, and D. McMIndes (1997). Phytoremediation at military installations. The 4th International Petroleum Environmental Conference; Environmental Issues and Solutions in Exploration, Production, and Refining, September 9-12, 1997, San Antonio, TX.

Nellessen, J.E. and J.S. Fletcher (1992). A computer database on residues of xenobiotic organic chemicals and heavy metals in plants. *J. Chem. Inf. Comput. Sci.* 32, 144-148.

Nellessen, J.E., and J.S. Fletcher (1993). Assessment of published literature on the uptake, accumulation, and translocation of heavy metals by vascular plants. *Chemosphere.* 27:1669-1680.

Nellessen, J.E., and J.S. Fletcher (1993). Assessment of published literature on the uptake/accumulation, translocation, adhesion and biotransformation of organic chemicals by vascular plants. *Environ. Toxicol. Chem.* 12:2045-2052.

Nelson, S. (1996). Use of trees for hydraulic control of groundwater plumes. In W.W. Kovalick and R. Olexsey (eds.), Workshop on Phytoremediation of Organic Wastes, December 17-19, 1996, Ft. Worth, TX. A USEPA unpublished meeting summary.

Nemeth, T. and I. Kadar (2005). Leaching of microelement contaminants: a long-term field study. *Zeitschrift fur Naturforschung. Section C, Biosciences.* T. Nemeth. 60: 260. A field experiment with microelement loads was set up on loamy textured, calcareous chernozem soil formed on loess. The ploughed layer contained ca. 5% CaCO₃ and 3% humus. The soil was well supplied with Ca, Mg, Mn and Cu, moderately supplied with N and K, and weakly supplied with P and Zn. The water table is at the depth of 15 m, the water balance of the area is negative, and the site is drought sensitive. Salts of the 13 examined microelements were applied at 4 doses in the spring of 1991. Treatments were arranged in a split-plot design, in a total of 104 plots with two replications. Loading rates were 0, 90, 270 and 810 kg/ha per element in the form of AlCl₃, NaAsO₂, BaCl₂, CdSO₄, K₂CrO₄, CuSO₄, HgCl₂, (NH₄)₆Mo₇O₂₄, NiSO₄, Pb(NO₃)₂, Na₂SeO₃, SrSO₄, ZnSO₄. Soil profiles of the control and the 810 kg/ha treated plots were sampled in the 3rd, 6th and 10th year of the trial. The mixed samples, consisting of 5 cores/plot were taken in 30 cm steps to 60 cm (year 1993), 90 cm (year 1996), and 290 cm (year 2000). Ammonium acetate+EDTA-soluble element content was determined. The main conclusions of the study are: 1. In soils contaminated with 810 kg/ha load rates As, Hg, Ni, Cu, Pb, Ba and Sr displayed no significant vertical movement. There is little uptake and translocation in plants of these elements: their concentration in the above-ground plant parts usually remains below 5-10 mg/kg dry mass with the exception of Ba and Sr showing a somewhat higher accumulation. Under our conditions the above elements did not behave as dangerous contaminants to the soil, groundwater, or plants. 2. Moderate leaching of Zn and Cd was detected when applied at higher doses. Their accumulation was also moderate in the above-ground plants parts. Zn is not a dangerous pollutant for soil, plants or groundwater at our site. Cd, however, is a very dangerous element because of its high toxicity to mammals, soil life and crops. 3. Cr, Se, and Mo (in the form of chromate, selenate and molybdenate anions) exhibited great mobility in the soil and partly in the soil-plant system. Cr was hardly detectable in the above-ground parts of plants. Still, its rapid leaching can jeopardize groundwater quality. Se was strongly accumulated in all plant organs and was highly toxic for all crops. Mo, too, showed 2-3 orders of magnitude greater accumulation in plant parts, resulting in products unfit for animal or human consumption. Under our experimental conditions Cr(VI), Se, Mo can be classified as dangerous contaminants, since the anion forms remained stable for a long time in this well-aerated calcareous environment.

Nepovim, A., M. Hubalek, et al. (2004). In-vitro degradation of 2,4,6-trinitrotoluene using plant tissue cultures of *Solanum aviculare* and *Rheum palmatum*. *Engineering in Life Sciences* 4(1): 46-49. The degradation of TNT was tested in suspension cultures (*Rheum palmatum* and *Solanum aviculare*) and

was followed by the identification of degradation products and the determination of the phytotoxicity of TNT to both cultures. The concentration of TNT inhibited the growth of cell cultures by 50 %, i.e., 37.8 mg/ and 38.1 mg/L for *Rheum palmatum* and *Solanum aviculare*, respectively. The TNT uptake was studied by determining the concentration of TNT and its degradation products, such as aminodinitrotoluenes and diamionitrotoluenes, in the cultivation medium as well as in plant cells. The kinetics of the degradation showed that TNT was mostly taken up within 10 hours and 6 hours for *S. aviculare* and *R. palmatum*, respectively. Aminodinitrotoluenes were preferentially produced by cultures of *S. aviculare*, whereas diamionitrotoluenes and aminodinitrotoluenes were revealed in cultures of *R. palmatum*. The final concentrations of identified degradation products did not stoichiometrically correspond to the decreased concentration of TNT in the medium. The different concentrations of degradation products in each culture were an indication that the metabolism of TNT is controlled by different enzymatic systems. Therefore, it was concluded that studying different species for TNT degradation is necessary for the search of most suitable candidates for TNT phytoremediation.

Neralla, S. and R. Weaver (2000). Phytoremediation of Domestic Wastewater for Reducing Populations of *Escherichia Coli* and Ms-2 Coliphage. *Environ. Technol.* 21(6): 691-698.

Neralla, S., R. Weaver, T. Varvel and B. Lesikar (1999). Phytoremediation and on-Site Treatment of Septic Effluents in Sub-Surface Flow Constructed Wetlands. *Environ. Technol.* 20(11): 1139-1146.

