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Joint Research Group

# Processes for the Bioremediation of Soil

Compilation of Current Projects

**Federal Environmental Agency**
**BMBF-Project Management Agency**
**for Waste Management and Remediation**
**of Hazardous Abandoned Sites**

**Joint Research Group**

# Processes for the Bioremediation of Soil

Compilation of Current Projects of the
Joint Research Group
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Edited by:
**DR. JOCHEN MICHELS**

**DECHEMA**
**German Society for Chemical Apparatus, Chemical Engineering, and Biotechnology**

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# Contents

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# Introduction

The Joint Research Group “Processes for the Bioremediation of Soil” is headed by the BMBF Project Management Agency For Waste Management and Remediation of Hazardous Abandoned Sites (PT AWAS). It currently comprises seven Joint Projects with over 30 single projects (see figure, page 9). This interdisciplinary group is working on the development of innovative processes for the bioremediation of contaminated soils. After the laboratory phase, not only is their effectivity tested under application-oriented conditions, but their success is monitored by a complex control system that goes far beyond a conventional chemical analysis of pollutants.

***BMBF Project Management Agency for Waste Management and Remediation of Hazardous Abandoned Sites located in the Federal Environmental Agency (USA)***

The overall coordination represents the joint research group and supports its participation at international events at home and abroad for the duration of the project. The findings of the approx. 30 single projects will be incorporated in the Handbook “Processes for the Bioremediation of Soil”. This work is intended to assist the authorities and industry to find and select promising clean-up processes, thus contributing to the reusability of economically attractive sites. The handbook in German language is scheduled for publication in 1999.

***Overall Coordination***

*Joint Project No. 1: Coordination and Development of a Handbook*

One important class of pollutants, found in many contaminated sites, are the carcinogenic, polycyclic aromatic hydrocarbons (PAH). These tremendously varied substances occur in tar and, as waste products, they contaminate the soil of gas works and coking plants. One of the largest and most well-known contaminated sites of this kind in Germany is Rositz in Thuringia. Joint Project No. 2 “Humification of PAH” has been working on the biological degradation of these substances since 1994. The biological degradation of PAH is highly dependent on its bioavailability and thus on its water solubility. The latter generally decreases with the number of condensed rings in the molecule. PAH composed of two or three rings are considered to be easily biologically degradable, whereas five-ring PAH are not.

The research groups of the joint project have successfully investigated possibilities of mechanically disintegrating lumps of tar that were not treatable biologically and subsequently of degrading them by biological processes, composting or with white rot fungi. In recent years it has become apparent that the natural attenuation of soil assists microorganisms in their task. The microbiological attack modifies the substances so that they are firmly integrated in the humus part of the soil and thus are deprived of all their hazard potential. The processes developed here should next be tested on an industrial 1000-t scale.

***Process Development***

*Joint Project No. 2: Humification of PAH*

*Joint Project No. 3: Bioremediation of Hazardous Abandoned Armament Sites*

From 1939 to 1944 over 100,000 t of TNT were produced in one of the largest explosives factory in Germany codenamed “Werk Tanne” (Tanne Plant) regardless of the forced labourers or the environment. The environment was heavily polluted by this production and also by the post-war dismantling programme. Today, more than 50 years after the war, the soil on this 290-acre site is still to a great extent contaminated by TNT and other nitro aromatic compounds.

Additionally with every rainfall the toxic substances are washed into the nearby lakes, which means that costly safety measures, such as charcoal filter equipment, are needed to protect the groundwater. As TNT production requires a great volume of water, explosives plants were usually built in areas with abundant supplies of water; the result is a threat to our present-day drinking water resources. The contaminated soil now has to be cleaned, but with such large quantities of soil involved economic and ecologically friendly alternatives to incineration are in demand.

For this reason since 1993 scientific investigations into the biological degradation of TNT have received financial support in Germany. As TNT can be metabolised by practically all organisms, the eight single projects in Joint Project No. 3 “Bioremediation of hazardous abandoned armament sites” are developing very different bioremediation processes for TNT-contaminated soils, whereby both the natural soil microorganisms and the addition of fungi or plants are applied. With the right selection of additives and the environmental conditions (e.g. pH value, water content, oxygen supply or exclusion) the contaminants could be almost completely neutralised by biological means in the laboratory.

*Joint Project No 7: Biological Remediation of Hydrocarbon Contaminated Sites*

In Joint Project No. 7 “Bioremediation of hydrocarbon-contaminated sites” possibilities of in situ cleaning of soil contaminated with volatile chlorinated hydrocarbons has been the subject of investigations since 1996. For example 1,1,1-trichloroethane is used to clean oil tanks. Consequently large areas of waste oil refinery sites are contaminated with chlorinated hydrocarbons.

The biological degradation of these substances can be divided into two phases: the biological dehalogenisation of trichloroethane to dichloroethane is more successful under anoxic conditions, i.e. with the exclusion of oxygen. However, the further dehalogenisation and subsequent complete degradation of the substance takes place with an adequate supply of oxygen. One in-situ process is currently being carried out at the Pintsch site in Hanau, a former waste oil refinery. This process takes both phases into consideration by alternately adding oxygen-consuming and oxygen-supplying substrates to activate natural soil microorganisms.

A further single project deals with the microbiological degradation of waste oil fractions using fatty acid-modified amino acids. This increases the bioavailability of organic pollutants in the soil and thus accelerates the degradation process.

*Back-Up* ***Research***

*Joint Project No. 4: Ecotoxicological Test Batteries*

Admittedly, the research groups that investigate biological degradation cannot make firm statements on the hazard potential of contaminants after biological soil treatment. This can be explained partly by the fact that it is tremendously difficult to detect the metabolites occurring due to the biological degradation, and partly by the fact that these metabolites are not even known. For this reason Joint Project No. 4 “Ecotoxicological test batteries” is a back-up project for the three above-mentioned process developments, investigating the hazard potential of treated soil using test organisms.

At the same time both genotoxicological tests on the soil retention function and chronic plant tests are being developed. Other research groups are working on the suitability of reporter bacteria for detecting acute hazard potential.

In this comprehensive research project the necessary framework conditions are being investigated, i.e. storage conditions for samples and various soil extraction methods. A strategic position is taken by the direct feedback between process developers and this research project. The investigations use authentically polluted soil before and after the biological treatment in order to investigate the suitability of tests for the various classes of pollutants on the one hand and in order to obtain new tools for determining the effectiveness of the individual treatment methods on the other.

Joint Project No. 5: *Long-Term Stability and Remobilization* Potential

The behavior of bound residues in biologically redeveloped soil will be investigated by climatic stress conditions by Joint Project No. 5 “Long-Term Stability and Remobilization Potential”. Seasonal climatic deviations of temperature and soil moisture of a 50 year period can be simulated. These investigations are necessary since biological remediation of PAH- and TNT-contaminated soil will lead to so called bound residues. Most probably these residues will be generated by biogenesis and are bound covalently to the organic soil fraction. They are not extractable at all. But no knowledge is available about the long-term remobilization capacity of bound residues and about their hazard potential. Investigations will additionally be made with real contaminated soil samples after biological redevelopment. Isotopically labelled substances will be used in control experiments.

Hitherto knowledge about long-term and remobilization behavior of contaminants in biological soil remediation will be compiled within the terms of the project and then evaluated in a literature study.

