



**Akademie věd České republiky**

Teze disertace  
k získání vědeckého titulu "doktor věd"  
ve skupině věd biologicko-ekologických

**Chlor v lesním ekosystému: biogeochemické cykly  
(radiochemická studie)**

*Chlorine in the forest ecosystem: biogeochemical cycles  
(A radiotracer study)*

Komise pro obhajoby doktorských disertací v oboru ekologická biologie

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Místo a datum:

Praha, listopad 2007



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## **Chlorine in the forest ecosystem: biogeochemical cycles**

### **(A radiotracer study)**

#### **Summary**

In nature, chlorine does not occur only as chloride or bound in substances of anthropogenic origin in the polluted environment but also in many hundreds of compounds of natural origin. Chloride deposited in the forest ecosystem from the atmosphere reacts with soil organic matter (SOM) under the mediation of enzymes and/or microorganisms (to a lower degree also abiotically), forming chloroacetic acids (CAA), chloromethane, chloroform, other aliphatic and aromatic compounds and chlorinated humic substances. Chloride, to which Norway spruce is sensitive, is thus mostly locked in organochlorines, which are even more common in soil than chloride. Chlorine is thus involved in SOM degradation, leading to smaller SOM decay products and finally to their mineralization. Closely related microbial processes were studied in methodologically similar soil bioremediation studies conducted earlier. The aim of the investigations was (i) first to elucidate the uptake, effects and fate of phytotoxic trichloroacetic acid (TCA, previously considered to be only a secondary atmospheric pollutant) in the Norway spruce/soil-system, (ii) to address the role of CAA, and finally (iii) to consider the global role of chlorine in coniferous forest ecosystems. Radiotracer methods were used with advantage.

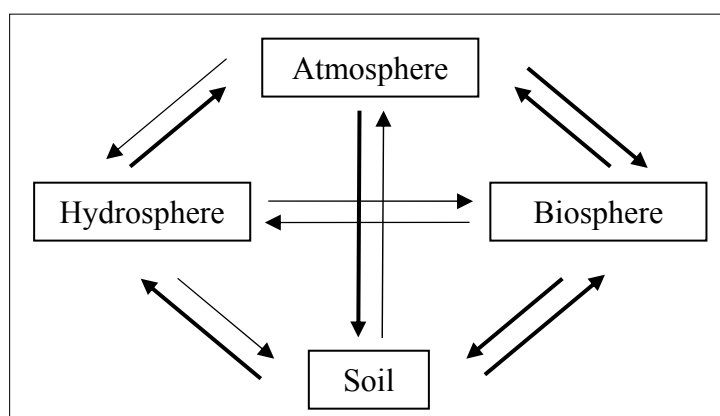
Using carbon 14, the fastest uptake of TCA was found to occur in the youngest spruce shoots due to a higher transpiration stream; TCA is then rapidly biodegraded in the forest soil. This microbial degradation depends on TCA concentration, soil humidity and temperature as well as on the soil character and microorganisms. The biodegradation of TCA took place also in the phyllosphere of the needles; TCA elimination in needles may be caused by slow decarboxylation. On the other hand, TCA may be formed by biooxidation of absorbed atmospheric tetrachloroethylene in needle chloroplasts.

Using chlorine 36 it was shown for the first time that chlorination of SOM yields TCA and also dichloroacetic acid (DCA), which is biodegraded even faster than TCA. The chlorination process was shown to proceed more microbially than abiotically, basidiomycetes being suggested as the responsible microorganisms. It is not clear whether the process is mediated by extracellular enzymes or intracellularly, to what degree the abiotic chlorination proceeds, whether dehalogenation of chlorinated substances takes place, and what further influences affect the ecosystem under study.

A connection between the carbon and chlorine cycles was clearly shown: products of chlorination of SOM are further mineralized and contribute to the litter decay and some chlorine is released from the forest ecosystem again. The previous chlorine level is thus restored. Moreover, the monitoring of chloride, TCA, TOX (total organic halogens) and other substances in forest soil, needles and soil solution in forest stands, especially near salted roads, showed an adverse effect of these substances on coniferous trees, especially spruce. These new, otherwise hardly attainable, results were obtained mostly using radiotracer methods.

