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BRIEF COMMUNICATION

Biodegradation of trichloroacetic acid in Norway spruce/soil system

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Abstract

Trichloroacetic acid (TCA) belongs to secondary atmospheric pollutants affecting the forest health. Distribution of [1,2-^{14}C]TCA-residues and TCA biodegradation were investigated in 4-year-old nursery-grown trees of Norway spruce [*Picea abies* (L.) Karst.] in the whole plant/soil system. Radioactivity was monitored in needles, wood, roots and soil as well as in the air. During two weeks of exposure TCA was continuously degraded, especially in the soil. Estimates of radioactivity balance showed loss of radioactivity into the atmosphere in the form of $^{14}CO_2$; unincorporated [1,2-^{14}C]TCA, chloroform, carbon monoxide and methane were not detected at all. TCA degradation to CO_2 was indicated also in the spruce needles. Moreover, it was found that soil litter contained [1,2-^{14}C]TCA unavailable to microorganisms.

Additional key words: chloroform in forest soil, ^{14}C-labelling, dechlorination, *Picea abies*, plant/soil system, radioindicator techniques, TCA distribution in spruce.

The action of trichloracetic acid (TCA) on plants has been studied for several decades. It has been first used as herbicide, but later it was found as a ubiquitous pollutant (Frank 1984). The origin of TCA in the environment has been proposed to be the atmospheric photooxidation of C_2-chlorocarbons emitted from anthropogenic sources and the effect of TCA has been also connected with forest decline (Frank *et al.* 1990, 1994, Schröder and Plümacher 1998). The natural formation of TCA and short-chain halocarbons in soil has been recently reported (DeJong and Field 1997, Hoekstra *et al.* 1998, 1999a, 1999b, Keppler *et al.* 2000) thus elucidating the TCA found in nature. In addition, there has been a significant natural background level of TCA in precipitation over the past 200 years (Sydow *et al.* 2000). Several genera of higher fungi have a capacity for biosynthesis of organohalogens, but they also can cause their reductive dechlorination (DeJong and Field 1997). The anaerobic microbial TCA biodegradation in digester sludge has been demonstrated by Chen *et al.* (1999). Uchiyama *et al.* (1992) reported the microbial conversion of trichloroethylene to glyoxylic acid, dichloroacetic acid (DCA), TCA, CO and CO_2. Microbial degradation of TCA to CO was supposed also by Weightman *et al.* (1992). Decarboxylation of TCA leading to chloroform in soil was supposed by Frank (1988), Plümacher *et al.* (1993), Frank *et al.* (1990), Uhlířová *et al.* 1996), and Haselmann *et al.* (2000). Short-chain chlorocarbons and chloroacetic acids are thus important minor contaminants (and because of their natural origin also products) of the environment, which undergo complex reactions and assume dynamic equilibrium within the system.

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Previous studies on TCA effects on the conifer/soil-system did not deal with the biodegrading effect of the soil (Sutinen *et al.* 1995, 1997). It is well known from the literature that TCA is biodegraded to CO_2 by soil microorganisms (Lignell *et al.* 1984, Süß and Eben 1977). We have previously examined the uptake, translocation and effects of TCA on Norway spruce using [1,2-^{14}C]TCA of high specific activity (3.7 GBq mmol^{-1} = 22 Bq ng^{-1} TCA) enabling thus to follow TCA appearing in nature, *e.g.* 5 - 200 ng(TCA) g^{-1}(needles). It was found that translocation of TCA occurs from atmosphere into soil by precipitation water, followed by uptake by roots and then movement into needles via transpiration stream, where TCA at concentrations higher than 60 ng g^{-1} destroys the photosynthetic apparatus (Uhlířová *et al.* 1996, Matucha *et al.* 2001). Since our radioactivity balance studies could not fully explain the observed losses of [1,2-^{14}C]TCA-derived radioactivity and since the role of the soil and of the plant in the TCA metabolism of the studied plant/soil system was not fully understood (Forczek *et al.* 2000, Matucha *et al.* 2000, 2001), the present study was carried out to address these questions.

Four-year-old potted trees of Norway spruce [*Picea abies* (L.) Karst.] grown at the nursery of the Forestry and Game Management Research Institute were used. The soil collected from the pots, after removal of the spruce trees, larger roots and pebbles, was carefully homogenized (all microbial and fungal components remained intact).