Neubauer, U., G. Furrer, A. Kayser and R. Schulin (2001). Siderophores, Nta, and Citrate: Potential Soil Amendments to Enhance Heavy Metal Mobility in Phytoremediation. *International Journal of Phytoremediation* 2(2).

Neuman, D.R., F.F. Munshower, D. Dollhopf, S. Jennings, and J. Goering (1994). Revegetation of streambank tailings, Silver Bow Creek, Montana. P. 150. In L.E. Erickson, D.L. Tillison, S.C. Grant, and J.P. McDonald (eds.), *Proceedings of the 9th Annual Conference on Hazardous Waste Remediation*, June 8-10, 1994, Bozeman, MT.

Neuman, D.R., F.F. Munshower, S.R. Jennings, and D.J. Dollhopf (1997). Phytostabilization of copper smelter tailings near Anaconda, Montana. Presentation 33. In *12th Annual Conference on Hazardous Waste Research - Abstracts Book*, May 19-22, 1997, Kansas City, MO. A phytostabilization treatability investigation was conducted at the Anaconda Smelter Superfund Site in southwestern Montana. Results of this metals immobilization/revegetation study will help define the alternatives for the cleanup of this area impacted by nearly 100 years of copper smelting. Laboratory tests to assess ameliorative effects of several chemical and biological amendments and greenhouse trials to select tolerant plant species were conducted prior to construction of field demonstration plots at five different areas impacted by the smelting processes. Two of these experimental plots were located on the acid producing, low pH smelter tailings known as the Opportunity Ponds and the Anaconda Ponds. Combinations of lime products (CaCO₃ and Ca(OH)₂), composted organic matter, and smelter slag were incorporated into the tailings to a depth of approximately 20 inches (50 cm) using specialized equipment. Selected grasses and legumes were seeded in the spring of 1995. In addition, shrub and tree seedlings were planted. Monitoring consisted of measurements of root zone hydrology, amended tailings chemical environment, erosion, and vegetation response. Measurements of vegetation cover as well as shrub and tree mortality were recorded. Measurements of tailings acidity and water soluble metal and arsenic levels both on and off the experimental plots have been determined. Instruments to measure water content and movement within the root zone were installed. Simulated rainfall/runoff tests defined the quality and quantity of surface waters affected by the phytostabilization of these tailings.

Neunhauserer, C., M. Berreck and H. Insam (2001). Remediation of Soils Contaminated with Molybdenum Using Soil Amendments and Phytoremediation. *Water Air & Soil Pollution* 128(1-2): 85-96.

Newman, L. A. and C. M. Reynolds (2004). Phytodegradation of organic compounds. *Current Opinion in Biotechnology* 15(3): 225-230. The phytodegradation of organic compounds can take place inside the

plant or within the rhizosphere of the plant. Many different compounds and classes of compounds can be removed from the environment by this method, including solvents in groundwater, petroleum and aromatic compounds in soils, and volatile compounds in the air. Although still a relatively new area of research, there are many laboratories studying the underlying science necessary for a wide range of applications for plant-based remediation of organic contaminants.

Newman, L. A. and C. M. Reynolds (2005). Bacteria and phytoremediation: new uses for endophytic bacteria in plants. *Trends in Biotechnology* 23(1): 6-8. The use of plants and bacterial to clean up environmental pollutants has gained momentum in past years. A limitation to phytoremediation of solvents has been toxicity of the compounds to plants, and the uncertainty as to the fate of many of the compounds. In a recent study, engineered endophytes have been shown to increase plant tolerance to toluene, and to decrease the transpiration of toluene to the atmosphere. This type of work has the potential to increase the use of phytoremediation by decreasing toxicity and increasing degradation of toxins.

Newman, L. A., X. P. Wang, et al. (1999). Remediation of Trichloroethylene in an Artificial Aquifer with Trees: A Controlled Field Study. *Environ. Sci. Technol.* 33(13): 1157-2265.

Newman, L., A. Wilson, S. Strand, P. Heilman and M. Gordon (2000). Phytoremediation of Methyl-T-Butyl Ether (Mtbe). *Abstr. Pap. Am. Chem. Soc.* 219: 232-ENVR.

Newman, L., C. M. Reynolds, et al. (2004). Separating RDX phytodegradation from photodegradation. *Proceedings of the First Sustainable Range Management Conference, New Orleans, USA, 5-8 January 2004.* L. Newman. Columbus, Battelle Press: 3.

Newman, L., S. Doty, et al. (1998). Phytoremediation of Organic Contaminants: A Review of Phytoremediation Research at the University of Washington. *J. Soil Contam.* 7(4): 531-542.

Newman, L.A. and C.M. Reynolds. (2005). Phytoremediation and Bacteria: New Uses of Endophytic Bacteria in Plants. *Trends in Biotechnology*, 23: 6-8.

