Metabolism of Organic Pollutants by Plants: Potential and Limitations

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Whilst a number of plant species have been studied in recent years for their ability to hyperaccumulate inorganic pollutants, such as toxic heavy metals, similar information on plant uptake/ degradation of organic chemicals is either lacking or patchy. There is a vast amount of literature on plant physiology, biochemistry and biotechnology. This knowledge could be utilised in assessing the potential of plants for cleaning up polluted environments.

Developments in quantitative-structure-activity-relationships (QSAR) provide a useful tool to predict the levels of organic chemicals that should be absorbed/ degraded by plants.

Genetic manipulation of plants has so far been aimed at increasing crop yields, protecting plants from pests, or enhancing taste, colour or shelf life of plant products. The knowledge could, however, be utilised in enhancing the ability of plants to cleanup polluted environments.

The article presents an overview of ways to combine existing knowledge to assess the potential and limitations of using plants for metabolism of organic pollutants.

Mycorrhizal Influence on Phytoremediation of Polycyclic Aromatic Hydrocarbons

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Mycorrhizal fungi are forming symbioses with a large majority of plant species, both under natural conditions and in anthropogenically-influenced soils. The main benefits of mycorrhiza are linked to the improvement of plant stress tolerance, including nutrient deficiency, salinity, metal toxicity, drought and adverse physical soil conditions. Recently, an improved plant tolerance to adverse soil conditions associated with organic pollutants in the presence of mycorrhiza has been established (Leyval and Binet, 1998). This may potentially allow or improve re-vegetation of polluted sites, and thus limit soil erosion and resulting diffusion of organic pollutants. Based on these positive indications, we have assessed the potential for phytoremediation of polycyclic aromatic hydrocarbons (PAHs) as influenced by mycorrhiza. Initial experiments with spiked soil showed both enhanced degradation and reduced toxicity of high molecular weight PAHs in the presence of mycorrhiza (Joner and Leyval 2001; Joner et al., 2001). Further experiments with two industrially polluted soils confirmed these findings, though the largest effects were seen for light, non-volatile PAHs. Mechanistic explanations may be found in mycorrhizal influence on the activity of oxidative enzymes in soil (Criquet et al., 2000), influence on bacterial community development (Joner et al., 2001), nutritional constraints to bacterial degradation (Joner et al., 2002), and different rhizosphere gradients resulting from the interactions of these factors.

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Activity and Adaptability: Plant Performance criteria

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Basic criteria of plant performance/vitality are their activity under certain environmental conditions and their adaptability to stress caused by changes of the environmental conditions. The general aim of our research is the formulation of a theoretical approach of stress – stress adaptability and an experimental access and evaluation of both criteria.

The term “stress” comes from Physics where it has been precisely defined and related to the strain it provokes. However, concerning Biology it has been given widely differing meanings. Probably due to an extension of the physical meaning, many of them converge in attributing stress to any environmental factor “unfavourable” for the living organism under consideration.

Our approach is different in principle (Strasser, 1985; 1988; Tsimilli-Michael *et al.*, 1996; Tsimilli-Michael and Strasser, 2002). Based on inferences from open system thermodynamics (Prigogine, 1967) the adaptation of plants to a continuously changing environment, and more generally the adaptive strategies and evolutionary behaviour of organisms, are approached as optimisation strategies dictated by the thermodynamic demand for minimal entropy production. When a thermodynamically optimal state is achieved, the system is characterised as being in harmony with its environment. Though optimal states are stability states, the thresholds of stability can be exceeded upon changes in the environmental conditions and the system is then forced to suboptimality. Any such change that disturbs the achieved harmony is regarded as a stressor, i.e. a stressful event. Hence, non-stress corresponds to optimality, whereas stress, defined as a deviation from the non-stress situation, corresponds to suboptimality. Because of the thermodynamic demand for optimality, the resulting suboptimality creates a state change force, under which the system undergoes state changes searching for a new optimal state, the attraction point, characterised by a new constellation of structural/conformational parameters. Any change of these parameters is denoted as strain.

