

## Literature Overview

# Dendroremediation of Trinitrotoluene (TNT)

## Part 1: Literature Overview and Research Concept

### Part 1: Literature Overview and Research Concept – Part 2: Fate of Radio-labelled TNT in Trees

**Preamble.** Phytoremediation technologies are of rapid growing concern for the cost effective sanitation of large areas polluted with low to middle or even an unknown degree of contamination. The interest in phytoremediation research is extensively mirrored by ecomed publisher's ScientificJournals (<http://www.scientificjournals.com>) [see JSS – J Soils & Sediments 3 (2) 72 (2003)]. Mainly, because the current number of phytoremediation reports from internet sources nearly exploded to an information jungle, the term 'dendroremediation' is preferred if trees are used or tested as sustainable remediation tools or if the role of trees in natural attenuation processes should be assessed. Using the example of the environmental TNT problem, a holistic approach was tried for the dendroremediation of trinitrotoluene and its concomitant hazards. The TNT dendroremediation research concept, presented in Part I, should be an interdisciplinary compromise between basic research and practical application. Part II reports on results regarding the fate of [<sup>14</sup>C]-TNT in compartments of older trees of willow and Norway spruce.

Bernd W. Schoenmuth\* and Wilfried Pestemer

Institute for Ecotoxicology and Ecochemistry in Plant Protection, Federal Biological Research Centre for Agriculture and Forestry (BBA), Koenigin-Luise-Str. 19, D-14195 Berlin, Germany

\* Corresponding author ([berndschoenmuth@yahoo.de](mailto:berndschoenmuth@yahoo.de))

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### Abstract

**Background, Aim and Scope.** For decades, very large areas of former military sites have been contaminated diffusely with the persistent nitroaromatic explosive 2,4,6-trinitrotoluene (TNT). The recalcitrance of the environmental hazard TNT is to a great extent due to its particulate soil existence, which leads to slow but continuous leaching processes. Although improper handling during the manufacture of TNT seems to be a problem of the past in developed countries, environmental deposition of TNT and other explosives is still going on unfortunately, resulting from thousands of unexploded ordnance or low order explosions at munitions test areas and at current battlefields.

**Objective.** Sustainable phytoremediation strategies for explosives in Germany, which intend to use trees to decontaminate soil and groundwater ('dendroremediation'), have to consider that most of the former German military sites are already covered with woodlands, mainly with conifer stands. Therefore, parallel investigation of the remediation potential is necessary for both of the selected hybrids of fast growing broadleaf trees, which are waiting for planting and forest conifers, which have already proven for decades that they are able to grow on explosive contaminated sites.

**Main Features.** A short literature review is given regarding phytoremediation of TNT with herbaceous plants and some general aspects of dendroremediation are discussed. Furthermore, an overview of our TNT-dendroremediation research network is introduced, which has the strategic goal to make dendroremediation more calculable for a series of potent trees for site-adapted *in situ* application and for the assessment of tree remediation potentials in natural attenuation processes.

**Results and Discussion.** Some of our methods, results and conclusions yet unpublished are presented. For a preliminary calculation of area-related annual TNT dendroremediation potential of five-year-old trees, the following values were assessed: *Salix* EW-13 6.0, *Salix* EW-20 8.5, *Populus* ZP-007 4.2, *Betula pendula* 5.2, *Picea abies* 1.9 and *Pinus sylvestris* 0.8 g m<sup>-2</sup> a<sup>-1</sup>. For a 45-year-old spruce forest, an annual natural attenuation potential of 4.2 g TNT m<sup>-2</sup> a<sup>-1</sup> was found.

**Conclusion, Recommendations and Perspective.** Our main results deliver quantitative proposals for dendroremediation strategies *in situ* and provide decision aids. Also aspects of growth of raw materials for energy production are considered. Our dendroremediation research concept for TNT and its congeners can be easily completed for other trees of interest and it can also be applied to herbaceous plants. Knowing the current bottlenecks of phytoremediation and considering the known environmental behaviour of other contaminants, elements of our methodological approach may be easily adapted to those pollutant groups, e.g. for pesticides, pharmaceuticals, PAHs, chlorinated recalcitrants and, with some restrictions, to inorganics and to multiple contaminations. Our dynamical dendrotolerance test systems will help to predict tree growth on polluted areas. To provide some light into the black box of TNT dendroremediation, experimental data regarding the uptake, distribution and degradation of [<sup>14</sup>C]-TNT in mature tree tissues will be reported in the second part of this publication.