Newman, L.A., C. Bod, N. Choe, R. Crampton, R. Cortellucci, D. Domroes, S. Doty, J. Duffy, G. Ekuan, D. Fogel, R. Hashmonay, P. Heilman, D. Martin, I.A. Muiznieks, T. Newman, M. Ruszaj, T. Shang, B. Shurtleff, S. Stanley, S.E. Strand, X. Wang, J. Wilmoth, M. Yost, and M.P Gordon (1997). Abstract: Phytoremediation of trichloroethylene and carbon tetrachloride: Results from bench to field. Presentation 55. In 12th Annual Conference on Hazardous Waste Research - Abstracts Book, May 19-22, 1997, Kansas City, MO. Remediation of contaminated sites using plants, or phytoremediation, is one of the most promising new technologies for remediation. As with any new technology, solid data concerning the efficacy of this needs to be produced before commercial groups are willing to implement the technology. This work shows that axenic poplar cell cultures produced from hybrid poplar H-11-11 (*Populus trichocarpa* x *P. deltoides*) are capable of independently oxidizing trichloro-ethylene (TCE) to expected metabolites. It also demonstrates that young rooted cuttings, when placed in metabolic chambers, are capable of taking up and transpiring both TCE and carbon tetrachloride (CT). These tests were then stepped up to a pilot scale remediation project that allows for the testing of multiple species of trees and contaminants under field conditions closely simulating what would be seen on a contaminated site. Two different trees are currently tested on the site, H-11-11 and Black Locust sp. The hybrid poplars were exposed to TCE for two growing seasons, and to CT for one growing season. After two years exposure to TCE, the data shows that hybrid poplars were able to remove over 97% of the TCE from the water stream. Additionally, at dose concentrations up to 50ppm, there is no apparent affect on the growth of the trees. Tissue analysis shows only low levels of accumulation of TCE metabolism intermediates. At the end of the one-year exposure to CT, the trees were capable of removing 95% of CT from the water stream. However, there was a higher susceptibility to the toxic effects of CT. Measurements of transpiration levels of both compounds are much lower than seen in the bench scale studies. Black locusts have been exposed to TCE for one growing season, and these studies will continue to better determine their field

applicability. Continued use of bench and pilot scale facilities will allow the testing of different species of plants challenged with a wide range of chemicals.

Newman, L.A., R. Cortellucci, R.S. Crampton, D. Domroes, J. Duffy, G. Ekuan, M.P. Gordon, R.A. Hashmonay, P. Heilman, G. Karscig, I.A. Muiznieks, T. Newman, M. Ruszaj, X. Wang, M.G. Yost and S.E. Strand. (1999). Remediation of Trichloroethylene in an Artificial Aquifer with Trees: A Controlled Field Study. *Environmental Science and Technology*. 33:2257-2265.

Newman, L.A., S.E. Strand, D. Domroes, J. Duffy, G. Ekuan, G. Karscig, I.A. Muiznieks, M. Ruszaj, P. Heilman, and M.P. Gordon (1997). Removal of trichloroethylene from a simulated aquifer using poplar. Fourth International In Situ and On-Site Bioremediation Symposium, April 28 - May 1, 1997, New Orleans, LA. 3:321.

Newman, L.A., S.E. Strand, N. Choe, J. Duffy, G. Ekuan, M. Ruszaj, B.B. Shurtleff, J. Wilmoth, P. Heilman and M.P. Gordon (1997). Uptake and biotransformation of Trichloroethylene by hybrid poplars. *Environ. Sci. Technol.* 31:1062-1067. Poplar trees were found to be capable of taking up trichloroethylene (TCE) and degrading it to several known metabolic products: trichloroethanol, trichloroacetic acid, and dichloroacetic acid. Poplars were also shown to transpire TCE in measurable amounts. To eliminate the possibility that the degradation we observed was produced solely by rhizosphere organisms, axenic poplar tumor cell cultures were tested; the cultures produced the same intermediate metabolic products. When dosed with [¹⁴C]TCE, cell cultures also produced low levels of radiolabeled carbon dioxide and a labeled insoluble residue. These results show that significant TCE uptake and biotransformation occurs in poplar, which demonstrates the potential for the use of poplars for in situ remediation of TCE.

Newman, L.A., S.L. Doty, K.L. Gery, P.E. Heilman, I. Muiznieks, T.Q. Shang, S.T. Siemieniec, S.E. Strand, X. Wang, A.M. Wilson and M.P. Gordon. 1998. Soil Contamination Phytoremediation of Organic Contaminants: A Review of Phytoremediation Research at the University of Washington. *Journal of Soil Contamination*. 7:531-542.

Newman, L.A., S.L. Doty, K.L. Gery, P.E. Heilman, I. Muiznieks, T.Q. Shang, S.T. Siemieniec, S.E. Strand, X. Wang, A.M. Wilson, and M.P. Gordon (1998). Phytoremediation of organic contaminants: A review of phytoremediation research at the University of Washington. *Journal of Soil Contamination*. 7 (4): 531-542, July 1998.

Newman, L.A., S.L. Doty, S.E. Strand, P.E. Heilman, and M.P. Gordon (1998). An overview of phytoremediation of both halogenated and non-halogenated organic compounds. 14th Annual Conference on Contaminated Soils. October 1998. University of Massachusetts at Amherst, Amherst, MA.

Ney, R.E., Jr. (1995). Fate and transport of organic chemicals. Government Institutes, Washington, DC. 2nd ed. pp. 43-44.

Ni, C. Y., J. Y. Shi, et al. (2004). Co-culture engineering for enhanced phytoremediation of metal contaminated soils. *Pedosphere* 14(4): 475-482. A co-culture of two plant materials, *Astragalus sinicus* L., a leguminous plant with concomitant nodules, and *Elsholtzia splendens* Naki-a Cu accumulator, along with treatments of a chelating agent (EDTA), root excretions (citric acid), and a control with *E. splendens* only were used to compare the mobility of heavy metals in chelating agents with a co-culture and to determine the potential for co-culture phytoremediation in heavy metal contaminated soils. The root uptake for Cu, Zn, and Pb in all treatments was significantly greater ($P < 0.05$) than that of the control treatment. However with translocation in the shoots, only Cu, Zn, and Pb in plants grown with the EDTA treatment and Zn in plants co-cropped with the *A. sinicus* treatment increased significantly ($P < 0.05$). In addition, when a co-culture in soils with heavy and moderate contamination was compared, for roots in moderately contaminated soils only Zn concentration was significantly less ($P < 0.05$) than that of heavily contaminated soils, however, Cu, Zn, and Pb concentrations of shoots were all significantly lower ($P < 0.05$). Overall, this co-culture engineering could be as effective as or even more effective than chelating

agents, thereby preventing plant metal toxicity and metal leaching in soils as was usually observed in chelate-enhanced phytoremediation.