The method of Bubner *et al.* (1992) was used to obtain [1,2-^{14}C]TCA with a specific activity of 3.7 GBq mmol^{-1}. After chlorination of the [1,2-^{14}C]acetate the radioactive mixture was purified by HPLC on a 250 × 8 mm polymer *IEX* column (8 μm) in H^+ form (*Watrex*, Prague, Czech Republic) yielding a radiochemical purity over 98 %. The TLC method used was similar to that of Süss and Eben (1977), using cellulose F (*Merck*, Darmstadt, Germany) and *n*-butanol : NH_4OH : H_2O (85:1:14) for development. The [1,2-^{14}C]TCA preparation was diluted to 55.5 kBq cm^{-3} and adjusted to pH 5.0.

Laboratory experiments were conducted in a growth chamber (of own provenance) at temperature of 21 ± 3 °C and 10-h photoperiod (irradiance of 80 μmol m^{-2} s^{-1}; 40 W *Nd Phytolamp*, *Osram*, Budapest, Hungary). The chamber in the first radioactivity balance study consisted of a polyethylene foil fixed hermetically to a metal frame and to the tubes (42 dm^3). A cylindrical laboratory vessel (12.5 dm^3) was later used as experimental chamber. Radioactive determinations of the ambient air in the chamber were made by passing a continuous air flow (40 cm^3 min^{-1}) through the chamber and 2 to 4 absorbers filled with 6 cm^3 1 M KOH (Stork *et al.* 1997). A combustion furnace (*Model 325*, *Packard Instr.*, Downers Grove, USA) was inserted into the system for detecting oxidizeable gaseous products like CO and methane. To detect chloroform, the first absorber was filled with chilled (-20 °C) non-radioactive chloroform. The content of the absorbers was changed and taken for measurements (after addition of 5 cm^3 of *LKB OptiPhase 'HiSafe' 3* (Loughborough, England) scintillation cocktail to 1 cm^3 absorption solution) daily. To distinguish soil- and spruce-derived radioactivity, the pot of the spruce was sealed with polyethylene foil.

[1,2-^{14}C]TCA of the given specific radioactivity, radioactive concentration and radiochemical purity was applied to the surface of the soil (30 cm^3 to 100 g soil placed in a 500 cm^3 Erlenmeyer flask). The soil was kept humid by moistening the flow-through air.

In order to determine the distribution of [1,2-^{14}C]TCA-residues in spruce needles without distortion of the results by assimilation of the released $^{14}CO_2$, a field experiment was carried out with a spruce tree. After application of 1.86 MBq (in 30 cm^3) carrier-free [1,2-^{14}C]TCA solution into the soil, the potted spruce was regularly irrigated, and after two-week long exposure in September needle samples were taken.

A sample oxidizer *Zinsser Analytic, model OX-500* (Frankfurt a. M., Germany) at the Institute of Clinical and Experimental Medicine, Prague, and a liquid scintillation spectrometer *Beckman LS 6500* (Fullerton, USA) were used for radioactivity determinations.

In the experiment with a potted spruce in the large chamber, only 22.44 % of the applied radioactivity was recovered (in the soil 17.0 %, in spruce 2.0 %, and in the air as CO_2 3.4 %) (Table 1), and therefore we were obliged to look after possible losses. At first, the possibility of TCA-degradation to carbon monoxide (Uchiyama *et al.* 1992, Weightman *et al.* 1992) or to methane was examined in the whole plant/soil system using a combustion train inserted after the CO_2 absorbers. No formation of CO or methane was observed, *i.e.* less than 1 % rel. as follows from radioactivity measurements (sample background 2.0 Bq). Also [^{14}C]chloroform was not detected in the air or soil (by extraction with $CHCl_3$ at the end of the experiment, conducted in the small chamber), which has been assumed to be formed (Frank 1988, Frank *et al.* 1990,

Table 1. Distribution of [1,2-^{14}C]TCA residues in the whole plant/soil system. Distribution of 373.4 kBq [1,2-^{14}C]TCA residues in the individual compartments and spruce parts after 19-d exposure to 1.67 MBq carrier-free [1,2-^{14}C]TCA; radioactivity of TCA absorbed in old wood fragments was not involved in the 22.44 % (laboratory experiment, see text).