## Introduction

Chlorine is one of the most abundant elements on the surface of the Earth and some authors (e.g. Winterton 2000 and Öberg 2003) compare its biological importance to that of phosphorus. In forest ecosystems, it is mainly present as chloride ion ( $\text{Cl}_{\text{in}}$ ) in the soil and in the biosphere or bound to organic matter ( $\text{Cl}_{\text{org}}$ ). As will be shown, it contributes to the degradation of soil organic matter (SOM), which represents the major sink of carbon in the forest ecosystem. Forest SOM is formed from litter, which is decomposed by basidiomycetes (de Jong and Field 1997). An abiotic chlorination of SOM has also been suggested (Keppler et al. 2000, Fahimi et al. 2003). Soils contaminated by chlorinated substances like PCBs may be remediated by degrading microorganisms (de Jong and Field 1997, Šašek et al. 1993, Vyas et al. 1994a).



**Fig. 1:** The chlorine cycling in forest ecosystems (bold arrows) is closely connected with the main chlorine cycles and reservoirs shown - mainly with oceans (Clarke et al. 2007).

The main source of chloride ions in the forest ecosystem is the oceans (in spite of the fact that more chlorine is contained in the Earth's crust and mantle – Winterton 2000), whereas  $\text{Cl}_{\text{org}}$  originates from various sources. The content of  $\text{Cl}_{\text{in}}$  in the environment depends on the geographic situation and  $\text{Cl}_{\text{org}}$  is connected with the biogeochemical cycling (Öberg 1998, 2002, 2003). Chlorinated compounds were formerly considered to be of anthropogenic origin only; however, the occurrence of more than two thousand natural chlorinated compounds has now been reported (Winterton 2000, Gribble 2003). Well known are toxic xenobiotics such as PCBs – we have previously studied their degradation by white-rot fungi using PCBs labelled with carbon 14 and suitable analytical methods (Šašek et al. 1993, Vyas et al. 1994a, Zachař et al. 1996, Kubátová et al. 1996a,b, 1998) - or pesticides, e.g. DDT and lindane, or solvents, e.g. tetrachloroethene and methylchloroform. Less known is the natural

origin of chlorinated dibenzodioxins and dibenzofurans (Silk et al. 1997), of the antibiotic chloramphenicol and of the methylating agent chloromethane (de Jong and Field 1997, Wintertone 2000). Several research fields have addressed this issue. These include studies of water disinfection by chlorination showing, among other things, the main chlorination products of humic substances (e.g. Christman et al. 1983, de Leer et al. 1985). Investigations have also been made concerning the effect of trichloroacetic acid (TCA) and chlorinated solvents (Frank and Frank 1989, Frank 1991, Frank et al. 1994, Weissflog et al. 2003, 2004 and 2005) on coniferous forest, the occurrence of AOX or Cl<sub>org</sub> in the forest soil (Öberg 1998, Öberg et al. 2005), chlorination and the litter degradation ability of basidiomycetes (de Jong and Field 1997), the fate of chlorine 36 as a uranium fission product in soils (Lee et al. 2001) and the bleaching of kraft pulps (Solomon 1996 and Juuti 1997).