***True to scale test***

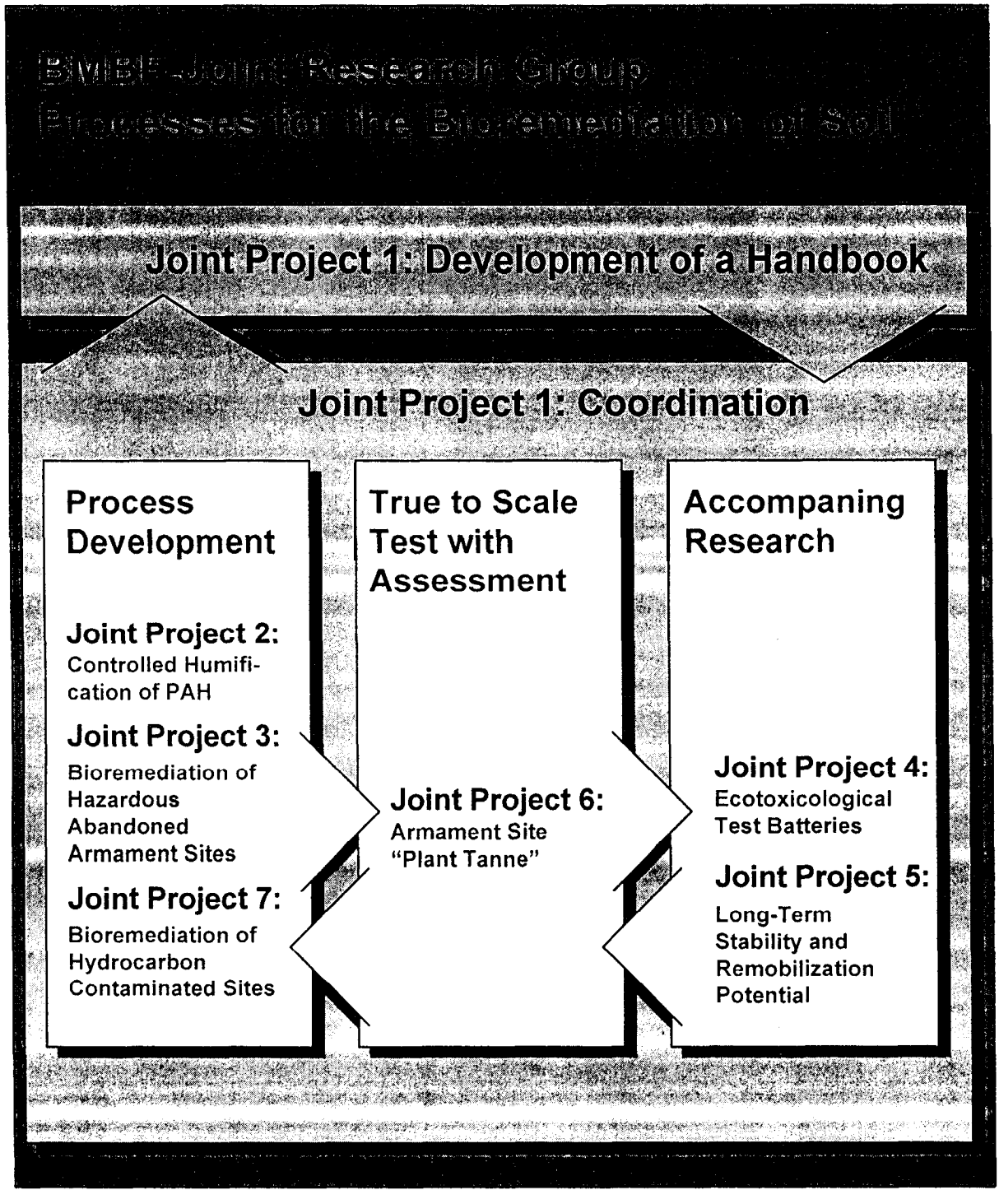
*Joint Project* No. 6: *True* to scale test *of* biological *processes with assessment*

The former ammunition plant “Tanne” will be the test area for Joint Project No. 6 “True to scale test of biological processes with assessment” Hundreds of tons of TNT-contaminated soil will be excavated, homogenized, and provided for selected biological soil remediation techniques. The preparations for the project have been ongoing for one year. A public announcement has produced different competing tenderers of biological remediation processes for TNT-contaminated soil. Now, three on-site and two in-situ processes will be able to demonstrate their power at the former ammunition plant. Especially in-situ remediation techniques seem to be very suitable for the vast contaminated areas. The research projects introduced No. 4 “Ecotoxicological Test Batteries” and No. 5 “Long-Term Stability and Remobilization Potential” will back up the redevelopments. An extraordinary documentation of the progress in remediation with assessments and costs will ensure an accurate evaluation of the experiments. Moreover a cost assessment for the biological redevelopment of hazardous abandoned armament sites will be available.

The high status of German biological remediation technologies can offer scientifically reliable, ecologically and economically meaningful, innovative gentle biotechnological procedures for the cleanup of TNT-contaminated soil worldwide. Processes have to be adapted to individual toxic waste sites and the requirements of the countries concerned. The innovative tools for assessment and long-term-behavior are essential for quality control.

Further information of the joint research group is available from the coordinating staff (Joint Project No. 1). Updated versions of this booklet can soon be found on our homepage under:

<http://www.dechema.de/biotech/bioall.htm>


BMBF Joint Research Group
Processes for the Bioremediation of Soil
Joint Project 1: Development of a Handbook
Joint Project 1: Coordination
Process Development
Joint Project 2:
Controlled Humification of PAH
Joint Project 3:
Bioremediation of Hazardous Abandoned Armament Sites
Joint Project 7:
Bioremediation of Hydrocarbon Contaminated Sites
True to Scale Test with Assessment
Joint Project 6:
Armament Site "Plant Tanne"
Accompaning Research
Joint Project 4:
Ecotoxicological Test Batteries
Joint Project 5:
Long-Term Stability and Remobilization Potential

# Coordination of the Joint Research Group "Processes for the Bioremediation of Soil" and Development of a Handbook

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The Joint Research Group is funded by the Federal Ministry of Education, Science, Research and Technology (BMBF). The aim is the redevelopment of soils contaminated by resistant (biologically slow degradable) pollutants. Hazardous abandoned industrial production sites are often contaminated by:

- polycyclic aromatic hydrocarbons (PAH), found in the soil of former coking plants and gasworks (Joint Project (JP) 2, pp. 12-16, individual project p. 17)
- residues from the production of explosives on hazardous abandoned armament plants, e.g. TNT, hexogen (RDX) and hexyl (JP 3, pp. 18-29)
- volatile chlorinated hydrocarbons (VCH) as used in dry cleaning and waste oil refining plants and resistant aliphatic hydrocarbons from crude oil accidents (JP 7, pp. 43-44).

Besides the remediation techniques suitable control methods will be developed within the scope of the scientific accompanying research programme. This includes

- the development of ecotoxicological test batteries (JP 4, pp. 30-40) and
- the long-term stability and remobilization potential of bound residues (JP 5, p. 41).

The transfer of the laboratory findings to practical remediation techniques has to be ensured by the appropriate scale-up. Concerning the redevelopment of hazardous abandoned armament sites

- the true-to-scale test of biological remediation processes with an assessment at the site "Werk Tanne" near Clausthal-Zellerfeld in the Harz mountains will start in spring 1998 (JP 6, p. 42).

The Joint Research Group consists of about thirty Single Projects (SP) and is headed by the BMBF Project Management Agency for Waste Management and Remediation of Hazardous Abandoned Sites (PT AWAS) in the Federal Environmental Agency (UBA). The DECHEMA is the official representative of the Joint Research Group and supports the participation of its members at international congresses, both domestic and foreign, for the duration of the project term. The findings will be published in the "Handbook for the biological cleanup of contaminated sites" which is intended to serve as a tool for federal agencies and companies to find suitable cleanup technologies for contaminated sites in order to regenerate these economically attractive areas. The first edition of the handbook will be published in 1999.

# Coordination of Joint Project No. 2: Controlled Humification of PAH

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The development of a new decontamination technology for PAH contaminated soils is financially supported by the ministery of education and research (BMBF) by the implementation of an interdisciplinary research and development project, where seven research groups and companies are involved as Single Projects (SP). The new decontamination procedure is called “controlled humification of PAH”.

The target of this R&D Joint Project is to use decomposition and humification processes to reduce measurable concentrations of PAH. The development of the technology for the application in a competitive market is done by laboratory experiments, up-scaling in technical experiments and application experiments in a pilot-state. This technological development is accompanied by an intensive research programme done by a variety of university and private research institutes.

The developed technology is based on the cracking of the PAH clusters in the soil. The availability of the PAH is increased by cracking this structures and spreading the PAH on the available particle surfaces in the soil. Due to this optimized distribution biological and chemical degradation processes are facilitated (SP 2.1). A special cracking procedure was developed by SP 2.4 for this conditioning of the contaminated soil. This procedure is patented (patent number 196 03 089.7)

In parallel scientific research is done on the fields of (eco-)toxicology (SP 2.7*), chemical long-term stability of the treated soil (SP 2.2*, SP 2.6) usage of mushrooms to increase PAH decomposition and humification processes (SP 2.3”) chemical oxidation (SP 2.1) and development of an in-situ field method based on the use of plants which are inoculated with special mykorrhizal fungi (SP 2.5).