Ni, W. Z., Q. Sun, et al. (2004). Growth and zinc accumulation of *Sedum alfredii* Hance -- a Zn hyperaccumulator as affected by phosphorus application. *Bulletin of Environmental Contamination and Toxicology*. 72: 756. A pot experiment under greenhouse condition was conducted to investigate the impact of phosphorus (P) application on the growth of *Sedum alfredii*, and to identify Zn accumulation response of the species to P fertilizer application. Stems of *S. alfredii* were collected from their naturally growing areas in an old mining soil in Quzhou City, Zhejiang, China. The treatments were 0 (CK), 31, 62, 124, and 248 mg P/kg soil (added as NaH₂PO₄). The supplied doses of N (in urea) and K (in KCl) were equal for all treatments in the pot experiment: 56 mg N/kg soil and 39 mg K/kg soil applied as basal fertilizer application. Results showed that shoot dry weight of *S. alfredii* were significantly affected by P application, with the highest growth occurring at 31 mg P/kg soil supplied. Shoot biomass was significantly higher for P application (except the 248 mg P/kg treatment) than for no P application. Zinc concentration in shoots of *S. alfredii* were also significantly affected by P application, with the highest concentration occurring at 31 mg P/kg supplied. These results indicated that P application could significantly increase Zn concentrations in shoots of *S. alfredii* at appropriate doses of P supply, and excessive P application could significantly reduce Zn concentrations. Phosphorus application can enhance the growth of *S. alfredii* (a Zn-accumulator), and increase Zn concentrations and Zn accumulation in shoots. The appropriate P concentration in shoots of the test plant is approximately 2.0 mg/g DW, which can be used as an index for P application in practice.

Ni, W.-Z., X.-e. Yang, et al. (2004). Comparative studies on zinc tolerance and accumulation between two ecotypes of *Sedum alfredii* hance in southeastern China. *Journal of Plant Nutrition* 27(4): 627-635. Phytoremediation is becoming an established technology for environmental clean-up and protection. Hyperaccumulator plants are potential tools to remove excess metals from contaminated soils. As an efficient operational routine technology, phytoextraction requires a better knowledge of tolerance and accumulation for excess metals by particular plants. Based on the vegetation survey, a new zinc (Zn)accumulating ecotype of *Sedum alfredii* Hance naturally-growing in an old Zn/lead (Pb) mining region in Quzhou City (29degree17' N, 118degree56' E) and a non-Zn-accumulating ecotype of *Sedum alfredii* Hance in the suburb of Hangzhou City (30degree06' N, 120degree12' E), Zhejiang province were found. To identify Zn tolerance and accumulation characteristics of the two ecotypes of *Sedum alfredii* Hance, a solution culture experiment was carried out with four treatments: 0.0325 (CK), 20, 80, 160 mg Zn L⁻¹ in the basic nutrient solution. The biomass of root, stem and leaf for the ecotype of *Sedum alfredii* from Zn/Pb mining area, as well as of leaf of non-Zn-accumulating ecotype of *Sedum alfredii*, expressed as dry weight, were not significantly different among the treatments after 12 days. However, the biomass of root and stem for non-Zn-accumulating ecotype of *Sedum alfredii* in treatments with 80 and 160 mg Zn L⁻¹ were significantly lower than that of the control treatment (CK). The results indicated that Zn concentrations in leaves, stems and roots of the test plants increased significantly with increasing Zn concentration in the nutrient solution. Zn concentrations in leaves and stems of the ecotype from Zn/Pb mining area were 25-34 and 7-48 fold higher than those in leaves and stems of the non-Zn-accumulating ecotype. The amounts of Zn accumulated in shoots (leaves and stem) of the ecotype from Zn/Pb mining area in 20, 80 and 160 mg Zn L⁻¹ treatments were significantly larger than those of the non-Zn-accumulating ecotype. As the Zn concentrations on the dry weight in leaves and stems of the ecotype from Zn/Pb mining area were 16,740 and 19,567 mg kg⁻¹ in the 80 mg Zn L⁻¹ treatment, this ecotype can be regarded as a Zn hyperaccumulator.

Nichols, T.D., D.C. Wolf, H.B. Rogers, C.A. Beyrouy, and C.M. Reynolds. Rhizosphere microbial populations in contaminated soils. *Water Air Soil Pollut.* 95(1):165-178.

Nicholson, S.A., and N.M. Safaya (1993). Restoring hazardous waste sites. *Environ. Sci. Technol.* 27:1022-1025.

Nicks, L.J., and M.F. Chambers (1995). Farming for metals? *Mining Environ. Manage.* 3(3):15-18.

Nie, J., X. Liu, et al. (2004). Influence of N, P, K nutrient levels on Pb uptake ability of hyperaccumulators. Transactions of the Chinese Society of Agricultural Engineering. J. Nie. 20: 262. In the greenhouse experiment, 3 varieties of hypertolerant plants: L. *Amaranthus tricolor* [*Amaranthus tricolor*], *Sophora japonica* [*Styphnolobium japonicum*] and *Bidens maximowicziana* were grown at different levels of nitrogen (0, 0.06, 0.12, 0.18, 0.24, 0.30 g/kg), phosphorous (0, 0.06, 0.12, 0.18, 0.24, 0.30 g/kg), and potassium (0, 0.08, 0.16, 0.24, 0.32, 0.40 g/kg) to study their performance with regard to lead uptake ability, while the other two-element levels were 0.12 g/kg and soil lead was 800 tonnes/kg. The results showed that N, P, K level had significant effect on plant total lead translocation. The ratio of maximum to minimum value for *B. maximowicziana*, *A. tricolor* and *Sophora japonica* were 11.6, 10.6, 11.9, respectively. But the influence of three elements were not uniform. A little amount of nitrogen and potassium can increase the dry weights of plant, advance the lead uptake of hyperaccumulators, but with the increasing of nitrogen and potassium concentration, the lead uptake of the plants dropped, and dry weights decrease constantly. The little different effect between nitrogen and potassium on lead uptake was that the restraint of the former was more significant than the latter. Additionally, the phosphorus applied to the soil can decrease the lead uptake of the hyperaccumulators dramatically. It is concluded that if the plants are used on the purpose of phytoremediating the lead polluted soil, the total lead translocation can be increased by regulating the soil N, P, K level.

