Uptake and distribution of [^{14}C]-TNT in conifers

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Introduction

This contribution deals with some never results about „Uptake and distribution of [^{14}C]-TNT in conifers“. The results are part of the KORA-associated Project entitled: „Dendrotolerance to explosive specific substances in soils and long-term fate of [^{14}C]-trinitrotoluene and [^{14}C]-RDX in conifer trees.“ The experiments were carried out in close co-operation between the Humboldt University and the BBA (Fig. 1).

Fig. 1: Location of the section phytomedicine at the Humboldt University (left) and the main building of the BBA at Berlin-Dahlem (right).

Background

Background is the fact, that many formerly as well as currently used military sites are contaminated with TNT.

Often these areas are covered with woodlands. In Germany, and other European countries these woodlands are dominated by conifers, especially by Norway spruce (*Picea abies*) and by Scots pine (*Pinus sylvestris*).

Moreover, we recently found that coniferous trees are more tolerant to TNT and other explosive specific substances than broadleaf trees or herbaceaous plants.

It is necessary to investigate the potential of (conifer) trees for their ability for natural reduction of TNT soil contamination.

While for broad-leaved trees some information about TNT uptake and TNT metabolisation is available, up to now, for conifers almost no results may be found.

Plant Material

Investigated plant objects were 4-5-year-old trees of *Pinus sylvestris* (Norway spruce, or in German: Gemeine Kiefer) and *Picea glauca* „Conica“,a dwarf mutant of Canadian white spruce (in Germany known as Zuckerhutfichte) (Fig. 2).

Plants were transferred from field soil to quartz sand pots.

Fig. 2: Pinus sylvestris (left) and Picea glauca (right) at the nursery of the BBA.

Application System

For application of water-solved, bioavailable radio-labelled TNT we used glass fibre wick application systems (Fig. 3). Application was done as a pulse of 45 mg/l TNT, spiked with uniform ring-labelled [^{14}C]-TNT. The plants drank out the application solution within five days.

After that, an after period of additional 4 weeks followed, where only nutrient solution was applied. This after period should allow the trees to metabolise the TNT which was taken up. The after period should also avoid interferences with TNT metabolites which are only temporarily present in the tree tissue.

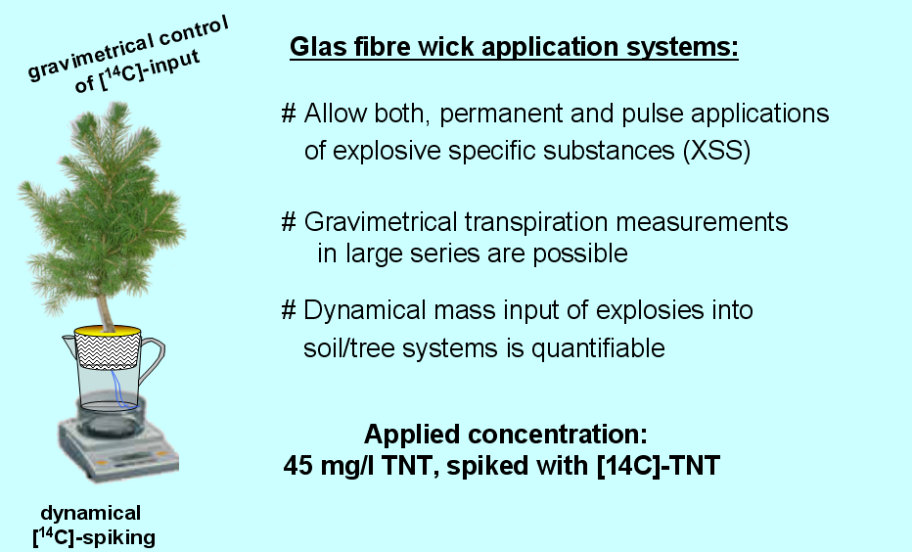

Fig. 3: Application system for [^{14}C]-TNT

Mass Fractionation of TNT Uptake

Tree harvest was carried out 5 weeks after single pulse of [^{14}C]-TNT) of TNT application was started. After harvest, potted plants were separated into their main parts, that means needles, wood, roots and substrate as well as ambient materials (quartz, pots, wicks, remaining solution) (Fig. 4).