	Mass [g]	Radioactivity [Bq]	Specific radioactivity [Bq g^{-1}]
air		56737	
soil	1224	282826	231
roots	45	12641	281
stem	29	3157	108
branches	12	3402	278
needles	33	14625	449

Plümacher *et al.* 1993, Uhlířová *et al.* 1995). This is in accordance with the finding of Hoekstra *et al.* (1999b) showing that TCA and chloroform come from humic acid. The investigation of Haselmann *et al.* (2000), however, has shown slight formation of chloroform from TCA in forest soil, which also was not confirmed in our study.

Table 2. Distribution of [1,2-^{14}C]TCA residues in the spruce foliage in field experiment. Distribution of 11.64 kBq [1,2-^{14}C]TCA derived radioactivity in spruce needles after two-week exposure of spruce seedling to 1.86 MBq carrier-free [1,2-^{14}C]TCA (*relative radioactive concentration in needles, *C* - current year needles, C+1 - one-year old needles, C+2 - two-year old needles).

	Mass [g]	Radioactivity [Bq]	Specific radioactivity [Bq g^{-1}]	[%]*
C	3.64	4220	1160	100.0
C+1	7.54	6580	873	75.3
C+2	1.17	840	722	62.2
1st whorl	1.53	1580	1033	100.0
2nd whorl	5.25	5000	952	92.2
3rd whorl	5.57	5070	911	88.2

The losses of [1,2-^{14}C]TCA-derived radioactivity from the plant/soil system (Table 1) may be also explained by the soil homogenization method, from which the most radioactive pieces of bark, branches and roots (coming from litter), as well as of compost and pebbles, were excluded. This radioactivity was observed to be, in most cases, substantially higher than that of the homogenized soil. The TCA in these fragments was not available for immediate microbial degradation (and surely also was not available in the soil pores). Our preliminary experiments showed that radioactivity of $^{14}CO_2$ adsorbed in soil was minimal (about 1 % of total released $^{14}CO_2$). After recalculation the radioactivity balance of the whole plant/soil-system was very close to 100 % even with carrier-free TCA (which was attained first in the experiment with non-homogenized soil using higher isotopic dilution).

The part of $^{14}CO_2$ released by the spruce foliage was low but evident. From the distribution of TCA-derived radioactivity in various spruce tree parts (Table 2) is obvious that the highest radioactivity was clearly found in the current-year needles of the first whorl. Lower levels were detected in older needles and whorls.

The results of biodegradation experiment in soil using carrier-free TCA (the lowest curve in Fig 1) demonstrated a high rate of biodegradation. To compare it with earlier published results (Lignell *et al.* 1984, Süß and Eben 1977) the same amount of radioactivity was used but 3 and 21 times isotopically diluted with non-radioactive TCA. The curves obtained with these dilutions demonstrate strong microbial activity of the soil and dependence of the TCA half-life in soil on its concentration (Fig. 1).

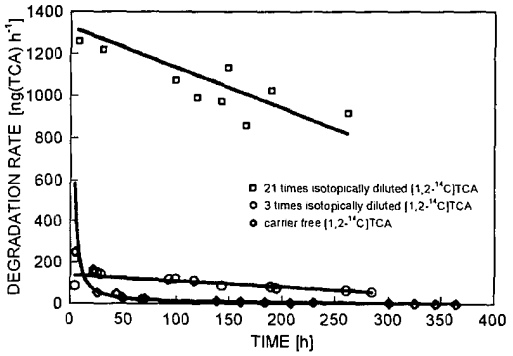

Fig. 1. Microbial degradation of [1,2-^{14}C]TCA in soil: a sample of 100 g humid soil substrate from forest nursery was treated with 830 kBq of carrier-free, 3 and 21 times isotopically diluted [1,2-^{14}C]TCA. Its biodegradation rate at room temperature was followed by absorption of released $^{14}CO_2$ (expressed in stoichiometric amount of TCA).

In conclusion, it is possible to say that *1*) the distribution of TCA in Norway spruce follows crown gradient being the highest in current year needles and the first whorl; *2*) TCA degradation to CO_2 occurs in two compartments of the studied plant/soil system: in spruce needles and in the soil; *3*) the rate of the microbial degradation of TCA in soil is high and concentration dependent; *4*) the hypothesis of chloroform, CO or methane formation from TCA in soil was not confirmed; and *5*) radioactivity balance studies should consider the heterogeneous character of the soil, especially those conducted with carrier-free preparations, *e.g.* older wood fragments in the soil may contain TCA unavailable to microorganisms.

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