Nie, J., X. Liu, et al. (2004). Screening out of Pb hypertolerant plant species. Transactions of the Chinese Society of Agricultural Engineering. J. Nie. 20: 255. A greenhouse experiment was conducted to select hypertolerant plants to heavy metals for the phytoremediation of heavy metal-polluted soils. Thirty-six species growing on Pb and Zn tail mining areas have been chosen for the study. The treatment concentration of Pb was 400 mg litre⁻¹. Three indexes, which are chlorophyll content of plant leaves, the plant height and the content of Pb in plants, were used as standards to choose Pb-tolerant plant species. Six plant species, i.e. *Vetiveria zizanioides*, *Schizonepeta tenuifolia*, *Amaranthus tricolor*, *Xanthium sibiricum*, *Sophora japonica* [*Styphnolobium japonicum*] and *Bidens maximowicziana*, met the standards for chlorophyll content, plant height and lead content, and they could be used for further testing in the phytoremediation of soils polluted with heavy metals.

Nie, L., S. Shah, et al. (2002). Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiology and Biochemistry 40(4): 355-361. It was previously observed that transgenic tomato plants that express the *Enterobacter cloacae* UW4 1-aminocyclopropane-1-carboxylate (ACC) deaminase (EC 4.1.99.4) gene, and thereby produce lower levels of ethylene, were partially protected from the deleterious effects of six different metals. However, since tomato plants are unlikely to be utilized in the phytoremediation of contaminated terrestrial sites, transgenic canola (*Brassica napus*) plants that constitutively express the same gene were generated and tested for their ability to proliferate in the presence of high levels of arsenate in the soil and to accumulate it in plant tissues. The ability of the plant growth-promoting bacterium *E. cloacae* CAL2 to facilitate the growth of both non-transformed and ACC deaminase-expressing canola plants was also tested. In the presence of arsenate, in both the presence and absence of the added plant growth-promoting bacterium, transgenic canola plants grew to a significantly greater extent than non-transformed canola plants. (C) 2002 Editions scientifiques et médicales Elsevier SAS. All rights reserved.

Nielsen, H. (1996). In Situ and Laboratory Determined First-Order Degradation Rate Constants of Specific Organic Compounds in an Aerobic Aquifer. Environmental Science and Technology 30(1): 31-37.

Nisbet, A.F. (1993). Effect of soil-based countermeasures on solid-liquid equilibria in agricultural soils contaminated with radiocesium and radiostrontium. Sci. Total Environ. 137:99-119.

Nishiyama, Y., J. Yanai, et al. (2005). Potential of *Thlaspi caerulescens* for cadmium phytoremediation: comparison of two representative soil types in Japan under different planting frequencies. Soil Science and Plant Nutrition. Y. Nishiyama. 51: 827. To assess the potential of the Cd hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) for Cd phytoremediation in Japan, we compared the changes in the soil Cd concentration between a Fluvisol and an Andosol and the efficiency of Cd removal under different planting frequencies in a pot experiment. The soils were artificially contaminated with Cd(NO₃)₂ to the

level of about 5 mg Cd kg⁻¹. The cultivation system of *Thlaspi caerulescens* applied to both soil types continued for 4 months (4 x 1 treatment) and the other system only applied to the Fluvisol consisted of two plantings, at the beginning and after 2 months (2 x 2 treatment). Control without plants was also prepared. The cumulative amount of Cd absorbed by shoots for 4 months was 385 microg pot⁻¹ for the 4 x 1 treatment of the Fluvisol, 582 microg pot⁻¹ for the 2 x 2 treatment of the Fluvisol, and 112 microg pot⁻¹ for the Andosol. In the Fluvisol, *Thlaspi caerulescens* plants in the 2 x 2 treatment could remove 1.5 times more Cd than in the 4 x 1 treatment. A much higher Cd concentration in the shoots of the second cropping in the 2 x 2 treatment mainly led to this difference in efficiency. The total Cd concentration of the unplanted Fluvisol and Andosol was 5.0 and 6.1 mg kg⁻¹, and after 4 months, that of the Fluvisol in the 4 x 1 and 2 x 2 treatments and the Andosol reached 4.0, 2.9 and 5.1 mg kg⁻¹, respectively. The difference between the rhizosphere and the non-rhizosphere soils was not evident in the Fluvisol, while the concentration in the rhizosphere was lower than that in the non-rhizosphere soil in the Andosol. In the Fluvisol, the amount of available (1 M NH₄NO₃-extractable) Cd decreased only in the rhizosphere, while in the Andosol, it was halved in both rhizosphere and non-rhizosphere soils. Furthermore, the ratio of the amount of available Cd to total Cd did not decrease in the Fluvisol, whereas it decreased in the Andosol, which indicated the presence of a smaller pool of potentially available Cd in the Andosol. Based on the shoot biomass and bioaccumulation factor obtained in the 4 x 1 treatment, it was estimated that only about 2 crops for the Fluvisol and 6 crops for the Andosol could decrease the Cd concentration to 50%. In conclusion, (1) short-rotation is recommended because of the high Cd concentration in the shoots of the second cropping and the increase in the volume of the rhizosphere soil, (2) the amount of available Cd in soil should be monitored in addition to the concentration of total Cd to evaluate the effectiveness of phytoremediation, and (3) the potential of *Thlaspi caerulescens* was clearly demonstrated and the use of this hyperaccumulator could become a promising alternative for the remediation of Cd-contaminated soils in Japan.