Aboveground parts, that means needles and wood are further sub-divided into three classes according to their age. Overall radioactivity of aliquots of all the plant parts was determined using an Biological Oxidizer. Further aliquots were used to quantify the extractability of plant material. Tested extractants were 50% acetic acid, methanol and acetonitrile. Extraction efficiency was calculated by Liquid Scintillation Counting. After that, aliquots of tree tissue extracts were analysed by thin layer chromatography.

The procedure for biochemical compartmentalisation is shown in Fig. 5.

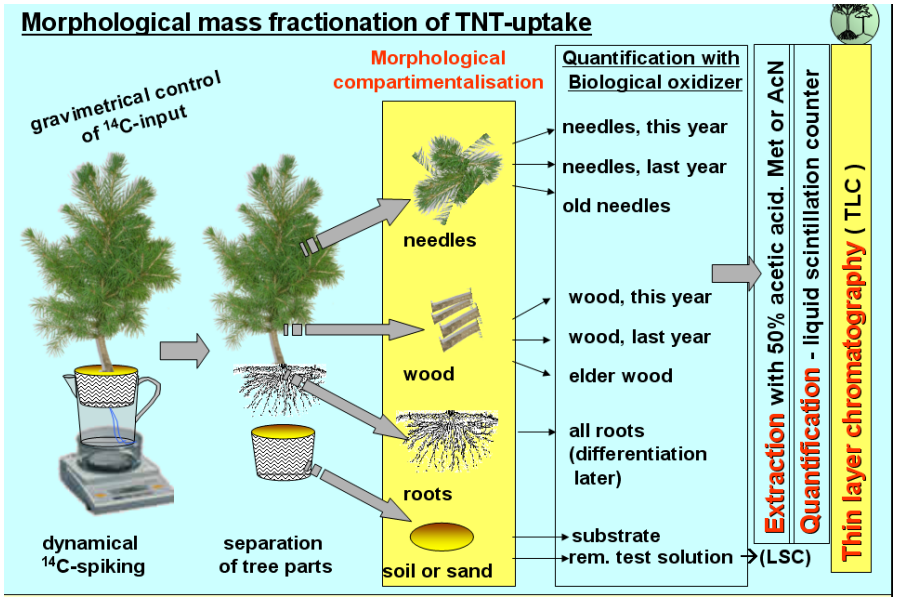

Fig. 4: Morphological compartmentalisation

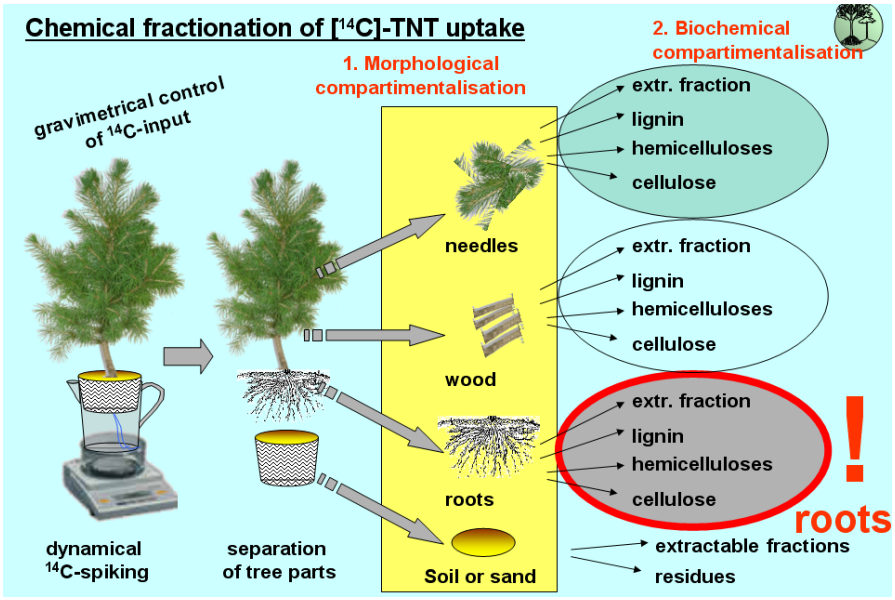

Fig. 5: Chemical compartmentalisation

Results

Morphological [^{14}C]-TNT distribution in conifers

The uptake experiments with radio-labelled TNT show a high degree of confirmation between pines and spruces (Fig. 6, Fig. 7, Fig. 10). If we consider the concentrations of TNT equivalents in Fig. 6, we