* Projects are already completed

# Humification of PAH - Technological Optimization, Validation

Aim of the project is to use humification processes technologically for the reduction of harmful chemicals realized through the pilot scheme of Polycyclic Aromatic Hydrocarbons (PAH). Works for the optimization and validation of the previous method for controlled humification of PAH contaminated soil were implemented in accordance with the development of a combinable decontamination programme (Modular principle). Components of the so called "Controlled Humification System" e.g. the use of PAH degrading fungi, the use of chemical oxidation processes, further optimization of the incubation and degradation conditions are developed. For utilization of remediated soil material a recultivation experiment is planned.

During the optimization phase of the development of the PAH remediation-technology further examinations and modifications of the process in view of long-term behaviour of the incubation settings as well as for the increasement of the biological availability of the remaining PAH are made. Further progress is done on more technological upscales and laboratory experiments to optimize the method. This includes an alternation of environmental conditions (aerobic-anaerobic-aerobic), a temperature management and the optimization of the degradation in the rhizosphere of plants. The efficiency of chemical oxidants to reduce recalcitrant PAH in soils and the balance of these chemical oxidations are now examined in final laboratory experiments.

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# Humification of PAH - Conditioning of the Soil

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Aim of the project part is to optimize the conditioning step of PAH-contaminated soil technically and economically in order to get suitable starting material for the decomposition and humification processes. The developed technology results in an ecological and sustainable remediation method for contaminated soil. Furthermore a concept will be worked out for the competent calculation, choice of location and an implementation plan for the pilot scale testing. The so far developed pretreatment technology for PAH contaminated soils is now validated in upscaling experiments (0.2 to 1 $m^3$) for the optimization of the most suitable conditioning method for the contaminated soil.

A special pretreatment of the contaminated soil was developed to ensure an enhanced bioavailability of PAH for the degradation and humification processes (decomposition) and on the other hand an optimized distribution of organic supplements which support the degradation and the humification of PAH. This new conditioning method was patented (patent number 196 03 089.7). For the decomposition as well as for the application of the organic additives a turbulent granulating machine proved its suitability. Validation and optimization of the technology is now under treatment. The modulated programme, worked out in the former part of the joint project, serves as a schedule for the implementation of a pilot project in which PAH loaded soil should be decontaminated by controlled humification.

# Degradation of PAH by Mycorrhizal Fungi

Mechanically, biologically and chemically refined recycling soil with inserted PAH in the humus complex is to be spread on recultivation grounds meant for reforestation. In this context, the project investigates the role of ectomycorrhizae in the release and degradation of PAH and the influence of PAH on the development of plants and mycorrhizal fungi.

Liquid culture experiments with mycorrhizal fungi and pyrene and $^{14}C$-phenanthrene as PAH model substances have shown that the fungi considerably vary in their PAH degradation capacity, depending on the enzyme equipment. Here, the content of phenol oxidases is a decisive factor. We were able to select one genus whose PAH metabolism leads to considerable $^{14}CO_2$ release. its PAH degradation potential is bigger than that of soil fungi. The planting of special mycorrhizae trees offers an economically and ecologically acceptable remedial technique for large contaminated areas with low PAH contents or continuous PAH immission. The advantages of this in-situ technique are the rooting of deeper soil layers and the high competitiveness of mycorrhizal fungi in the soil.

PAH contents of 635 mg/kg at the start of a greenhouse experiment with oaks and actually contaminated soil inhibited plant development, but the inhibition was relatively low. Applying recycling soils (with much lower PAH contents) to recultivation areas seems therefore to be harmless from the plant physiological point of view. The influence of PAH on the development of mycorrhizae can only be assessed after the completion of analyses.

***Grant No.:***
***1480928***

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# Formation and Long-Time Stability of Humificated PAH in Biologically Treated Recycling Soils

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The intentional stimulation and use of the humification of Polycyclic Aromatic Hydrocarbons (PAH) as bioremediation strategy is based on the assumption that the bound xenobiotica behave like other natural soil-bound aromatics and become a stable and indistinguishable part of the natural soil organic matter.

However, since this immobilization- or clean-up-method is not well established it is necessary to elucidate the relevant processes by mass balance experiments with $^{14}$C-labelled PAH more closely. Both, the formation of non-extractable $^{14}$C-PAH-residues and the fate and long-term stability of the non-extractable residues is therefore investigated with different organic supplements and soil. It is also evaluated for risk assessment purposes whether and in which term the non-extractable residues will remain stable within soil.

The fate and stability of non-extractable PAH-residues is studied under different 'worst case'-conditions. The experimental concept includes biological (influence of PAH-degrading and humus-decomposing microorganisms), physical (influence of alternating temperature, water conditions, mechanical disruption of soil structure) and chemical (influence of complexing agents or Fenton's reagent) stress treatments.

The implementation of soils from contaminated sites in the experimental 'worst case'-simulation will help to relate the results to other contaminated sites and remediation programs.

# Bioremediation of PAH contaminated Soils in a Fluidized Bed Bioreactor (1 $m^3$ Scale)

For the bioremediation of silty soils or soil fractions no decontamination techniques tested in practice are available. Therefore, the objective of the project is to test and optimize the DMT-BIODYN-process in pilot scale for the biotreatment of fine grained, PAH contaminated soils.

The process is consisting of a slurry reactor where the soil is fluidized by means of an upward slurry flow. The effluent slurry is saturated with oxygen in a gasing container and pumped back into the bioreactor. The process is characterized by the following parameters:

- the reactor configuration allows a high maximum solid content (up to 50 % w/w)
- low energy input necessary to maintain the suspension
- optimal bioavailability of pollutants for biodegradation based on the separation of all soil particles
- short bioremediation periods (days to few weeks)

The practical suitability of the process was demonstrated by the treatment of different PAH contaminated soils from coking plants. As an example biotreatment was finished after 6 days resulting in a reduction of the PAH content from 222 mg/kg soil down to 10 mg/kg soil. On the other hand bioremediation of a soil with higher amounts of non bioavailable 5- and 6-ring PAH (sorption of contaminats to the fine grained soil fraction) failed with respect to the remediation target. For treatment of these soil qualities the process was optimized by combination of biotreatment in the slurry reactor with wet-mechanical process steps.

The realization of the DMT-BIODYN process in industrial scale takes presently place in cooperation with a swedish partner, the company EcoTec in Skelleftehamn.

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# Coordination of Joint Project No. 3: Bioremediation of Hazardous Abandoned Armament Sites

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03.96 - 08.98

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2,4,6 trinitrotoluene (TNT) and its derivatives are considered to be toxic contaminants on former armament production sites. The ability to metabolize TNT seems to be ubiquitous in animate nature, although its biodegradability is low. In general, microorganisms, plants, and animals are able to reduce TNT to its primary transformation products, the aromatic amines. The biological decay of TNT is limited by its low water solubility and the fact that it could not be used as sole source of energy and carbon. Therefore, organisms have no advantage over competitors by the utilization of TNT. Thats another reason why TNT and its primary transformation products are still detectable on former armamant plants fifty years after the second world war. To override the limitating factors of the degradation of TNT the joint project has discovered substantial new knowledge already in the first period supported by the BMBF from 1993 to 1996. The actual investigations during the second period are concerning with the transfer of knowledge into applied remediation technology. The main topics of the joint project investigated in the Single Projects (SP) are:

- Optimization of the bioavailability of TNT in combination with the stimulation of the autochthoneous soil microorganisms under aerobic and anaerobic conditions (SP 3.1, SP 3.2').
- Addition of cultured, not genetically modified fungi with high ability in the degradation of xenobiotics (SP 3.3, SP 3.4).
- Landfarming using deep rooting copse or annual plants.(SP 3.6**, SP 3.7).
- Degradation of the explosives Hexyl and RDX (SP 3.5, SP 3.8).

* project is already completed
** an succeeding proposal has been applied

# Development of a Bioremediation Process for TNT-Contaminated Soil at the Former Armament Plant 'Tanne' next to Clausthal-Zellerfeld

Main objective of the project is the development of a microbiological process for cleansing up soil contaminated with TNT- and other nitro and amino aromatic compounds and a process water treatment. Three different variants of the process have been investigated:

- Biological mineralization of TNT by anaerobic or aerobic bacteria,
- microbial transformation of TNT in an anaerobic stage and an aerobic follow up treatment based on a conversion of TNT to polymerized substances fixed in the humic matter of soil (SP 3.1.1) and
- microbialogically induced reduction of TNT to extract toxic constituents from soil and combined chemical-biological follow up treatment of the extracts (SP 3.1.2).