When the system succeeds to reach the attraction point, the state change force vanishes and a new optimality - stability is established. Stress adaptation is therefore defined as the sequence of processes that realise the state changes until a new non-stress situation is established.

Different adaptive strategies are employed to regulate different functional and structural parameters of the system. The deformation of any parameter can be plastic (irreversible) or elastic (reversible). This is recognised by the appearance or not of a residual strain, i.e. of a strain caused by a cyclic environmental change. Generally, not only the adaptability of a plant, but also the plasticity or elasticity of the deformations it undergoes, appear to be determined by the evolution of the plant in its natural habitat.

Our conceptual approach is centred on the dynamic character of the relation between organism and environment, keeping from the physical approach the concept of “action-reaction”. No environmental factor is considered *a priori* as unfavourable and the plant has not to “resist”, but simply reacts, in different ways. As far as the system manages to adapt, which means that the attraction point is within realistic limits, stress is harmless and can, even more, be constructive, because it results in improved adaptability and hence adaptive evolution. But, if the adaptability of the system is overtaxed, then stress is destructive, leading to permanent damages or even to death (Larcher, 1987). However, the optimal states before and after a stress (when stressors do not overtax the adaptability of the plant), can differ concerning the plant performance. Hence, stressors (not destructive) can be distinguished in beneficial (e.g. rhizosphere/mycorrhizosphere influences) or not beneficial (e.g. high temperature, heavy metals), depending on whether they trigger the achievement of an optimal state with improved or poorer performance, i.e. with better or worse compromise between activity and adaptability (Tsimilli-Michael and Strasser, 2002). In this frame, a beneficial stressor may be also regarded as improving the buffer capacity of an organism, in the sense that it reduces the extent of the suboptimality to which the system is forced by a co-stressor and, hence, the possibility that the latter would overtax the adaptability of the organism. Antagonism and synergism of stressors are similarly integrated in this conceptual frame (Tsimilli-Michael *et al.*, 2000).

Different structural and physiological levels in the plant are expected to undergo deformations of different extent under stress. The photosynthetic apparatus – and especially photosystem II (PSII) - is well known to be very sensitive to different stresses, before other symptoms appear. Our proposition for the experimental evaluation of stress and adaptation, as well of the plant activity at optimal states, is a method providing a quantification of the PSII behaviour. The method utilises a well-elaborated procedure, the JIP-test, based on the measurement of the O-J-I-P kinetics of the fluorescence emitted by plants (Strasser *et al.*, 1995). This test, which has been developed in the laboratory of Bioenergetics of Geneva (for a review see Strasser *et al.*, 2000), is today used worldwide. The JIP-test can be easily used for the description of plants under different stress conditions, which result in the establishment of different physiological states, and can thus serve as a tool for screening different phenotypes and the performance/vitality of plants in any biotope.

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Critical Success Factors for Optimal Interaction between Plants and Micro Organisms in Phytoremediation

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In the Netherlands, many sites are contaminated with a mixture of organic compounds as PAH's, hydrogen carbons of different oils and heavy metals. For efficient degradation of complex organic compounds in the rhizosphere by microbial activity, detoxification of heavy metals is necessary. In principle, most functions essential to bioremediation are present in the cryptobiotic soil complex. We have intensively studied bioremedial relations between soil, plants and the cryptobiotic soil complex on kerosene-, oil- and PCP-polluted soils.

We have spiked a soil with a kerosene plume. It was shown that the degradation of kerosene began immediately and that O_2-consumption and CO_2-production was proportional to the presence of kerosene. During degradation O_2 and N appeared to be limiting factors. When plants were grown on the plume, root growth tends to approach the centre of the plume with in ca. one year. Especially Alfalfa could be grown succesfully on the pollution.

We have studied oil-polluted soils from contaminated sites in the Netherlands. It was shown by PCR-DGGE that during the process of oil degradation specific components of the natural microbial communities will be selected on their functionality in this respect. Also in this case plants may play an important role. Criteria on which plants are suitable in this respect, are:

- capable of deeply rooting;
- good host for micro-organisms;
- causing a selective advantage for degradating microbials.