can observe that the highest concentrations were found in roots. For instance, concentrations up to 300 mg TNTeq per kg dry matter are possible in pine roots.

If we calculate the absolute mass distribution as microgram TNT eq (Fig. 7), it becomes obvious, that pines as well as spruces are able to lower the content of TNT eq in the growth substrate. Tree planted quartz sand (blue columns in figure Fig. 7) shows considerably lower TNTeq masses than unplanted controls.

The relative mass distribution of TNTeq in aboveground needles (Fig. 8) and wood (Fig. 9) shows differences between pines and spruces.

The overall relative mass distribution of TNT eq shows, that 96% of tree localized radioactivity remains in the root. Only 3% were transported into wood and 2% into the needle fraction (Fig. 10).

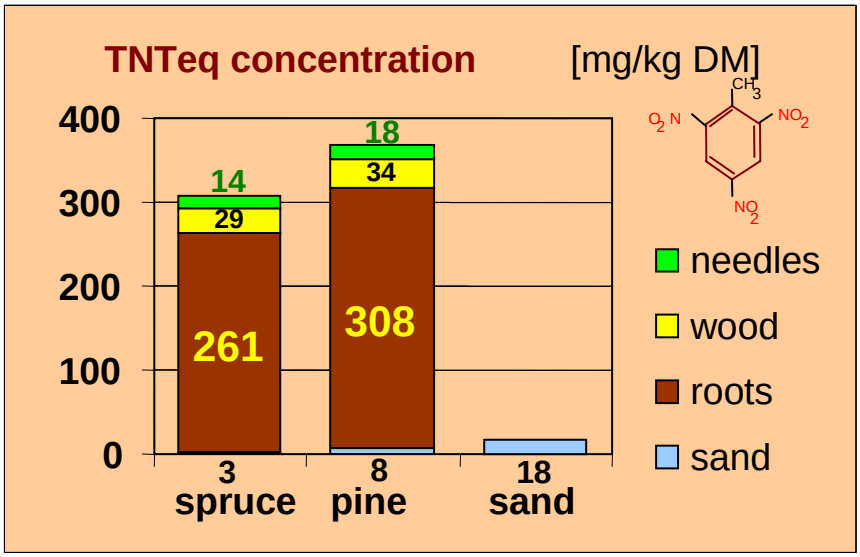

Fig. 6: Distribution of TNT equivalent (TNTeq) concentrations

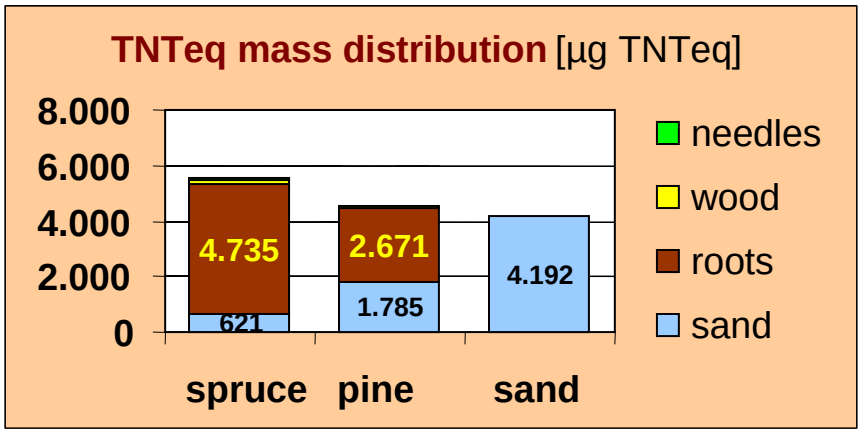

Fig. 7: Absolute mass distribution of ^{14}C-TNT equivalents (TNTeq)