**Keywords:** Coniferous tree; deciduous tree; dendroremediation; explosives; natural attenuation; nitroaromatic compounds; phytoremediation; soil decontamination; TNT (2,4,6-trinitrotoluene)

### 1 Background: The TNT-Problem

For decades, many former military sites in Germany have been contaminated with the explosive 2,4,6-trinitrotoluene (TNT) and its co-contaminants, mainly resulting from World War II [1]. Besides known contamination hot spots at former production and filling sites for explosives, there are larger areas around these sites, where TNT pollution of soils is rather diffuse and hot spots are often undetected. Similarly diffusely contaminated are former battlefields and former or current firing ranges, where residues of low order ordnance explosions (partial detonations) are found and unexploded munitions are slowly corroding. Persistence of ex-

plosives from production in World War One [2] and from massive delaboration after WW I illustrates the longevity of the TNT-problem.

Manifold TNT-binding to clay minerals and/or to organic matrices [3], and especially particulate existence of TNT in the form of microcrystalline solids [4], grain particles [5], and nuggets [6] cause us to be afraid that slow TNT-leaching into soil solutions will continue for the next hundred years. For instance, solid fragments of TNT in contaminated soil had to be pre-treated in a TNT-dissolving slurry process with enormous amounts of acetone, before composting of slurry was successful [7] and TNT soil samples had to be mill-ground in order to achieve subsample homogeneity [4,8].

For Germany Schröder et al. (2003) [9] reported that former military sites (ammunition plants and military training areas) represent 2.8% (9997 km<sup>2</sup>) of the entire territory. Unfortunately, the large area TNT-problem is still growing on currently used firing ranges and military training areas, which were approximated to cover several thousand square kilometres in the U.S.A. [8].

The hazardous potential, inclusive mammalian toxicity, mutagenic and carcinogenic features of TNT, of TNT transformation products and other explosive specific compounds, are reviewed by Talmadge et al. (1999) [10]. Newer results of TNT-toxicity to microbes are presented by Frische and Höper (2003) [11]. Toxicological data of military range contaminants are summarised in a database [12]. Schäfer (2002) [13] presented toxicity of explosives regarding the habitat function of TNT-soils and further ecotoxicological and genotoxicological assessments were made by Hund-Rinke et al. (2002) [14]. Phytotoxicity of TNT and of the explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) to *Lactuca sativa* and *Hordeum vulgare* was determined in forest soils [15].

## 2 Main Features

### 2.1 Phytoremediation of TNT

Forced by economic reasons, on the search for cost effective biological soil decontamination methods for large areas, the importance of phytoremediation is growing as a public-accepted strategy for *in situ* sanitation of soil and water contaminated with explosives. Reviews, where phytoremediation of explosives and other organics are evaluated, were recently published [16–23] and proposals for metabolic pathways of TNT degradation in plant tissues were made [24–27].

TNT-uptake and transformation was proven in numerous terrestrial herbaceous crop plants like *Allium schoenoprasum* [28], *Cyperus esculentus* [29,30], *Phaseolus vulgaris* [31–35], *Triticum aestivum* [36], *Medicago sativa* [28], in the fibre plant kenaf (*Hibiscus cannabinus*) [37], in aquatic plants like *Myriophyllum aquaticum* [38–41], *M. spicatum* [42], and other submersed and emerged wetland plants [43–45], in the blue-green algae *Anabaena* [46] and in axenic cell suspension cultures of *Datura innoxia* [47]. TNT uptake may vary in species dependent on herbaceous plants [48] and trees [49]. Also, for other common explosives, herbaceous plant uptake and accumulation has been shown, e.g. for RDX (hexahydro-

1,3,5-trinitro-1,3,5-triazine) [43,50–52], HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) [37,51–53], and tetryl (2,4,6-trinitrophenylmethylnitramine) [54].