As a result it can be stated that biological mineralization of TNT can not be proved successfully for clean up techniques. The research on bio-remediation of TNT contaminated soil results in a combined anaerobic/ aerobic process leading to a detoxification of soil by transformation of TNT to polymers which are fixed as undegradable organics (SP 3.1.4). Waste water arizing from this process can be treated chemical-biologically (SP 3.1.3). This process will now be developed to a large scale technique in the current second project phase. Further investigation will focus on the mass balance of the transformed and immobilized TNT.

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# Aerobic and Anaerob-Aerobic Treatment of TNT-Contaminated Soil from the Site 'Tanne' (Immobilization)

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Aim of the project is to develop a microbial redevelopment technique for TNT-contaminated soil. At the University of Marburg basic research and on-stream laboratory work will be done. Aerobic and anaerobic-aerobic processes will be examined as possible redevelopment methods,

In the first already completed project phase, soil composting and the aerobic infiltration of nutrients into contaminated soil were investigated intensively. It could be shown, that a two-step composting system, beginning with an anaerobic phase and ending with an aerobic, was highly effective to decontaminate soil contaminated with >I000 mg TNT/kg soil. Furthermore the aerobic infiltration of nutrients turned out to be a possibility to decontaminate soil containing <300 mg TNT/kg soil. Both processes are based on a microbial transformation of TNT to aromatic amines, which are bond covalently to the soil-humus matrix by oxygen depending mechanisms.

In the current project-phase, besides further process optimizations, the mechanisms involved in the immobilization of the aromatic amines in the soil-humus matrix shall be illuminated. $^{14}$C-TNT and $^{15}$N-TNT will be used in this survey. Furthermore HPLC-MS and NMR-analysis will help to understand the immobilization processes. Besides chemical analyses a toxicological supervision of the redevelopment process will be done. Last but not least the infiltration of nutrients will be tested as possible in-situ redevelopment method.

# Anaerobic Extraction in Percolation Reactors

Studies on the removal of 2,4,6-trinitrotoluene (TNT) from contaminated soils under anoxic conditions indicated that the indigenous microbial community of contaminated soil material transformed TNT into its reduction products. These are aminodinitrotoluenes and diaminonitrotoluenes, respectively. 2,4,6-triaminotoluene (TAT) was found only in minor amounts. Due to the relatively high water solubility of the formed products, up to 95 % of the original concentration of about 2.5 g TNT/kg of soil dry weight could be removed using a reactor based on the principle of percolation and water as the washing solution. This process was significantly enhanced by adding a supplementary carbon and energy source, like molasses, whereas addition of different electron acceptors (e.g. nitrate or sulfate) caused an inhibition of the reduction process. The addition of e.g. straw (4 % [w/w]) led to an enhanced long-term stability of the process. These results indicate that TNT can be removed from contaminated soil under anoxic conditions, especially when there is an appropriate electron donor in the soil material stimulating the microbial activity.

TNT did not serve as an energy and carbon source nor as a nitrogen source and its removal was apparently achieved by microbial co-metabolism and a change of the physico-chemical properties of the soil material. A significant adsorption of TNT or its transformation products to soil constituents could be excluded because of proving mass balances. A decrease in the toxicity of the obtained effluent water as well as the soil material applied to the luminescent bacterium *Vibrio fischeri* was coupled with the transformation and elimination of TNT.

Strains of *Clostridium* spec. isolated from the investigated soil material co-metabolically transformed the products of TNT metabolism in the obtained waste water into TAT. Within the stationary phase of growth this product was further converted at least via two metabolites into products that no longer exhibited absorption of UV-light (200 - 450 nm). The formation of a variety of products so far described in the literature could be excluded by means of several analytical techniques.

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*14507911*

***Term:***
*06.96 - 05.98*

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# Wet-Chemical Treatment of TNT-Polluted Processwater

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*06.96 - 05.98*

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The oxidation of organic contaminants in the process waters from the biological treatment of TNT-contaminated soils is investigated. Three oxidation methods, the Fenton reaction (oxidation with hydrogen peroxide in presence of dissolved iron compounds), the photo-Fenton reaction (oxidation with hydrogen peroxide in presence of dissolved iron compounds under illumination with ultraviolet light), and the photocatalytic oxidation with irradiated titanium dioxide are used.

The aim of these investigations is the development of a low-cost procedure, which is suitable to oxidize mono-, di-, and trinitrated aromatics in process waters from the biological soil treatment to less harmful, biodegradable compounds or to the thermodynamically favored products.

# Medium-scale Tests to Optimize and Scale Up the Clean-up Processes

The aim of this part of the project is to develop low-cost, large-scale microbiological processes for cleaning up former sites of military production and military operations. Plambeck ContraCon GmbH handles the following tasks:

- Transfer of promising development methods at the Universities of Marburg and Oldenburg and the Institute of Solar Energy Research in Hameln/Emmerthal (cooperation partners in SP 3.1) to the medium scale, and optimisation of these methods taking special account of process conditions relevent to large scale.

- Estimation of the clean-up capacity achievable on a large scale and of the soil treatment costs under various conditions of large-scale clean-up.

Based on the results achieved so far this part of the project entails the following methods:

- Method for anaerobic/aerobic immobilisation of nitroaromatics (collaboration with the University of Marburg, SP 3.1.1)

- Method for anaerobic extraction of nitroaromatics from soils (collaboration with the University of Oldenburg, SP 3.1.2)

- Method for treating process water (collaboration with ISFH, SP 3.1.3)

One of the lines of development of anaerobic/aerobic immobilisation of nitroaromatics has led to a variant of the dynamic heap, the use of which for cleaning up soils with low to medium concentrations of pollutant is highly promising. Development of this variant on a laboratory and medium scale may now be regarded as completed.

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# Composting Explosives Contaminated Soil by White Rot Fungi - Mass Balance Analysis of Xenobiotica and Quality of the Bioremediated Soil

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*1450779*

***Term:***
*07.96 - 06.98*

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Using the white rot fungi *Bjerkandera adusta* for bioremediation of TNT-contaminated soil resulted in an effective decontamination of the soil. The products, deriving from the biological breakdown of TNT should now be elucidated by using radioactive labeled TNT for mass balance analysis of TNT-metabolism in soil. This analytical procedure will separate the radiolabeled metabolites deriving from the biological breakdown of TNT into several fractions: $CO_2$, intermediates associated with the aqueous fraction, intermediates extractable by organic solvents and radiolabeled metabolites which were not extractable at all. Those non-extractable complexes, consisting of xenobiotica and soil fraction (bound-residues) should be investigated in detail. Therefore, investigations on long-term stability and remobilization of those bound-residues should be carried out.

TNT-derived residues, remaining in the soil after bioremediation, may represent a potential risk as indicated by toxicological investigations. Therefore, the Ames-test should be used to demonstrate the mutagenic potential of soil eluates and the establishment of cytotoxicological tests (like the MTT-test) for nitroaromatic compounds should be helpful for investigations on genotoxicity.

Our company carried out a large scale bioremediation process for TNT-contaminated soil. During this process, each of the three different white rot fungi decreased the initial TNT-concentration to comparable low amounts and detoxfication was indicated by means of the bioluminescence bacteria test. The cytotoxicological methods mentioned above should now be applied to control any harmful risk of bioremediated soil for eucaryotic cells.

# Bioremediation of TNT Contaminated Soil With Fungi; Investigations in Metabolization, Mineralization, and Humification

The project is dealing with investigations in the remediation of soil of former armament plants by the use of fungi. The goal is the multivalent exploitation of the potential of fungi in composting and phytoremediation processes of TNT contaminated ground. Fungi of different ecological groups are already selected which are able to mineralize or metabolize nitro-aromatics with high rate. Obligatory for a bioremediation process is the establishment of the fungi in soil. By transfer of the laboratory findings with fungi-wood-soil-systems and the trail of the stability of the microbial activity in the mixed culture of a composting system the necessary conditions for the establishment of the fungi could be determined. For that purpose authentically contaminated soil is treated on-site with fungi-straw-mixtures in one cubic meter scale. In cooperation with the last project mentioned above different processes for the establishment of fungi in that soil are tested. Concurrent for the process occurs the chemical analytical and toxicological assessment of the remediation process.