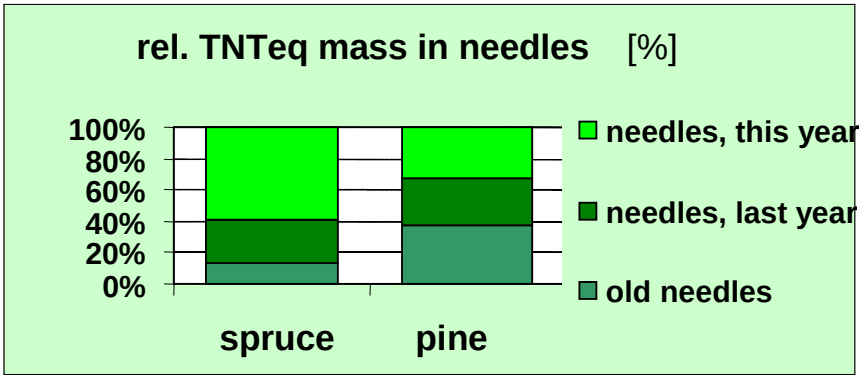

Fig. 8: Relative mass distribution of ^{14}C-TNT equivalents (TNTeq) in conifer needles

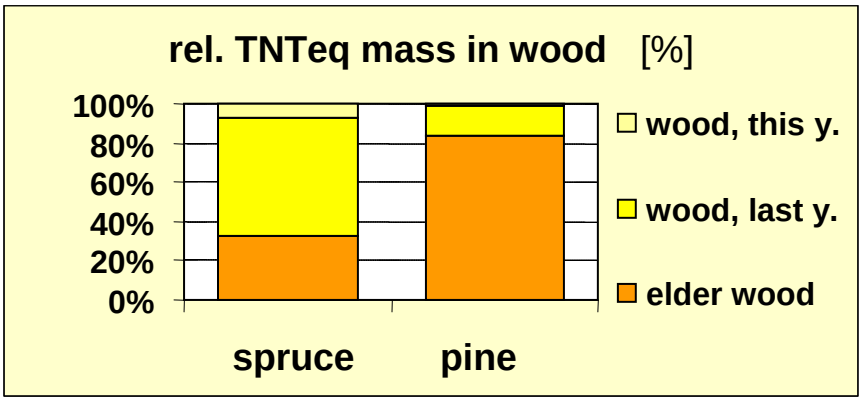

Fig. 9: Relative mass distribution of ^{14}C-TNT equivalents (TNTeq) in conifer wood

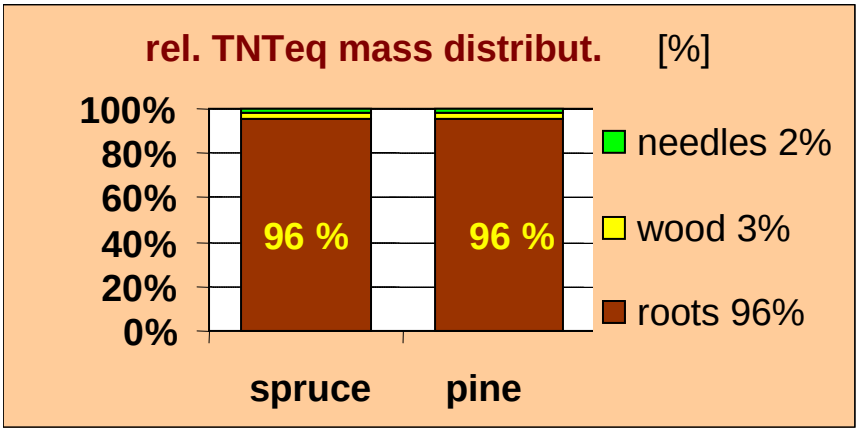

Fig. 10: Relative mass distribution of ^{14}C-TNT equivalents (TNTeq) (overall)

Extractability

The extractability tests showed that acetic acid was most efficient, followed by methanol. Acetonitrile is not suited as extractant (Fig. 11).