The role of chlorine in the forest ecosystem drew increased attention after apparent problems with coniferous forest decline in central and northern Europe in the eighties and the nineties. Injuries to conifers such as chlorosis and needle loss, without evident damage caused by coal burning, were observed in regions marked by a higher Cl content in needles and were ascribed to chlorinated solvents (Figge 1989 and 1990) produced industrially and used in large amounts at that time, especially to TCA, their atmospheric photooxidation product (Sidebottom and Franklin 1996, McCulloch 2002, Hoekstra 2003). It was presumed that TCA, a compound with phytotoxic properties, arises by atmospheric photooxidation of chlorinated C<sub>2</sub>-hydrocarbons - methylchloroform, tri- and tetrachloroethylene - used on a broad scale in large amounts (about 1 million tons/yr at that time) as cleaning and degreasing solvents in the electronic, metallurgical and textile industries, affecting the health state of coniferous forest (McCulloch 2002, Matucha and Uhlířová 2002). The effect of TCA on conifers was studied in Germany (Figge 1989 and 1990, Frank 1984, 1988, 1989 and 1991, Frank and Frank 1985 and 1986, Frank et al. 1989, Frank et al. 1990b, 1991, 1992a,b, and 1994, Plümacher et al. 1993, Plümacher and Schröder 1994, Schröder and Plümacher 1998, Schröder et al. 2003), in Finland (Juuti et al. 1994, 1995 and 1996, Juuti 1997, Norokorpi and Frank 1993 and 1995, Sutinen et al 1995 and 1997), in the Czech Republic (Uhlířová et al. 1995, 1996 and 2002, Matucha et al. 2001, Forczek et al. 2001) and later in Scotland (Cape et al. 2003, Dickey et al. 2004, Heal et al. 2003, Stidson et al. 2004a,b). Natural occurrence of Cl<sub>org</sub> was indicated many times (e.g. Hoekstra and de Leer 1995, Hoekstra et al. 1995, Gribble 2003). It was found that TCA is also of natural origin and occurs in forest soil among other places (Hoekstra et al. 1999, Matucha et al. 2007a) as was predicted by Schöler et al. (2003). This later led to

investigations of chlorination of SOM, formation of AOX and associated problems (Öberg et al. 2002, Bastviken et al. 2007, Gryndler et al. 2007, Rohlenová et al. 2007).

Related studies by Weissflog et al. (2001) on the influence of tetrachloroethene on conifers in highly polluted regions of Germany and Russia showed natural formation of PER by microbiological processes in salt lakes (Weissflog et al. 2005), its transformation to TCA in needles and chloroplasts and its effect on conifers (Weissflog et al. 2007, Forczek et al. 2007).

Of similar importance are the studies of Cl<sub>org</sub> (chlorinated compounds or SOM) in the aquatic and forest ecosystems carried out by the group at Linköping University (Asplund and Grimvall 1991, Öberg et al. 2002, Öberg 2003, Johansson et al. 2001, Öberg and Sandén 2005), showing an occurrence of amounts higher than 200 mg Cl<sub>org</sub>/kg forest soil. They showed that relatively low chloride content in precipitation or forest soil may lead to a higher Cl<sub>org</sub> content in forest soil and run-off water (estimated as AOX or TOX – adsorbable or total organohalogenes, resp.; an aromatic character of some part of AOX was also indicated).

The approach of the group at Wageningen Agricultural University, whose attention was drawn to chlorination of litter or SOM by basidiomycetes, suggested an ecologically significant role of these microorganisms in decomposition of forest organic matter connected with chlorination activity of the fungi (Field et al. 1995, de Jong and Field 1997, Verhagen et al. 1998). It was shown that the chlorination products are smaller molecules of aromatic and aliphatic character, such as methyl chloride, chloroform, TCA and chlorinated anisyl derivatives (CAMs), as well as chlorinated humic acids of smaller size (Lee et al 2001). They may behave like intermediates, i.e. they may be at the same time microbially degraded and mineralized (Verhagen et al. 1998). A possibility of intracellular microbial chlorination was also mentioned (Steinberg et al. 2006). An abiotic chlorination of SOM (similar to microbial chlorination) was suggested by the group from the University of Heidelberg (Keppler et al. 2000, Fahimi et al. 2003).

## **Methodology**

Forest is a very complicated ecosystem and it is thus very difficult to sample its compartments, e.g. soil and needles. It is much easier to study processes in vitro than to investigate them in the field or by lysimeter experiments. An additional problem with field studies is that it is almost impossible to find a representative, 'mean' part of the ecosystem because of its diversity and inhomogeneity, as indicated by Rodstedth et al. (2003), Öberg et al. and Öberg and Sandén (2005). They attempted to solve this problem by analysing many

samples or investigating many soil cores, which is laborious, expensive and perhaps not representative enough. Previous experience and the instrumentation available led us to an application of radiotracer techniques, which have several advantages.