Nishizono, H., S. Sukui, and F. Ishii (1987). Accumulation of heavy metals in the metal tolerant fern, *Athyrium yokoscense*, growing on various environments. *Plant Soil*. 102:65-70.

Nixon, W.B., R.J. Murphy, and R.I. Stessel (1997). An empirical approach to the performance assessment of solid waste landfills. *Waste Management and Research*. 15, 607-626.

Nogales, R. and E. Benitez (2006). Absorption of zinc and lead by *Dittrichia viscosa* grown in a contaminated soil amended with olive-derived wastes. *Bulletin of Environmental Contamination and Toxicology* 76(3): 538-544. This study was conducted in Spain to determine, in an artificially contaminated soil (Typic Calcixeroll), the effect of the application of 3 olive-derived wastes (biphasic olive pomace, and compost and vermicompost from biphasic olive pomace) on the growth and concentration of Zn and Pb in *Dittrichia viscosa* [*Inula viscosa*], as well as on the extractability of these metals in the soil after harvesting. The availability of Zn and Pb in the artificially contaminated soil was significantly reduced after the application of compost or vermicompost from biphasic olive pomace. In addition, the olive derived wastes augmented the amounts of humic acids and nitrogen in the soil, thereby implying improved fertility and quality of the artificially contaminated soil. As a consequence, the aerial biomass of the shrub *D. viscosa* was significantly improved, maintaining, in addition, a high capacity to accumulate Zn and Pb in the roots. Therefore, the combined use of biostabilized olive-derived wastes and *D. viscosa* could constitute an alternative and appropriate tool in phytostabilization processes in soils contaminated by heavy metals, especially in the Mediterranean area, where the shrub is distributed widely and olive waste is produced in huge quantities.

Norina, E., O. Norina, et al. (2004). Influence of cereal plants on microorganisms of leached chernozem polluted by hydrocarbons. In situ and on-site bioremediation – 2003 . Proceedings of the Seventh International In Situ and On-Site Bioremediation Symposium, Orlando, Florida, USA, 2-5 June, 2003. E. Norina. Columbus, Battelle Press: 03. The application of plants for remediation of soils contaminated with petroleum hydrocarbons is one of the promising cost and environment effective approach. Microbiocenosis of planted with maize and oat and unplanted leached chernozem artificially polluted with methyl-naphthalene and tetradecane was studied. The positive rhizosphere effect of both plants on microbiocenosis of the only contaminated soil in comparison with uncontaminated planted soil was

detected. The maize has provided a more stimulating influence on the microbial community of polluted leached chernozem in comparison with the oat. The quantity and quality changes in microbiocenosis of polluted chernozem under plants and in unplanted soil were similar. As to the oat, an increasing of tetradecan and 1-methylnaphthalene destructors in root zone soil were detected even in the absence of the pollution.

Nouairi, I., W. Ben Ammar, et al. (2006). Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. *Plant Science (Oxford)* 170(3): 511-519. The term phytoremediation is used to describe the clean-up of heavy metals from contaminated soils by plants. In this study, we examined cadmium (Cd) accumulation by *Brassica napus* in comparison with the known Cd-hyperaccumulator *Brassica juncea*. in a hydroponic medium. Cd treatment was applied as a concentration series between 0 and 50 μ M for 15 days. Most of the Cd taken up was retained in roots in both species, however, *B. juncea* accumulated more Cd in the shoots compared to *B. napus*. Excess metal supply resulted in an increase in the lipid content of *B. juncea* leaves grown under cadmium treatment, but did not affect fatty acids composition. In contrast, an alteration in the lipid composition of *B. napus* leaves was observed together with a decrease in the lipid contents. The amounts of chloroplastic lipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfolipids (SL) and phosphatidylglycerol (PG) decreased drastically under the effect of metallic treatments. Whereas, amounts of extrachloroplastic lipids: phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were significantly increased. The latter finding suggested that PC and PE synthesis was enhanced by metal application. Moreover, the levels of polyunsaturated fatty acids mainly linolenic acid (C18:3) and hexadecatrienoic acid (C16:3) and that of trans palmitoleic acid (C16:1t) from PG declined. So, cadmium seems to affect preferentially chloroplastic lipids containing higher levels of polyunsaturated fatty acids. Lipid changes in *B. juncea*, the well known Cd-hyperaccumulator specie, revealed a more stability of its cellular membranes to cadmium-stress as compared to Cd-sensitive specie, *B. napus*. (c) 2005 Elsevier Ireland Ltd. All rights reserved.

Novak, J. and S. Trapp (2005). Growth of plants on TBT-contaminated harbour sludge and effect on TBT removal. *Environmental Science and Pollution Research* 12(6): 332-341. Background. Worldwide, large amounts of sediments have to be dredged annually from waterways and harbours. These sediments are sometimes polluted with a variety of toxic compounds. In some countries, including Belgium, the load with the biocide tributyltin (TBT) from ship coatings prohibits the dumping of harbour sludge into the sea. Land-based dumping is a commonly used alternative. Objective. This research investigated the feasibility to use land-deposited harbour sludge for plant production. In a field trial, the growth of 38 more or less salt-tolerant plant species on low and high TBT-contaminated sediments was studied. The elimination of TBT from sludge with and without vegetation was compared. The uptake of TBT and its degradation products di- and monobutyltin (DBT and MBT) into harvest products under field conditions was determined. Experimental Set-up. Sediments dredged in May 2003 from the brackish waters of the port of Antwerp were analysed in the laboratory for soil texture, pH, electroconductivity, sodium, magnesium, potassium, calcium, ammonium, nitrate, total nitrogen, chloride, sulphur and the organotins TBT, DBT and MBT. The sediments were lagooned for one year to dewater, desalinate and improve their structure. Salt-tolerant domestic and wild plants were selected and sown in May 2004. In August 2004, plants were harvested and the produced biomass was determined. Samples were taken from vegetated and non-vegetated top and bottom sediments and from plants growing above soil and analysed for TBT, DBT and MBT. Results. The fresh sediments showed a good supply with nutrients and a neutral pH, but were rather saline (EC 14 mS cm⁻¹) of the saturated paste extract). The salinity decreased to 3.7 mS cm⁻¹ during lagoonation. The high and the low contaminated sediment had initially 43 and 1.6 mg TBT kg⁻¹ dry weight, respectively. Besides TBT, several other contaminants were present in the sediments at critical levels. The biomass production of the plant species from the field trial ranged from 0.2 to 13 tons dry mass per hectare. Plants performing excellently were barley, *Sorghum*, rape seed, a clover/grass mix and reed. If at all, a positive influence of TBT on plant growth was seen. TBT was degraded significantly faster (>40%) below barley. The uptake of TBT, DBT and MBT into stem and leaves of reed, grass and clover was very low, but measurable and not related to concentrations in soil. NO uptake of TBT or its metabolites into corn of barley was found. Discussion. This study confirmed former results: the toxicity of TBT to higher plants is low, and even high levels in soils would not be a hindrance for crop production.