If we now consider only the acetic acid extracts in Fig. 11, it becomes clear that approx. 90 % of TNTeq are non-extractable bound in roots. Relative extractability is increased in aboveground portions, but the quantities of upwards transported TNTeq remain relative low, because of the low mass distribution values, repeated in the right columns.

Thus, the possibility for an acropetal transport via transpiration stream is low for TNTeq.

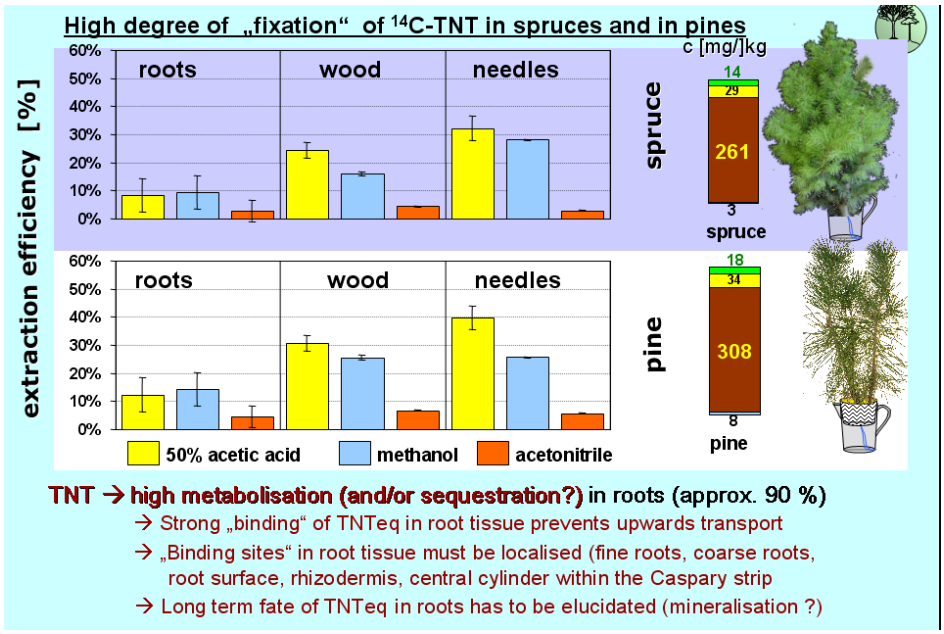

Fig. 11: Extraction efficiency in *Picea glauca* and *Pinus sylvestris*

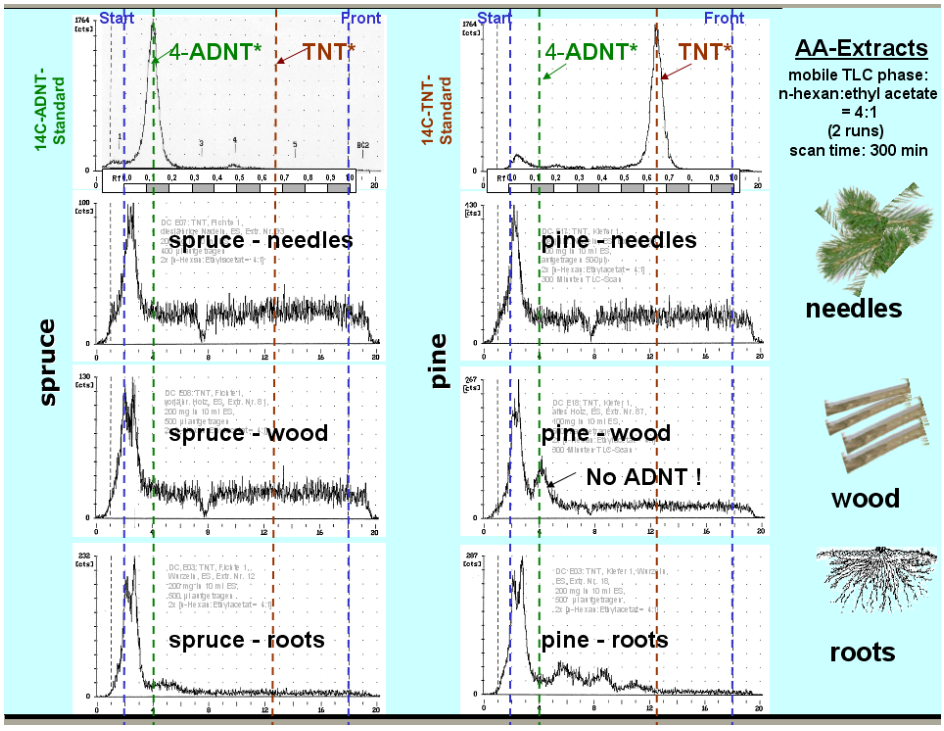

Fig. 12: TLC radiochromatograms of acetic acid extracts (AA) in *Picea* and *Pinus*

Extractability with organic solvents

Radio-TLC chromatograms of acetic acid extracts in Fig. 12 show, that the very low extractable portions do not contain TNT nor other known TNT metabolites such as ADNTs or DANTs.