### 2.2 Dendroremediation

It is obvious that the only plants capable of confronting the longevity of recalcitrant soil pollutants *in situ* are long-living trees. Trees can meet the demands of sustainable phytoremediation, of long-term bioindication and of bio-energy production. Low nutrient and soil quality requirements and high tolerance to many soil pollutants enable trees to survive on real contaminated sites where most crop plants fail to grow. Woody plants, equipped by evolution with a very long life at the same site, and reaching sexual maturity often after decades must obviously be fitted with multiple adaptation mechanisms to withstand a variety of natural environmental hazards within their life cycle.

In this paper, the term 'dendroremediation', introduced by Nguyen et al. (2002) [55,56], is preferred for the use of trees and other woody plants to remediate contaminated soil, (ground) water (and air). We suggest that dendroremediation should consider the tree as a whole with the surrounding soil, with the associated microflora and as a part of the environment. Tree roots exsude various organics [57,58] and give a home for soil microorganisms, which are tree-associated, such as mycorrhizal fungi and rhizosphere bacteria [58–60]. A dendroremediation research concept should also consider microbial consortia, living more far away from the rhizosphere in bulk soil where tree-produced matter (dead roots, dead wood, litter, soil added bark or wood chips) is used as a substrate and as a microenvironment for pollutant degrading microbial activities.

Our dendroremediation research is directed on both, on beneficial effects of newly planted phytoremediation trees and on the natural attenuation potential of older trees, which already grew for decades on polluted sites (see Fig 1).

## 3 Results and Discussion

### 3.1 Dendroremediation of explosives

The first evidence for TNT uptake by trees was found in 1996 in a previous project in experiments with juvenile, rooted hardwood cuttings of various willow clones (*Salix spec.*) and hybrid poplars (*Populus spec.*) [49,61]. After tree planting of both TNT spiked sand and former ammunition plant soil (AP-soil), TNT was mainly found in form of its primary reduction products 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-4,6-dinitrotoluene (2-ADNT) in tree tissue. ADNTs and smaller amounts of TNT were accumulated in roots. ADNT tissue concentrations successively declined apicalwards up to the leaves, where TNT was only found in traces [49].

Thompson et al. (1998) [24] balanced uptake and degradation of TNT in freshly rooted poplar hardwood cuttings using [<sup>14</sup>C]-labelled 2,4,6-trinitrotoluene. Also, uptake of the explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) was shown for hybrid poplar trees (*Populus deltoides*

*x nigra*, DN34). Even under saturated conditions HMX showed no dendrotoxicity, although it was intensively taken up from hydroponic solutions [62]. Hydroponic translocation studies of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in poplar showed that up to 60% of [ $^{14}\text{C}$ ]-RDX taken up by the trees accumulated in leaf tissue [63]. Dobner (2003) [64] found no mycorrhiza effects of *Pisolithus tinctorius* on TNT-uptake of pine seedlings (*Pinus sylvestris*) and aspen seedlings (*Populus tremula*) were more effective in the elimination of ADNTs when the plants were mycorrhized with *Hebeloma spec.*

Transpiration of trees [65,66] plays a major and a dual role in dendroremediation. Firstly, transpiration is a solar-driven force for indirect transport of water and thus of pollutants within the soil and within the plant. On the other hand, pollutant-induced transpiration decrease may serve as a measure of dendrotoxicity [67,68]. For instance, TNT reduces transpiration in young poplar hardwood cuttings [69]. We utilized this TNT-induced tree transpiration decrease in parallel assessments of dendrotolerance and dendroremediation capacity with juvenile *Salix* and *Populus* and mature trees of *Salix*, *Picea* and *Pinus* (Schoenmuth and Pestermer 200X, in prep.). Tree transpiration systems for testing phytotoxicity of different xenobiotics are also established by others for cuttings of willow [67] and poplar [68–70].