Moreover, basic research oriented investigations on the balancing and optimization of the TNT degradation and on the biological processes of its humification are carried out with [$^{14}$C]TNT. In cooperation with the other projects the hazardous potential of remobilized bound residues, which could be caused by microbial activity, is estimated.

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*1450821A*

***Term:***
***03.96 - 08.98***

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# Degradation of Hexyl and RDX in Soil and Water - Development and Assessment of a Technical Process of Remediation

***Grant No.:***
*1450950 3*

***Term:***
*10.95 - 03.98*

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The aim of this study was to develop a technical process for the remediation of soil and water contaminated with the explosives Hexyl (Hexanitrodiphenylamine) and RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) for the former ammunition plant - WASAG - Torgau/Elsnig.

Different mixed microbial cultures capable of metabolizing the explosives Hexyl and RDX were obtained from soil enrichements. The enrichment, isolation and degradation studies were carried out in a mineral medium which were supplemented by Hexyl or RDX. The explosives were source of carbon and energy, source of nitrogen or both. Additionally the culture mediums were supplemented by different co-substrates. An effective degradation of Hexyl and RDX occures in the presence of a suiteable co-substrate only. For the technical cleanup process sucrose or melasse as co-substrates could be used.

To determine the ecotoxicological potential of contaminated soil and water samples of Hexyl, Hexogen and TNT were subject to different ecotoxicological testsystems. Determination of

- the inhibitory effect on the light emission of *Photobacterium phosphoreum,*
- the growth inhibition test of *Photobacterium phosphoreum,*
- the growth inhibition test of *Pseudomonas putida,*
- the inhibitory effect on the oxygen consumption of a mixed microbial culture in a closed respirometer, and
- the inhibitory effect on the growth of *Lepidium sativum* were used.

# Out Door Experiments Regarding TNT-Decontamination and [$^{14}$C]TNT-Uptake by Woody Plants

For economic reasons remediation of explosive contaminated soils by means of physical-chemical or by microbial procedures is focused on highly polluted places. For the large areas contaminated to a weak or middle degree with 2,4,6-trinitrotoluene (TNT) methods of decontamination with low expense have to be developed. For this reason the remediation potential of woody plants was studied.

After growth experiments with hydroponic solutions and in TNT-contaminated sand TNT-tolerant genotypes of willow *(Salix)* and poplar *(Populus)* could be selected. In container experiments stem cuttings of these TNT-tolerant clones were exposed to TNT-contaminated soils in the greenhouse. Plants and soils were analyzed by GC-ECD. Both in TNT-contaminated sand and in TNT/ADNT-polluted hazardous abandoned armament plant soil planting led to a decrease of the overall soil content of nitroaromatics. Furthermore, degradation of TNT to its primary reduction products 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amine-4,6-dinitro-toluene (2-ADNT) is enhanced by the plants tested. It could be shown that the decrease of nitroaromatics is partially due to plant uptake. This uptake of nitroaromatics preferentially occurs as root uptake of ADNT and translocation following the transpiration stream into the above ground region of the woody plants. Although radiotracer quantitation of the nitroaromatics portion metabolized and incorporated by the tree tissue is still open, woody plants have a sanitation potential for TNT-polluted soils.

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*(Proposal)*

***Term (expected):***
***01.98. - 12.00***

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# Uptake of TNT in annual Plants

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The project aims to use annual plants for phytoremediation of soils contaminated with nitroaromatic compounds. By use of 14C-labelled TNT the distribution of intermediates and metabolites in various plant compartments and in the rhizosphere is studied. At the start of the project, only a small percentage of TNT metabolites were identified. The following plant species will be used: *Alospecurus pratense, Bromus inermis, Festuca rubra, Helianthus annuus, Lolium perenne, Lupinus angustifolius, Medicago sativa, Phacelia* spec., *Phleum* spec., *Phaseolus vulgaris, Taraxacum officinalis, Trifolium pratense* and *Triticum aestivum.* A second main emphasize of the project is the reduction of detectable nitroaromatic compounds in the rhizosphere soil of the species studied. Similarities and differences between metabolic processes inside the plant and in the rhizosphere are also a major point in the project.

# Biodegradation of the Explosives Hexogen and Hexyl by Fungi

In 1992, a review was carried out by the Industrieanlagen-Betriebs-gesellschaft mbH (IABG) on suspected warfare-related environmental damage in the Federal Republic of Germany; identifying about 4336 sites. Some 941 sites showed a prior history of manufacture and handling of explosives and military ordinance, that may have led to extended contamination of the soil and water. To date this contamination exerts a great threat to the environment, in particular the groundwater. This study addresses the remediation of waterways contaminated with explosives by fungi.

Present studies have shown that fungi, such as the well-known american lignin-degrading fungi *Phanerochaete chrysosporium,* have the capabilities to degrade recalcitrant pollutants, such as DDT, PAK, TNT and RDX. This ability has been attributed to the non-specific extracellular lignin-enzymatic system, which can attack aromatic ring structures under oxidative and nutrient-limiting conditions.

The former explosives production site ,,WASAG-Elsnig" in Saxony was chosen as an exemplary site, due to the extended contamination with hexogen and hexyl. The microbial analysis of the soil determined numbers of colonies between $3*10^3$ and $7{,}7*10^5$ cfu/g DW. About 160 species of fungi were isolated from the site, most of which belonged to the class of fungi imperfecti and others to the zygomycetes and yeast. Around 60 were enriched and screened for their ability to degrade hexogen, showing an average rate of degradation of 240 µg/d/l. In addition, investigations were carried out with about 50 various species of european white rot fungi and other basidiomycetes, whose degradation rate were on average 300 µg/d/l.

Further research as part of this project will identify possible metabolites in the aerobic degradation pathway of hexogen in fungi through the use of radioactively-labelled hexogen. In addition, some of the screened fungi will be incubated with contaminated groundwater from the site to evaluate the degree of degradation. Furthermore the degradation process will be toxicologically supervised.

A follow-up project will allow to apply the fundamental knowledge obtained by these investigations and scale-up the process to develop a pilot plant for groundwater remediation at the site.

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***1451070***

***Term:***
***07.96 - 086.97***

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# Coordination of Joint Project No. 4: Ecotoxicological Test Batteries

***Grant No.:***
*1491031*

***Term:***
*05.96 - 04.99*

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Environmental risk assessment for contaminated sites and soil materials is often restricted to chemical analyses. Soil and soil materials are accepted to be clean if pollutant concentrations do not exceed the utilization limits. Ecotoxicological tests are a complement to chemical analysis. They provide information on the effects of all pollutants in soils which chemical analysis does not do because the bioavailability of pollutants can vary considerably depending on the chemical species and the environmental conditions. Moreover, biotests integrate combination effects of pollutants and effects of chemicals/metabolites which were not detected in routine analysis.

Existing ecotoxicological test methods were developed to describe the ecotoxic potential of a test substance added to a soil/soil material. These methods can be partly used for the ecotoxicological characterization of soils and soil materials. Thus the aim of the project is to develop and validate ecotoxicological tests for different soil functions with respect to their intended use considering the retention function (Ability of soils/soil materials to adsorb pollutants in such way that they cannot be mobilized via the water pathway) and the habitat function (Ability of soils/soil materials to serve as a substrate for plant growth and as a basis for biocoenosis). Criteria for the selection of tests are sensibility, reproducibility, costs and duration of tests. Besides the development and validation of test methods the storage of soil samples will be also investigated.

As a final result of this project a compilation of biotests “Ecotoxicological Test Batteries” will be provided. These methods can be used by official authorities and remediation companies for risk assessment and remediation control. The test battery will become part of the BMBF-handbook “Processes for the Bioremediation of Soil”.