The removal of TBT seemed to be increased by both lagooning and plant growth, although the target values for sea dumping in use in certain European countries were not reached. A plausible explanation for the faster degradation of TBT under vegetation is that oxygen is a limiting factor, and plants dewater the soil, thus aerating it. The uptake of the organotin TBT, DBT and MBT into harvest products is probably due to attached soil particles. Conclusions. To summarize, barley was the optimal species: it grew very well despite the salinity of the dredged sediments, it had a significantly positive effect on TBT removal; it showed no measurable uptake of TBT or the other butyltins into the harvested product; and it is a cash crop well established in European agriculture. Outlook. The amounts of dredged sediments are high, and good soils are becoming increasingly rare. The feasibility of using dredged sediments for non-food production, such as energy crops, should be investigated by a critical risk assessment.

Novak, J. and Z. Al-Ghazzawi (1997). Plant-Enhanced Remediation of Petroleum Contaminated Soil. Mid-Atlantic Industrial Waste Conference, Technomic Publ Co Inc.

Novak, J. M., D. W. Watts, et al. (2004). Copper and zinc accumulation, profile distribution, and crop removal in coastal plain soils receiving long-term, intensive applications of swine manure. Transactions of the ASAE. J. M. Novak. 47: 1513. Trace metals like copper (Cu) and zinc (Zn) are added to animal feed as dietary supplements, and trace metals not assimilated by the animals gut are excreted in manure. The accumulation and movement of Cu and Zn in soils has not been as well documented when compared to nitrogen (N) and phosphorus (P). In cases where plant-available Cu and Zn concentrations accumulate to phytotoxic levels, yield may decline for sensitive crops. We investigated the accumulation of Cu and Zn in the topsoil (0 to 15 cm) and the soil profile (0 to 183 cm deep) and estimated uptake by Coastal Bermuda grass (*Cynodon dactylon* L.) in a Coastal Plain spray field after 4 and 10 years of swine manure effluent application. Effluent was initially applied to a 1 ha portion of this field, which was later enlarged to 5 ha. Top and subsoil samples were collected at nearby control sites that received no swine manure. All soil samples were extracted using Mehlich 3 (M3) and double acid reagent for plant-available and total Cu and Zn concentrations. Comparison of trace metal concentrations in the topsoil after 4 and 10 years of effluent application with control soils showed that total Cu, and M3 and total Zn had accumulated. Mehlich 3 and total Cu and Zn accumulation rates both declined after an increase in spray field area and decrease in effluent application rates. In a 1 ha region of the field, total Cu and Zn concentration both increased between 45 and 90 cm deep after 4 years of effluent applications. Across the 5 ha field, higher subsoil total Cu concentrations relative to controls suggest that Cu leaching occurred; however, leaching of Zn into the total pool was not significant. Estimated times through Coastal Bermuda grass uptake to reduce topsoil M3 Zn concentrations to background levels ranged between 21 to 152 years. Results from this study show that after 10 years of swine manure effluent application, mean topsoil M3 Cu and Zn concentrations were far below concentrations considered phytotoxic to sensitive crops.

Novozamsky, I., T.M. Lexmond, and V.J.G. Houba (1993). A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Int. J. Environ. Anal. Chem. 51:47.

Noyd, R.K., F.L. Pflieger, M.R. Norland, and D.L. Hall (1997). Native plant productivity and litter decomposition in reclamation of taconite iron ore tailing. J. Environ. Qual. 26:682-687. Nuclear weapons' testing, mineral extraction industries and nuclear power generation are among the activities which have led to radioactively contaminated land. In the United Kingdom (UK), current activities such as the decommissioning of nuclear licensed sites and the sale of Ministry of Defence land require that the legislation, remediation and management of radioactively contaminated land be addressed. With an emphasis on the UK, this paper reviews potential management/remediation strategies for radioactively contaminated land, including consideration of the environmental mobility of potentially important radionuclides.

Nudunuri, K.V., R.S. Govindaraju, L.E. Erickson, and A.P. Schwab (1995). Modeling of heavy metal movement in vegetated, unsaturated soils with emphasis on geochemistry. Pp. 57-66. In L.E. Erickson, D.L. Tillison, S.C. Grant, and J.P. McDonald (eds.), Proceedings of the 10th Annual Conference on Hazardous Waste Research, May 23-24, 1995, Manhattan, KS.

Nwosu, J.U., H.C. Tatsch, and L.A. Kapustka (1991). A method for on-site evaluation of phytotoxicity at hazardous waste sites. Pp. 333-341. In J.W. Gorsuch, W.R. Lower, M.A. Lewis, and W. Wang (eds.), *Plants for Toxicity Assessment: Second Volume*. ASTM STP 1115. American Society for Testing and Materials, Philadelphia, PA.