With respect to PCP's, we have shown that the efficiency of degradation strongly depend on the type of plant species that were grown on the soil. We have clues that the success rate of plants in this respect correlates to the presence of certain secondary metabolites. At the same time these secondary metabolites, but also exudates and surfactants appear to detoxify heavy metals. This can be used as starting point for efficient measurements in managing polluted soils.

In general, we have shown that plants increase both the bioavailability of the pollution and the availability of O_2, C and N.

Usage of TNT Biodegradation for the Remediation of Disused Military Sites

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Former military sites contaminated with explosives, especially TNT, cover large areas. Soils contaminated with explosives presents hazards to the groundwater.

Therefore BioPlanta has established a biological *in situ* technology to remediate large areas of medium-to-low contamination of explosives. The technological sequence of operation for the microorganism-based process and the remediation performance achievable by it were tested on an production site. A birch-covered area, exhibiting a mean TNT concentration of

6,000 mg per kg, was selected as the test area. The birch trees present in the area were rooted out and crushed using a shredder. The shredded tree material was ploughed into the ground using a rotary hoe together with rotting birchwood chips. The results demonstrate the fundamental efficacy of the in situ process.

In the presence of a readily degradable carbon source, TNT is transformed under reducing conditions. The reduced transformation products are reactive and react with functional groups in the organic soil material. The products of the reaction with humid substances are complex, with various types of covalent bonds being formed.

The remediation period required, which depends on the aim that is set for the remediation process, for this area will be 2 years. The material introduced in the form of appropriate carbon sources and soil containing autochthonous microflora is able to bring about transformation of the TNT. A roughly 80% reduction in the TNT contamination of the soil was detectable over a 100-day period. ADNT concentrations were in the 25-50 mg per kg DW. At the same time, 4-ADNT content was somewhat higher - a fact that is indicative of active microbiological transformation.

A very powerful agent for bioremediation of TNT is molasses, which can be applied to soil or used as an additive in passive water treatment in constructed wetlands. If combined with elemental iron the process is even more effective. A small-scale study of the *in situ*-treatment of contaminated soil with different carbon sources and iron showed the superiority of the combined molasses/iron treatment.

Studies on constructed wetlands on pilot scale for treatment of explosives contaminated drainage water and groundwater were done. Relevant constituents of the waters include TNT, Hexogen and Hexyl. The investigations show that about 95 % of the TNT contamination can be reduced. BioPlanta will implement this cost efficient technology for the remediation of groundwater on an armament industry site in Germany 2002.

The Limiting Steps in a Cooperative Remediation Process between Plant and Microorganisms

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Xenobiotic movement and metabolism was studied in soil-plant systems, either under laboratory conditions, with labeled compounds, or in agrosystems, with non-labeled pesticides, after pre-emergence treatments. The distribution of the native compounds inside the system was the result of a competitive binding to several different types of matrices, which were clays, dead organic matter, living microbial biomass and plant roots. In each matrix, one can separate several different structures whose binding properties can differ significantly from one to the others, as is the case, for instance, for clays, such as montmorillonite, kaolinite or smectites. As a whole, the concentration of the compounds in soil water depended on the partitioning between the heterogeneous solid system and water. In plants, the first step of plant absorption was evidently the partition between soil water and the first lipidc biological membrane which was mostly represented by root hair plasmalemma.Most xenobiotic pollutants are only submitted to passive root penetration, which can be quantitatively described by Fick's law. The penetration flux from soil water to root cell water depended on the differential concentration between these two aqueous compartments. The transfer through a biological membrane was a limiting step for hydrophilic compounds but not for lipophilic ones. For highly lipophilic substances, water solubility constituted the limiting factor and explained that the powerful water movement from roots to stems could not displace high amounts of such compounds. Under these conditions, the absorption of the compound only concerned the root, not the shoot. Hence, the differential concentration between root and soil water decrease to 0 and absorption readily stopped. The role of the microbial biomass in the rhizosphere space would be to transform the initial substance into less lipophilic derivatives which could be absorbed by plants and transported to aerial organs, especially leaves, where they could accumulate or be metabolized.