# Validation of Ecotoxicological Test Methods (and Coordination)

The aim of the project is to develop and validate ecotoxicological tests for different soil functions with respect to their intended use considering the ability of soils/soil materials to serve as a substrate for plant growth and as a basis for biocoenosis (habitat function) and the ability of soils/soil materials to adsorb pollutants in such way that they cannot be mobilized via the water pathway (retention function)

Habitat function will be tested by

- plant growth inhibition test (ISO 11269-2) with oat, cress and turnip and
- microbial activity tests (respiration, nitrification)

Retention function will be tested by the ToxychromoPad test (ß-galactosidase activity). Water soluble pollutants endanger not only ground- and drinking water, they have also the highest bioavailability. The ToxychromoPad test should give informations about the ecotoxicological effects of soil eluates.

The results will become part of the BMBF-handbook " Processes for the Bioremediation of Soil ".

As a final result a compilation of biotests “Ecotoxicological Test Batteries” will be provided for risk assessment and remediation control.

***Grant No.:***
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# Investigations Regarding the Practicability and Standardization of Test Methods Using Terrestrial Invertebrates

***Grant No.:***
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The objective of remediation is the restoration of soil as habitat for plants and animals. The assessment of different methods of soil remediation using toxicity testing with terrestrial invertebrates has to be performed with indicator species, exposed to the full range of contaminants present in soil. Appropriate test systems must record the hazards and risks to the biocoenosis with sufficient sensitivity and precision, give reproducible results, be competitive and easy to perform.

Standard test methods for soil using such indicator species are the acute and sublethal toxicity test with *Enchytraeus crypticus,* a tiny soil annelid and *Folsomia candida,* a springtail. The toxicity endpoints are mortality after 1 to 7 days and reproduction after 28 days of exposure. The reproduction test corresponds to a life cycle test as the generation time for both species is only about 14 days. The endpoints represent structural parameters of the ecosystem such as abundance and population structure.

With both species toxicity tests can be performed in natural soil. Basis of a reliable recording of the effect of environmental chemicals is (a) the possibility to raise both species on uncontaminated substrate all year round in sufficient numbers, (b) the possibility to produce synchronized test populations and (c) the high number of offspring during the sublethal test. Of practical importance is the small amount of soil needed per replicate (10 to 15 g in the acute test, 25 to 30 g in the reproduction test).

Dose-response relationships provide the basis for assessment of hazards and risks presented by environmental chemicals .The results obtained so far with soils contaminated with mineral oil hydrocarbons, polycyclic aromatic hydrocarbons (PAH), trinitrotoluene (TNT) and heavy metals prove the superiority of the reproduction test to the acute test.

Functional ecosystem parameters like the activity of saprophage animals can be accounted for by the lamina bait test developed by VON TÖRNE. This test indicates the effects of environmental chemicals upon the decomposition of organic mater in soil in a very rapid and simple way. The adaptation and standardization of this outdoor test system for laboratory use is in progress.

# Development and Implementation of a Standardized Test System with Soil Protozoa

Protozoa represent an essential element in microbial loops and comprise the crucial link between bacteria and small metazoa. They inhabit the water films surrounding soil particles in the root as well as the litter zone. More than two thirds of metabolic activity (respiration) of the soil fauna can be ascribed to these unicellular organisms, making them fundamental in matter cycling and soil remineralisation processes. Despite their ecological key position for the soil environment, no ecotoxicological tests exist with representatives from this group of animals.

The aim of the project is to develop a practice oriented and versatile test using the cosmopolitan soil protozoa of the genus *Colpoda* (Ciliates). Representatives of this dominant genus in soil can be regarded as promising test organisms for ecotoxicological purposes. Due to their unicellular structure, without a protective cell wall, these naked protozoa are directly exposed to contaminants in water films. Their complex eucaryotic cell structure with physiological characteristics, normally found only in higher animals, enables the monitoring of a wide range of toxic injuries. As ,,r"-strategists, with a generation time of only a few hours, multiple generation assays can record chronic effects of contaminants within short periods.

The test should be included in a battery of tests for the biological assessment of soil remediation and is envisaged as a component of an integral biological evaluation of contaminated sites.

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# Use of Genetically Engineered Microorganisms as Bio-indicators for the Redevelopment of Hazardous Abandoned Sites

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*1490914*

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This project is concerned with the development and application of genetically engineered microorganisms as a new type of bioindicators for the detection of toxic or mutagenic compounds in soil. By the use of reporter genes encoding for traits not present in the natural soil bacterial community and easily detectable, e. g. bioluminescence, the effect of an exposure to toxic or mutagenic substances will be detected. Therefore, selected bacterial strains are genetically engineered by insertions of such reporter genes. The genes are either constitutively expressed or inducible by exposure to different stresses. Reporter bacteria, capable of surviving in the soil environment, are then inoculated into soil samples and, immediately after reextraction of the inoculated cells, the specific activity of reporter genes will be determined. This determination can be performed with either cultivated cells (colonies) or directly with luminometric cell activity measurements. In addition to research concerned with the use of genetically engineered microorganisms we are testing a microtitre plate based assay which aims at detecting the immediate metabolic response of heterotrophic, aerobic indigenous soil bacteria for the degradation of 95 different carbon sources. It is expected that in contaminated soil less substrates are being utilized as a result of a decreased biomass and a selection of less diverse microbial communities, Finally, other methods which will be developed in this project, aim at detecting the diversity of the soil microbial community at the level of heterogeneity of DNA, directly extracted from soil.

# Test of Positive Selection Vectors for the Detection of Mutagenic Substances by Using Soil Bacteria

Objective of the project is the development and test of genetically modified reporter bacteria, which can be used to detect the presence of mutagenic substances in soils. The test system will be developed by using soil bacteria, which can survive and multiply in soil microcosms, so that the bioavailability of contaminants can be measured without extraction of the substances.

The concept is based on the use of broad host-range vectors and indicator genes, the inactivation of which by any kind of mutation can be positively selected. Two systems are being tested: the rpsL-gene (phenotype after mutation is resistance against streptomycin) to be used with GRAM-negative bacteria and the sacB gene (phenotype after mutation is tolerance of high concentrations of sucrose) to be used with GRAM-positive bacteria.

The worktask comprises the following experimental steps:

- Introduction of the reporter genes using different vectors (multi- or low-copy number plasmids and transposons) into a number of soil bacteria which have been modified by appropriate chromosomal mutations.
- Test of the stability of the vectors as well as the expression of the reporter genes in laboratory models under various conditions.
- Analysis of the behavior of the reporter bacteria in soil microcosms (survival, multiplication, efficiency of recovery).
- Validation of the new bioindicator system by comparison with established methods (e.g. Ames test).

***Grant No.:***
***1490915***

***Term:***
*05.95 - 04.98*

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# Development and Investigation of Genetically Engineered Soil Bacteria as Bioindicators

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*05.95 - 04.98*

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The main objectives of the project are the development and use of bioindicators for the detection of toxic and genotoxic compounds in contaminated soils, For this purpose three different types of bacterial reporters will be used.

- Typ I reporters are unable to perform RecA-mediated homologous recombination (RecA) and consequently, display an enhanced sensitivity to DNA damaging agents.
- Typ II reporters carry promotorless reporter genes fused to the *recA* gene. The presence of inducing mutagenic compounds is indicated by the expression of the reporter gene.
- Typ III reporters carry two inactive copies of an antibiotic resistance gene. Any stimulation of the RecA-mediated homologous recombination is indicated by the frequency with which the two inactive copies of the antibiotic resistance gene are converted to one active copy.

Using isogenic bioluminescent *Rhizobium meliloti* strains L1 (RecA) and L33 (RecA*) as type I reporters, their survival in various non-contamined soil types was assessed. These analyses showed that strain L1 displayed only a slightly reduced survival ability compared to strain L33. Consequently, detection of genotoxic compounds by using isogenic strains L1 and L33 might be indicated by a pronounced reduction in survival ability of strain L1. Analysis of the survival of both strains in sterile soil in the presence of the model compound nalidixic acid again showed that RecA-strain L1 displayed a reduced survival capability. However, compared to the results obtained in experiments using liquid culture, the sensitivity of the strains to nalidixic acid was reduced. This result might be explained by a reduced bioavailability of the model compound in soil. *R. meliloti* strain IG1, a type II reporter which carries the promotorless *Escherichia coli gusA* gene mediating GUS activity fused to the *recA* gene was analysed for gene induction using the genotoxic compound mitomycin C. These analyses showed that the *recA* gene was induced in a dose-respone manner by a factor of five. Presently, experiments are under progress to construct type III reporters.