Carbon 14 and chlorine 36, radioisotopes with properties suitable for long-term radioanalytical studies of the role of chlorine in the complex forest ecosystem, i.e. nuclides with long half-lives (5760 and  $3.01 \times 10^5$  y, resp.) and sufficiently hard  $\beta$ -emitters ( $E_{\max} = 156$  and 710 keV) were used. Use of carbon 14 has a long tradition since its discovery and use in the studies of photosynthesis in the forties by Calvin et al., while chlorine 36 was used as a conservative tracer for translocation studies in plants by Gage and Aronoff (1960) and in hydrology by Nyberg et al. (1999).

Syntheses of [1,2- $^{14}\text{C}$ ]TCA and [1,2- $^{14}\text{C}$ ] DCA (Bubner et al. 1996 and 2001) of high specific radioactivity (100 mCi/mmol) and radiochemical (>98%) and chemical purity, and two analytical methods (Matucha et al. 2004 and 2006) as prerequisites of the investigations, were developed for the intended research. Another method developed by Bastviken et al. (2007) is still being optimized. The most important advantage of using radioisotopes in environmental research is the possibility of tracing the element or labelled compound examined (following its transport, translocation and distribution), its reactions and metabolism, and also checking the radioactivity budget in the system under study. Importantly, the isotopic exchange of chloride with  $\text{Cl}_{\text{org}}$  was also examined for typical compounds (Matucha et al. 2007b).

Common liquid scintillation was used for measurements of the  $\beta$ -radioactivity of both radioisotopes (Beckman LS 6500). The determination of carbon 14 in soil was conducted by combustion of the soil sample at 900°C in a stream of oxygen and absorption of the developed  $^{14}\text{CO}_2$  in a suitable liquid scintillator cocktail for radioactivity counting (Zinsser). A similar method was used for determining mineralized  $^{14}\text{CO}_2$  in the atmosphere after biodegradation of [1,2- $^{14}\text{C}$ ] chloroacetic acids (CAAs); the flow-through method using absorption in 1.5 M KOH (Stork et al. 1997) was adapted (Matucha et al. 2003).

Extraction of polar compounds from soil and needle samples was conducted by repeated extraction with water or potassium nitrate solution; apolar or less polar substances were extracted by *tert*-butyl methyl ether. Humic substances were extracted by 1M KOH (Stevenson 1981) or 0.1 M NaOH (Bastviken et al. 2007). The alkaline extraction of humic substances with their subsequent acidic precipitation led, however, to the loss of bound chlorine remaining in the solution as chloride and not as fulvic acids (Rohlenová et al. 2007).



Before extraction of chloride from soil microorganisms their cell walls were destroyed by freezing to  $-20^{\circ}\text{C}$  or below and slow thawing (Matucha et al. 2004, Bastviken et al. 2007).

Volatile substances such as chloroform (Hoekstra et al. 1998a,b, Svensson et al. 2007) and chlorocarbons were determined by capillary GC, using cryogenic or SPME techniques: detection was conducted mainly by ECD (Varian 3400) and MS (Laternus and Matucha 2007). For  $\text{CHCl}_3$  separation from soil samples before determination SPME technique was used (Rohlenová and Matucha, unpublished results). Identification was carried out by GC-MS (Ion trap Finnigan 800). TCA in environmental samples was determined after extraction and esterification also by capillary GC combined with ECD or MS detection (Frank et al. 1990 and 1995). AOX, TX and TOX were determined by coulometric titration of chloride after soil sample combustion at  $1000^{\circ}\text{C}$  in an oxygen stream (Euroglas). Thin-layer chromatography after preconcentration by evaporation was used for separation of CAAs. Distribution of radioactivity on TLC chromatograms was measured by a radioluminescence imaging technique (Bio-Imager BAS 5000 Fuji). The methods used are described in detail in the corresponding articles.

## **Results and discussion**

The applications of radioactively labelled compounds for environmental research, which were methodologically prepared over several years, were first connected with the microbial remediation of PCB contaminated soil by white-rot fungi (Klötzer et al. 1992, Matucha et al. 1998, Bubner et al. 1997 and 1998, Kubátová et al. 1996a,b). In these investigations we verified our capabilities to synthesize and analyze  $^{14}\text{C}$ -labelled substances as well as complex PCB mixtures (Šašek et al. 1993, Vyas et al. 1994a, Zachař et al. 1996, Kubátová et al. 1996a,b, 1998).