### 3.2 Dendroremediation research network for TNT

Trapp and Karlson (2001) [18] criticized that success of phytoremediation projects was not frequently controlled, and that the applicability and potential of phytoremediation were not assessable.

Within the Joint Project 'Bioremediation of Hazardous Abandoned Armament Sites' of the German Federal Ministry of Education and Research (BMBF) the subproject 'Tanne' was implemented as an *in situ* TNT-dendroremediation experiment with a comprehensive success control programme [71–76]. Mixed planting of Norway spruce (*Picea abies*), aspen (*Populus tremula*), and elder (*Sambucus nigra*) was combined with variants of mycorrhized plants and added white-rot fungi with a drastical soil homogenisation. Control of success was monitored chemically by soil core analysis and estimation of nitroaromatic soil solution concentrations. Ecotoxicological monitoring was performed using a biotest-battery and ecological field indicators. The results show that an initial rapid decrease of 80% of soil TNT-content is dominated by soil homogenisation within the first months and tree remediation effects are slowly emerging.

Our work was integrated into the above-mentioned BMBF joint project as well. Our strategic goal was to search for ways to determine quantities of tree mediated sanitation of TNT-polluted soil and thus, making tree-specific dendroremediation calculable (for overview, see Fig. 1). In a first step, screening procedures for growth tolerance to TNT and for TNT uptake as well delivered TNT-tolerant clones of *Salix* and *Populus* for further experimental use [49]. Since dendroremediation potential for soils is not sufficiently assessable *in situ* up to now, outdoor mass balances of nitroaromatic compounds (NAC) were performed in lysimeter pots using four-year-old trees of *Salix*, *Populus*, *Betula* and *Picea* for planting in TNT contaminated AP-soils with a definable volume of homogenized soil. The decline of TNT/ADNT amounts per soil mass unit was bio-indicated by monitored tree growth and these results agreed with