# Soil Extraction Procedures to Assess the Ecotoxicological Potential of Soils

The objective of the project is to elaborate elution methods suitable for evaluating potential risks of residual soil contaminations with respect to a hazard for groundwater and - with reduced evidence - for a reduction of the ecological soil function.

For solid materials a number of elution methods originally developed for different purposes are available, which do not yield directly comparable results. Moreover, most of the existing elution methods have to be modified for routine analysis of organic soil contaminations. Therefore, elution methods for soils urgently need to be validated and standardized to extend the spectrum of methods available for elaborating a handbook for the investigation of biologically decontaminated soils and normalization, respectively.

Elution methods for soils are needed to:

- determine the mobile portions of pollutants (exposure of groundwater and surface waters by metals and organic contaminants)
- determine the bioavailable portions of a contamination and their hazard potential in the soil eluates (exposure of the epigeic fauna).

The validation of the earthworm test for the objectives of the joint project will be a further contribution of the Fraunhofer Institute extending the scope of the study units ,,Investigation of the feasibility of ecotoxicological tests" (Prof. Wilke, Prof. Achazi). The results and handbook proposals will be included in the guidance "Processes for the Bioremediation of Soil".

***Grant No.:***
1491058

***Term:***
***10.96 - 08.99***

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# Development and Validation of Genotoxicological and Ecotoxicological Methods for the Valuation of the Retention Function of Soils

***Grant No.:***
*1491076*

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*17.96 - 70.99*

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The objective of the project is the development and the validation of biological test methods for the valuation of biological soil remediation success. Different biotests standardized for testing of water/wastewater and chemicals will be proofed for their suitability to characterize the bioavailable genotoxicological and ecotoxicological potential of contaminated soils. Investigations will be focused on the determination of the availability of pollutants for the aqueous path exposure route. Therefore, the test methods will be adapted to the specific conditions of the complex soil matrix.

Based on the above mentioned pre-investigations several soils of different origin contaminated with PAH, mineral oil and military chemicals will be examined before and after bioremediation by techniques of practical relevance. Furthermore, instructions for test application as well as proposals for the valuation of test results will be worked out and published in the part “Ecotoxicological Test Batteries” of the handbooks “Processes for the Bioremediation of Soil” planned by the BMBF.

The investigations of all project partners will contribute within a strategy for the valuation of the ecotoxicological potential of contaminated soils. These strategy will allow a practically biotest-based risk assessment of decontaminated soils. The results will be usefull for companies offering remediation techniques as well as for engeneering companies and public authorities which are responsible for the risk assessment of contaminated areas.

# Development of a Chronical Toxicity Test Using Plants

The project intends the development and validation of a terrestrial plant life-cycle test under laboratory conditions. The test can be used in risk assessment for environmental chemicals. After modification the test will be applied for soil quality assessment, e.g. after soil remediation. The work will be performed in three steps:

1. standardization of the measurement of chronic endpoints in plants,

2. adaptation of the method to plants growing in contaminated soil, and

3. preparation of a draft test handbook based on the experiences of the experimental parts of the project.

All tests will be performed using one monocotyledoneous and one dicotyledoneous plant species. The various activities will be done in close cooperation with other participants of the joint project.

***Grant No.:***
***1491077***

***Term:***
*01.97 - 12.99*

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# Determination of Optimal Storage Conditions for Reserve Soil Samples with Different Pollutants for Toxicological Examinations

***Grant No.:***
***1491080***

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*11.96 - 10.98*

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At present there is a high level of uncertainty whether or not soil samples, that are required to be toxicologically characterized, could be preserved without affecting their toxicological characteristics. In the existing DIN-ISO-norms for soil investigations there is no documentation of how soil samples should be preserved after collection. Furthermore, procedures for receiving justifiable and verifiable results from soil samples are quite vague.

Therefore, the stability and changes in the biological effects of different soil contents are to be investigated by varying the storage conditions, the storage times as well as the physical, chemical and biological qualities of the soil. The limitations of storing soils could therefore be subsequently outlined. From the results of the investigations, recommendations for the optimization of storage conditions for reserve soil samples would be put forward. These recommendations have the capability of finding entrance into DIN/ISO-norms and in the standards for investigations under the new federal soil protection laws of Germany.

# Coordination of Joint Project No. 5: Evaluation of the Long-Term Stability and Remobilization Potential of Residual Xenobiotica After Bioremediation

The intentional fixation or binding of xenobiotica into soil organic mater (controlled humification) shall become part of the bioremediation of contaminated soils. To assess the ecological sustainability of this intentional immobilization- or clean-up-method the long-term stability and the remobilization of the humificated xenobiotics should be investigated in detail. A scientific study comparing all available results from different investigations which allow to assess the long-time risk of humificated compounds is still missing. For this reason this project was initiated to collect and elucidate the experience about the long-term behaviour and the remobilization of bound xenobiotics in the course of biological soil clean-up. This knowledge will be described and evaluated in a literature study.

Another aim of the study is to determine existing deficits for risk assessment and to coordinate further research to close the gaps. Part of such experimental research will be carried within the project it self.

Thus it was not investigated yet which impact humic and loamy soils do have on the long-term stability of humified PAH. The influence of soil fauna on the remobilization of non-extractable $^{14}$C-PAH-residues was also not estimated yet. Both aspects will be studied in the experimental section of this project. The ecological stress conditions will be based on previous investigations (cooperation “Humification of PAH”, Single Project 2.6; grant no. 1480937).

The work of the project will result in the preparation of the chapter “Evaluation of the long-term stability and remobilization potential of residual xenobiotics after bioremediation” within the planned guide on “Processes for the Bioremediation of Soil”.

***Grant No.:***
***14810925***

***Term:***
***07.97 - 06.00***

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# Coordination of Joint Project No. 6: True to Scale Test of Biological Processes with Assessment at the Site 'Tanne' next to Clausthal-Zellerfeld

***Grant No.:***
*1451129/2*

***Term:***
*05.97 - 06.99 (extension to 12.99 planed)*

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The project should prove the biological remedy treatments at the former ammunition plant 'Tanne' in Clausthal-Zellerfeld (Lower-Saxony) and document the results as a success control. This results - specially concerning the universally validy aspects - should lead to a manual for biological remedy and quality standards. For authorities, concerning enterprises and investors this manual should offer an appreciate guide (and handle). In a first step the treatment was choosen by selected consultants and the remedy target was coordinated Now the last preparations for the realization of the project take place. The IABG takes over the central project management for the tasks of the site coordination, the scientific attendance and the planification for the required construction works. Mainpoint of the scientific attendance is the success control and the comparative valuation of the choosen treatments as well as the characterization of the results. Beside the classical analysis all new results of Joint Project No. 4 (ecotoxicological test batteries) and Joint Project No 5 (Long-Term Stability and Remobilization Potential) will be considered. Furthermore an important criteria will be the statements for biological security, profitability and efficience of the special treatments.

# Humifying Decontamination of Soils Containing Explosives by an Anaerobic/Aerobic Process (Composting Process)

The composting process tested at the ,,Tanne" location is based on a step-by-step transformation of the initial substance, TNT, under anaerobic conditions, resulting in the end product, triaminotoluene (TAT). The reduction products, such as DAT and TAT, react completely with the surrounding soil components, such as humus fractions and clay minerals, and are irreversibly bonded in an aerobic step. Bonded TAT cannot be dissolved from the soil even under radical extraction conditions (solvents, acidic or alkaline hydrolysis).

To achieve rapid decontamination of the soil material through a large-scale technical process, it is necessary

- to activate the already adapted local microbiology,
- to minimise the transport resistance due to the crystalline structure of the nitroaromatic compounds and
- to implement a two-stage anaerobic/aerobic process.

A composting process is deployed in which soil from which impurities have been removed is mixed with various, easily recoverable, oxygen-depleting substrates as well as structural material and placed into windrows.

By turning the soil at frequent intervals, oxygen pulses are inputted in the otherwise anaerobic (anoxic) soil/substrate mix, thus causing a rapid degradation of the contaminants. To test the process, 210 t of soil are available that will be processed in 3 batches.

According to previous preliminary tests, a contaminant reduction of over 98% can be expected within 5 weeks. In a further rerotting phase (aerobic step), complete contaminant turnover is possible. However, the remediation goal or remediation performance that can be achieved at the Tanne location depends to a great extent on the local conditions, such as type of soil, spectrum of contaminants, initial concentration and distribution of contaminants in the soil.