The still extractable radioactivity is dominated however by highly polar metabolites of TNT. These are the peaks near the TLC start line.

Extraction of „Organic Non-Extractable Residues“

A scheme for the extractraction of „Organic Non-Extractable Residues“ is given in Fig. 13.

The main focus here was laid on the cell wall constituents lignin, hemicellulose and cellulose fractions, which are quantified by LSC and analysed by TLC.

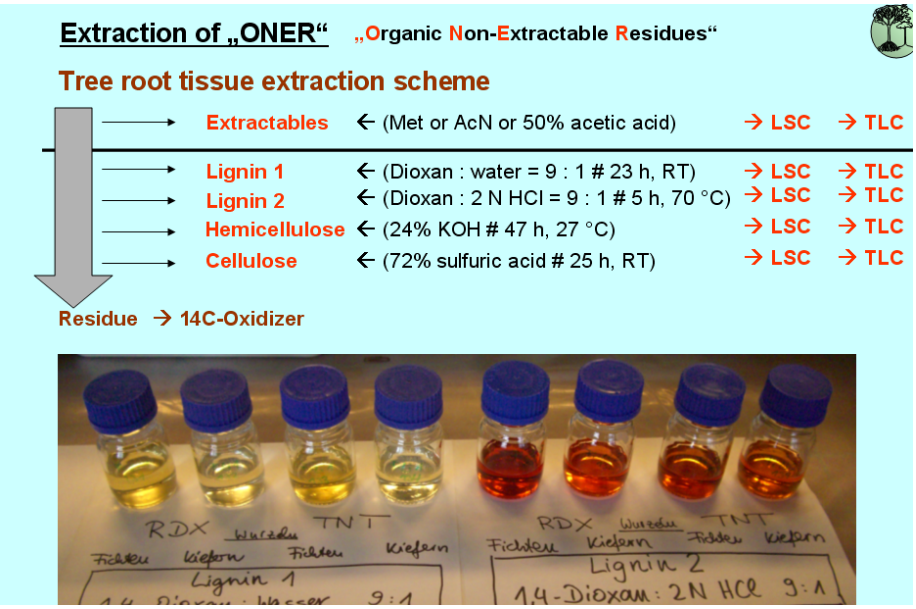

Fig. 13: Scheme for extraction of „Organic Non-Extractable Residues“ (ONER)

Chemical fractionation of non-extractable residues

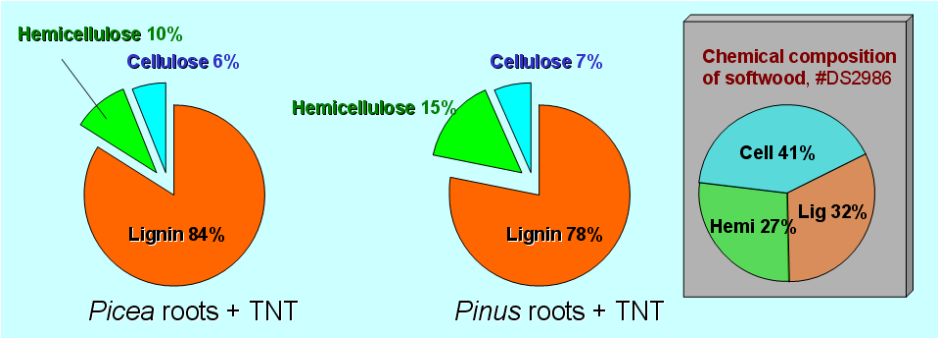

Fig. 14: TNT fate in root cell wall constituents

Although the extraction experiments for the biochemical distribution are still on the way, we want to present some preliminary results obtained with root tissue (Fig. 14).