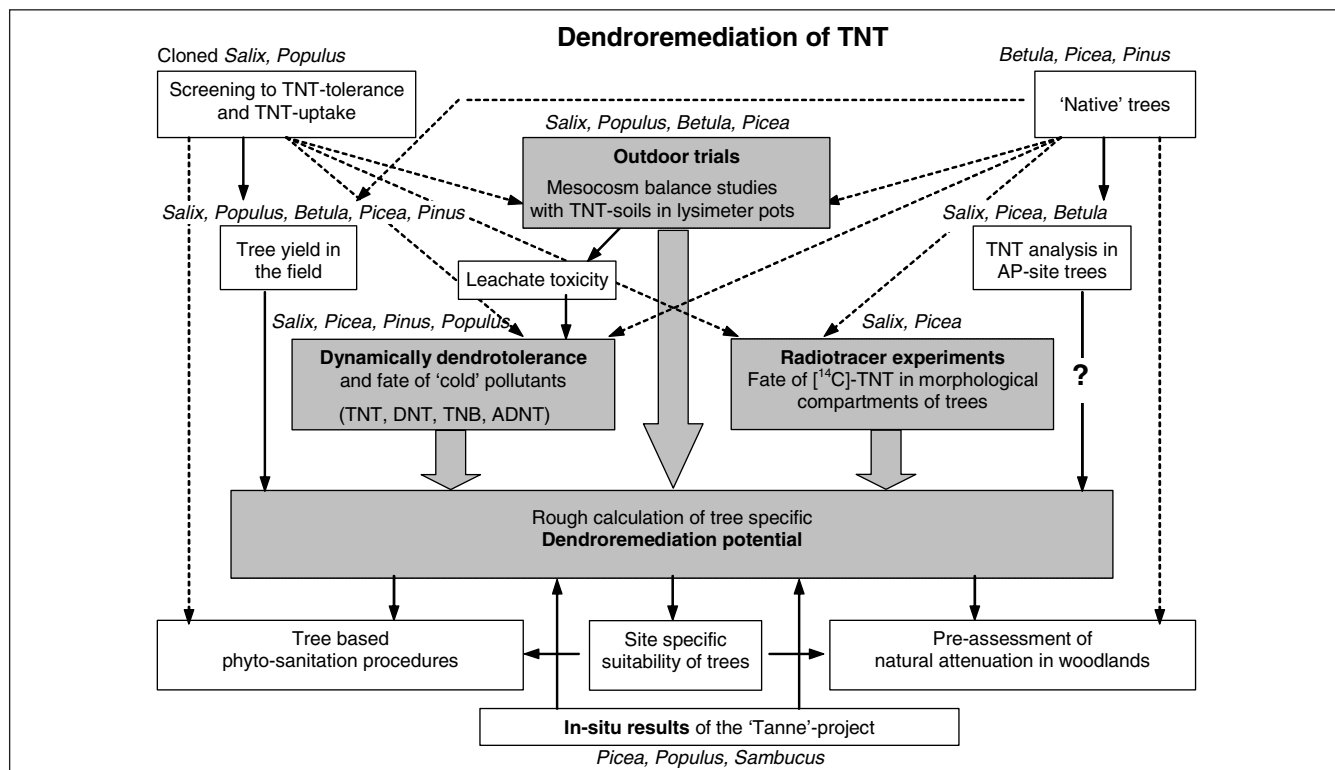


Fig. 1: Dendroremediation research network at the German Federal Biological Research Centre for Agriculture and Forestry (BBA). AP = former ammunition plant

leachate quantification. Analysis of sampled soil cores showed a general disappearance of TNT and ADNTs. However, rapid and drastic initial decline during the four-month tree establishment phase and spatial soil heterogeneity of the distribution of contaminants masked the success of dendroremediation in soil analyses (Schoenmuth and Pestemer 200X, in prep.).

Provocative, 'best case' greenhouse experiments were established to 'dynamically' assess dendrotolerance, experimental dendroremediation capacity, and fate of 'cold' TNT and related nitroaromatics. For quantification, soil-based tree test systems using continuous and repeated application of water solved pollutants were applied. Since area related field mass of leaves or needles can be correlated to their transpiration [58], monitored transpiration and greenhouse growth were related to field measurements of the organ specific yield of trees (Schoenmuth and Pestemer 200X, in prep.).

Result confirmation under different conditions with different tree species and cross-linkages between dendroremediation research components, as illustrated in Fig. 1, allowed preliminary rough calculation scenarios of dendroremediation potentials as a basis for later modelling. Knowing that TNT contamination *in situ* is mostly mixed with related NACs, experimental dendrotolerance limits for TNT, ADNTs, dinitrotoluenes (DNTs) and 1,3,5-trinitrobenzene (TNB) were considered, and measured TNT degradation capacities of soil/tree systems allowed the calculation of annual dendroremediation potentials. Calculated values for TNT for four-to-five-year-old trees were the following: *Salix* EW-13 6.0, *Salix* EW-20 8.5, *Populus* ZP-007 4.2, *Betula pendula* 5.2, *Picea abies* 1.9 and *Pinus sylvestris* 0.8 g m<sup>-2</sup> a<sup>-1</sup>. On the other hand, a 45-year-old spruce forest was estimated to possess an annual natural attenuation potential for TNT of 4.2 g m<sup>-2</sup> a<sup>-1</sup> (Schoenmuth and Pestemer 200X, in prep.).

#### 4 Conclusion, Recommendation, Perspective

Together with other features like drought resistance or flooding tolerance, area related transpiration power, interception of precipitation, ease of establishment, tree yield and growth requirements, a suitability list was obtained as a basic tool for proposals for the site-adapted remediation use of trees.

Main purpose of the second part of the present paper was to give an assessment support for the above-mentioned experiments and calculations (see Fig. 1). Here, TNT uptake, localisation of TNT-derived radiolabel in tree tissues, and extent and quality of TNT-degradation in four-year-old *Salix* and *Picea* were estimated [77]. Up to now, mass balances of radiolabelled TNT in trees are restricted to young poplar cuttings of Thompson et al. (1998) [24]. No data are available for the fate of TNT in older woody plants for either deciduous or coniferous trees.

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