The aim of the project is to determine the max. capacity of the process as well as ways of modifying the composting process. The focus will be on studies concerning long-term stability on treated soil that will be conducted by the Fraunhofer lnstitut für Grenzflächen- und Bioverfahrenstechnik.

***Grant No.:***
*1451129/5*

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*01.98 - 12.99*

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# Irreversible Transformation and Immobilisation of TNT for the Detoxification of Contaminated Soils (Dynamic Pile Process)

***Grant No.:***
1451129/1

***Term:***
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As part of the Integrated project 3, “Biological Remediation of Hazardous Wastes from Past Ammunition Manufacture”, project part TV 3.1, “Development of a Microbiological Remediation Process Using the Former Explosives Factory ‘Tanne’ at Clausthal Zellerfeld as an Example”, Plambeck ContraCon GmbH is pursuing the task of implementing interesting research work by their university collaborators to produce economical and exploitable large-scale microbiological processes for the remediation of former munitions plants and military operations sites (TV 3.1.4).

Together with the University of Marburg (TV 3.1.1), promising initial results from the development of a process for anaerobic/aerobic immobilisation of nitroaromatics to medium-scale standards have been achieved and will be tested true to scale. The process allows soils contaminated with explosives to be treated in conventional dynamic beds. The process is based on the principle of transforming harmful substances under anaerobic conditions to produce reduction products, which are then bound irreversibly to the soil matrix under aerobic conditions. According to present knowledge, the bonding reactions are comparable to those associated with the formation of humus. The biological processes represent an economical addition to the thermal soil-treatment processes which are currently regarded as the state of the art for remediation of explosives-contaminated soils.

# Bioremedeation of Hazardous Abandoned Armament Sites with the Aid of White Rot Fungi and Other Fungi (White Rot Fungi Process)

Soils of former armament production sites are often contaminated with nitroaromatics. Within the framework of the joint project No. 3 ,,Bioremediation of hazardous abandoned armament sites". WISSTRANS Umwelt GmbH company has evolved a process for the biological redevelopment of these soils with the aid of white rot fungi (TV3.3).

The adjustment of optimal conditions for degradation on a technical scale is of special interest. In particular, the nutrition and the supply of oxygen for the fungus, which has to grow in the soil, have to be maintained over long periods of time. Different thicknesses of layers of soil and various ratios of fungus and soil will be tested.

The fungus has to predominate over the soil-specific microflora for optimal detoxification. In order to achieve this, the humidity and aeration of the soil will be optimized. A watering installation, which serves also for the supplementation with nutrients is necessary for this purpose. The aeration of the soil is carried out by an exhauster, which removes surplus water as well. Exhauster air and effluent are decontaminated afterwards. Especially in the final phase of decontamination, the concentration of harmful substances in the soil is minimized by addition of fresh cultures of the fungus.

The procedure is performed at a nearly constant temperature in a redevelopment shed, which is especially rebuilt for this purpose. The white rot fungus Bjerkandera adusta and the straw decomposing fungus Stropharia rugosoannulata (in cooperation with the chair of technical microbiology of the university of Jena) are compared in their remediation performance. The fungus which proves to be more efficient in the depletion of harmful substances in the soil and which shows a higher detoxification rate will be optimized in a third experiment. The influence of the natural climate on the remediated soils is examined by applying them to an outdoor stack and by analyzing them for a possible liberation of residues.

During the whole phase of decontamination, the depletion of harmful substances and the detoxification as well as common soil parameters will be determined. The examination of the toxic potential is performed with fluorescent bacteria and algae tests. Moreover, since safety standards have to be observed, liberated volatile compounds will be recorded, and the interior as well as the exhausted air will be examined with respect to mono-nitroaromates and fungus spores.

***Grant No.:***
*1451129/3*

***Term:***
01.98 - 10.99

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# Invesigation of the microbial in-situ mineralization of volatile chlorinated hydrocarbons (VCH)

**Grant No.:**
*1481079*

**Term:**
*06.96 - 09.98*

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Besides the most frequently detected VCH tetrachloroethene (PCE) and trichloroethene (TCE), 1,1,1-trichloroethane (TCA) is also an important contaminant. The results of the analytical monitoring of hydraulical safety measures at the site Pintsch, Hanau, revealed that the concentrations of PCE and TCE were lowered by intrinsic bioremediation. TCA however, was recalcitrant. Within the research and development project (R&D) it will be investigated whether the microbial in-situ-degradation of all VCH can be accelerated. A cometabolic sequential anaerob-aerob-process was chosen as a preferred procedure. During the anaerobic stage, the higher chlorinated VCH are transformed to lower chlorinated metabolites which are mineralized during the aerobic stage. The different stages are separated by time instead of by space.

In laboratory investigations, the transformation kinetics will be optimized. This includes a proper choice of nutrient supply, environmental conditions as well as the identification and elimination of inhibitory factors and the enhancement of the desorption of VCH from the soil matrix. In column tests, the question whether anaerobic bacteria survive in micro-compartiments during a bulk aerobic stage and the development of anaerobic metabolic activities after initiation of the anerobic stage will be investigated. The results of the laboratory investigations will be transferred to a field test at the Pintsch site where the process can be optimized in this scale. Thus, the investigations will not only provide scientific findings but also an optimization of the technical scale in-situ remediation and a cost reduction,

# Investigations into the microbial degradation of persistent mineral oil fractions using fatty acid-acylated amino acids

The main problem in the remediation of soils long term-contaminated with mineral oils is that the process stops after reaching residual concentrations between 10 and 50% of the starting value - depending on the type and the age of the damage. The aim of the project is the minimization of residues which remain after microbial degradation of mineral oil (especially in sludges from soil washing) by the addition of special tensides: fatty acid-acylated amino acids.

The main reasons for the remaining of residues are (provided a suitable microbial community and adequate physiological conditions) the structure-based persistence of the hydrocarbons contained and an unsufficient bioavailability. The latter can be improved by the addition of tensides. In preceding experiments with the addition of fatty acid-acylated amino acids a further microbial degradation of the hydrocarbons of the fine-grain fraction from a soil washing plant could be achieved.

The project focusses on the search of suitable and efficient combinations of amino acids/fatty acids (including the variation of the chain length of the fatty acids, the influence of the configuration of the amino acids, the suitability of non-proteinogenic amino acids), the optimization of the physiological parameters for degrading the mineral oils with the most suitable tensid, the elucidation of the fundamentals of the action of tensides, the test of protein hydrolysates from vegetable materials as the source of amino acids as well as accompanying analytical investigations. In the case of positive results of our investigations, which represent a feasibility study, a technical test will be carried out in cooperation with companies running soil washing plants. We intend to lower the hydrocarbon content of soils and washing sludges to values which will allow to reuse them.

***Grant No.:***
*1471086*

***Term:***
*07.97 - 12.98*

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# Synthesis of fatty acid-acylated amino acids and protein hydrolysates as detergents for the microbial degradation of persistent mineral oil fractions

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This research project is carried out in close collaboration between our group at the Bergische Universität in Wuppertal and the Umweltforschungs-zentrum (UFZ) in Leipzig. Main goal of our co-operative research programme is the improvement of the microbial degradation of (mineral) oils in correspondingly contaminated soils and sludges, and here especially of otherwise nondegradable residues.

In earlier experiments our partners at the UFZ discovered that the addition of certain surface active compounds such as acylated amino acids (amino acids modified with fatty acids) - considerably enhances such microbial degradations. Consequently, we are now concentrating on the improvement and optimisation of this process by providing a broad spectrum of such acylated amino acids by systematic variation of (a) the type of amino acid and (b) the employed fatty acid according to chain length and degree of unsaturation.

Using native amino acids and typical fatty acids such as caprylic-, lauric-, palmic-, stearic- and oleic acid - all available from native oils - we are synthesizing a broad range of these biodegradable surfactants which will be tested at the UFZ for their efficiency in accelerating the microbial degradation of mineral oils in contaminated soil and sludge samples. Next to the improvement and optimisation of the degradation process this project is aimed also at the elucidation of the involved mechanisms by synthesising labelled compounds and employing unnatural amino acids.

Due their chemical structure - consisting entirely of natural components - these surfactants are fully biodegradable and could also be highly useful for other applications such as the treatment of oil spills in environmentally sensitive areas.