The bound TNT-derived radioactivity is mainly located (approx. 80%) in the lignin fraction. Hemicellulose and cellulose fractions play only a minor role.

This distribution does not reflect the chemical composition of conifer wood (Fig. 14).

Radio-TLC chromatograms of cell wall fractions

Finally three radio-TLC chromatograms of the following fractions lignin, hemicellulose and cellulose are presented (Fig. 15, Fig. 16, Fig. 17). The coloured circles in Fig. 15, Fig. 16 and Fig. 17 represent cold reference substances, that means non-radioactive standards.

All extracts are analysed with two different mobile TLC phases.

At first a lignin extract is analysed (Fig. 15). As shown in the right chromatogram in Fig. 15, two peaks may be detected. One is near the start line and may be a highly polar TNT-metabolite or a conjugated TNT metabolite of highly molecular weight. The second peak is a substance which is apparently liberated after lignin decomposition. May be it was sequestered into the conifer lignin molecule. As the left chromatogram in Fig. 15 shows, this compound is neither TNT nor ADNT or DANT.

For the hemicellulose fraction such a sequestration could not be observed (Fig. 16).

And also for the cellulose fraction such a sequestration seems not possible (Fig. 17).

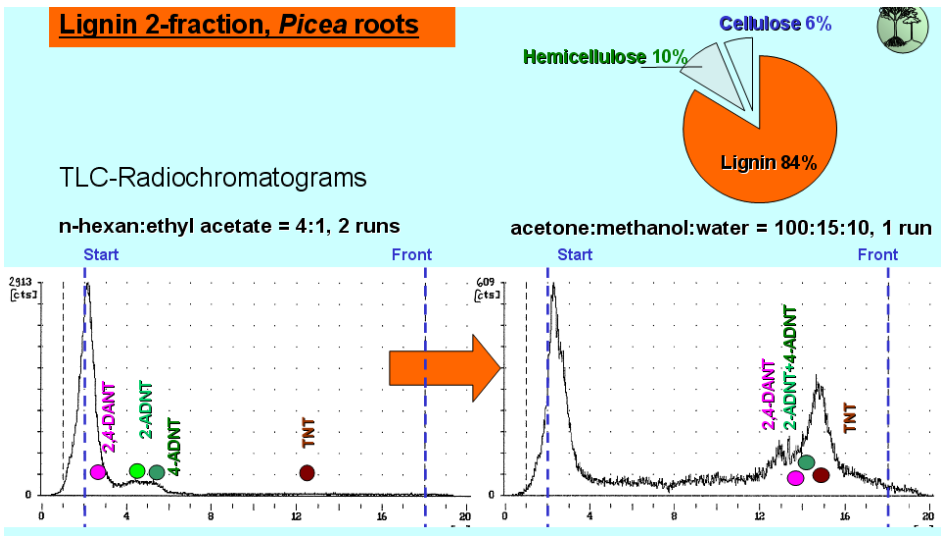