Nyer, E.K., and E.G. Gatliff (1996). Phytoremediation. *Ground Water Monitoring Remed.* 16(1):58-62.

Nyhan, J.W., T.E. Hakonson, and B.J. Drennon (1990). A water balance study of two landfill cover designs for semi-arid regions. *J. Environ. Qual.* 19:281-288.

Nzungu, V. A. and C. H. Wang (1999). Influences on Phytoremediation of Perchlorate-Contaminated Water. *Abstr. Pap. Am. Chem. Soc.* 218: 37-ENVR.

Nzungu, V. A. and S. C. McCutcheon (2003). Phytoremediation of perchlorate. *Phytoremediation: transformation and control of contaminants*. V. A. Nzungu. Hoboken, Wiley-Interscience: 863. Perchlorate is taken up from contaminated waters by a variety of plants under a wide range of conditions similar to those observed at contaminated sites. Several but not all terrestrial and wetland plants mineralize perchlorate to chloride and oxygen. Some plants accumulate perchlorate to more than 0.1 percent by weight. Some halophytes and phreatophytes adapted to the U.S. southwest (location of many sites of perchlorate contamination) do well in degrading perchlorate or spurring bacterial mineralization. Some trees spur the acclimation of microorganisms, and these bacteria mineralize perchlorate faster than plants. Chloride, nitrate, and other ions competitively affect perchlorate uptake and mineralization.

Nzungu, V. A., C. H. Wang and G. Harvey (1999). Plant-Mediated Transformation of Perchlorate into Chloride. *Environmental Science & Technology* 33(9): 1470-1478.

Nzungu, V. A., H. Penning, et al. (2004). Mechanistic changes during phytoremediation of perchlorate under different root-zone conditions. *International Journal of Phytoremediation* 6(1): 63-83. Two types of hydroponic bioreactors were used to investigate the mechanistic changes during phytoremediation of perchlorate under different root-zone conditions. The bioreactors included: (1) an aerobic ebb-and-flow system planted with six willow trees, and (2) individual willow trees grown in sealed root-zone bioreactors. Rhizosphere probes were used to monitor for the first time during phytoremediation of perchlorate, diurnal swings in oxidation-reduction potential (EH), dissolved oxygen (DO), and pH. Radiolabeled (Cl-36-labeled) perchlorate was used as a tracer in a subset of the sealed bioreactor experiments to quantify the contribution of phytodegradation and rhizodegradation mechanisms. Rhizodegradation accounted for the removal of 96.1 +/- 4.5% (+/-95% CI) of the initial perchlorate dose in experiments conducted in sealed hydroponic bioreactors with low DO and little or no nitrate N. Meanwhile, the contribution of rhizodegradation decreased to 76 +/- 14% (+/-95% CI) when nitrate (a competing terminal electron acceptor) was provided as the nitrogen source. Slower rates of phytoremediation by uptake and phytodegradation were observed under high nitrate concentrations and aerobic conditions, which allowed perchlorate to persist in solution and resulted in a higher fraction uptake by the plant. Specifically, the rate of removal of perchlorate from bulk solution ranged from 5.4 +/- 0.54 to 37.1 +/- 2.25 mg/L/d (+/-SE) in the absence of nitrate to 1.78 +/- 0.27 to 0.46 +/- 0.02 mg/L/d (+/-SE) at high nitrate concentration. The results of this study indicate that the rootzone environment of plants can be manipulated to optimize rhizodegradation and to minimize undesirable processes such as uptake, temporal phytoaccumulation, and slow phytodegradation during phytoremediation of perchlorate. Rhizodegradation is desired because contaminants resident in plant tissue may remain an ecological risk until completely phytodegraded.

Nzungu, V. A., L. N. Wolfe, D. E. Rennels, S. C. McCutcheon and C. Wang (1999). Use of Aquatic Plants and Algae for Decontamination of Waters Polluted with Chlorinated Alkanes. *International Journal of Phytoremediation* 1(3).

Nzungu, V. A., W. L. O'Niell, et al. (2003). Sequestration and transformation of water soluble halogenated organic compounds using aquatic plants, algae, and microbial mats. *Phytoremediation:*

transformation and control of contaminants. V. A. Nzungung. Hoboken, Wiley-Interscience: 499. Bench and greenhouse testing establish that aquatic plants, macrophytic algae, and microbial mats all separately transform and mineralize common halogenated contaminants such as trichloroethylene, tetrachloroethylene, hexachloroethane, and carbon tetrachloride. Even dead plants and algae continue to transform halogenated organic compounds -- a potential link between plants and dehalogenase activity in sediments. Plants, algae, and mats also are good biosorbents that may damp uneven loading. The phytotransformation pathways combine phyto reduction and phytooxidation informing carbon dioxide, chloride, and bound residues. The more highly halogenated contaminants (e.g., tetrachloroethylene) are phyto reduced, whereas phytooxidation dominates the transformation of trichloroethylene and less chlorinated organic contaminants. Microbial mats applied in bioreactors and treatment wetlands can take advantage of both reductive and oxidative reactions in easily engineered aerobic and anoxic zones. In addition, monitored natural attenuation in contaminated wetlands is feasible. Therefore, evaluation of on-site remediation must consider sequestration and phytotransformation along with microbial degradation of halogenated organic contaminants during treatment of contaminated waste waters, soils, and groundwaters. Based on these results, treatment wetlands and bioreactors are a viable option ready for field-testing. The next developments should include bench scale continuous flow testing and applications of microbial mats in created treatment wetlands.

Nzungung, V. and P. Jeffers (2001). Sequestration, Phyto reduction, and Phytooxidation of Halogenated Organic Chemicals by Aquatic and Terrestrial Plants. *International Journal of Phytoremediation* 3(1).