Fig. 15: TNT fate in the lignin 2 fraction of spruce root tissue

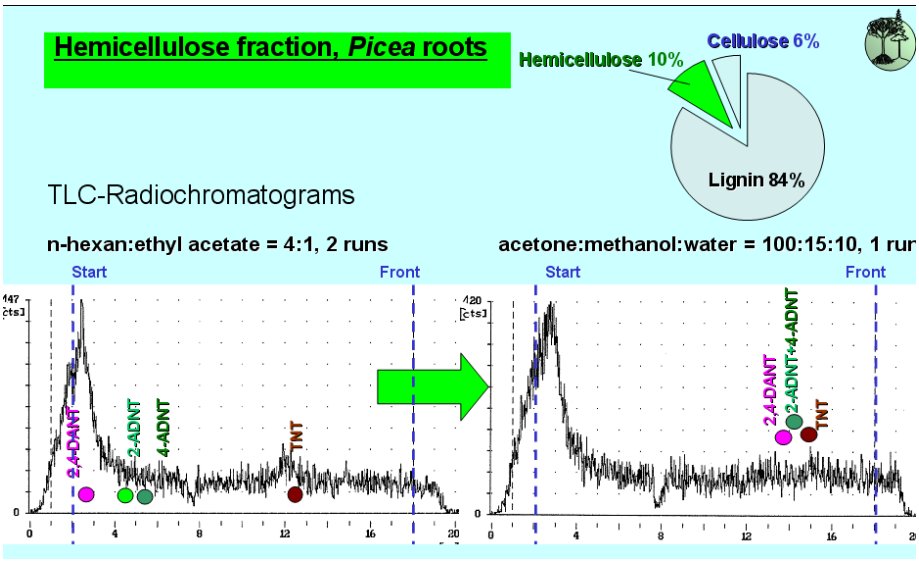

Fig. 16: TNT fate in the hemicellulose fraction of spruce root tissue

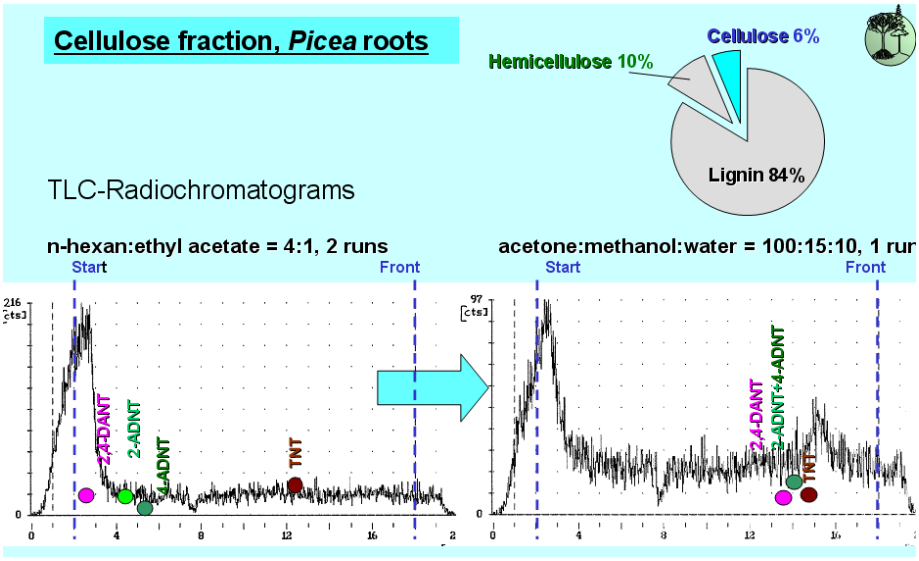

Fig. 17: TNT fate in the cellulose fraction of spruce root tissue

Summary and conclusions

In comparison to broadleaf trees and to herbaceous plants, conifers (spruce, pine) are more tolerant to TNT, RDX und other explosive specific compounds.

Both, spruces and pines are able to take up considerable amounts of TNT from soil solutions and TNT derivatives are accumulated in conifer plants.

Metabolized TNT-derivatives are mainly accumulated in roots where 90-95% of them are non-extractable „bound“.

The non-extractable bound TNT derivatives are mainly localized in the lignin fraction of conifer roots. This opens the possibility for slowly degradation of bound TNT-derivatives by soil born lignolytic fungi (e.g. *Phanaerochaete chrysosporium*), which are known to mineralise both, TNT and lignin.

Toxicological risks for wood utilization are (preliminary) considered as low.

Fig. 18: Spruce trees at the former ammunition plant site Clausthal-Zellerfeld (Lower Saxony)

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Further information regarding project 5 A1 is available at www.dendroremediation/KORA and at www.natural-attenuation.de.