Our studies using radiotracer techniques and  $[1,2-^{14}\text{C}]\text{TCA}$  in the field of forest research started with the investigation of TCA uptake by Norway spruce shoots and TCA distribution in needles (Uhlířová et al. 1996), and were followed by studies of the behavior of TCA in the spruce/soil-system (Matucha et al. 2003a, Forczek et al. 2001 and 2004) and then of chlorination of SOM with  $^{36}\text{Cl}$ -chloride (Matucha et al. 2003b).

The study of the role of chlorine in the forest ecosystems included investigations of uptake, effects and fate of TCA, first used as a herbicide (Ashton and Crafts 1973, Lignell et al. 1984, McGrath 1976) and then considered as a secondary atmospheric pollutant (Frank 1991, Frank et al. 1994, McCulloch 2002), in the Norway spruce/soil system (Matucha et al.

2001).  $^{14}\text{C}$ -labelled TCA and DCA were synthesized for this purpose (Bubner et al. 1998 and 2001).

It was shown that the phytotoxic TCA, a polar and highly water soluble compound, is readily taken up by roots from soil, then translocated by the transpiration stream and accumulated in spruce needles (Uhlířová et al. 1996, Matucha et al. 2000 and 2001, Forczek et al. 2001 and 2004, Schröder et al. 2003). Its higher concentrations cause chlorosis, necrosis and needle loss and lower shoot increments (Uhlířová et al. 2002). Microbial degradation of [1,2- $^{14}\text{C}$ ] TCA in soil leads to a release of  $^{14}\text{CO}_2$  into the atmosphere, thus influencing substantially the experiments with spruce plants in closed growth boxes by assimilation of the released  $^{14}\text{CO}_2$  (Matucha et al. 2001). Field experiments with the plant/soil-system (with plants in a pot) were therefore preferred. It was also found that the TCA from the soil translocated through the xylem by the transpiration stream and accumulated in needles (most quickly into current needles) is eliminated relatively slowly (Matucha et al. 2001, Forczek et al. 2001, Forczek et al. 2004, Matucha et al. 2006), whilst in the forest soil it is rapidly degraded by microorganisms; for this reason, TCA biodegradation in soil was investigated thoroughly (Matucha et al. 2001, 2003 and 2007b, Forczek et al. 2001, Schröder et al. 2003).

An investigation of TCA biodegradation in soil has shown that the biodegradation depends on TCA concentration (previously unknown in herbicide applications), humidity, temperature and origin of the forest soil (Forczek et al. 2001). The fastest (aerobic) microbial degradation was found in the fermentation horizon of mildly humid forest soils (Matucha et al. 2003, Schröder et al. 2003, Matucha et al. 2007b), the microbial activity in the inorganic horizon being lower (Matucha et al. 2007b). In sterilized soils TCA degradation was negligible – clear evidence of the microbial character of the degradation (Matucha et al. 2007b). In needles we determined TCA elimination by microbial degradation (Forczek et al. 2004), and in addition also by slow thermal decarboxylation of TCA (Matucha et al. 2006).

As shown recently, there is still another possibility of TCA formation and accumulation in needles: uptake of tetrachloroethylene – a primary air pollutant of both anthropogenic and natural origin and a precursor of TCA – and its penetration through the needle cuticula and all the cell membranes into chloroplasts, followed by its biooxidation there to TCA with an adverse effect on the photosynthetic apparatus as a result of its decay to toxic HCl and  $\text{CO}_2$ . Compared with the TCA transported from the soil through the xylem, the TCA in chloroplasts has a much stronger adverse impact on spruce health due to the effects of protons from toxic HCl formed in the most sensitive needle subcellular structures (Weissflog et al. 2007, Forczek et al. 2007).

Finally, it was found - and proved by our application of chlorine 36 (Matucha et al. 2007a) - that TCA was formed in forest soil together with DCA and chloroform by microbial chlorination of organic matter, i.e. of aromatic resorcinolic structures as suggested earlier (Hoekstra et al. 1999b). It is caused by hypochlorous acid (i.e. by chlorine radicals) formed by enzymatic oxidation of chloride at forest soil humidity; probably only a minor part of SOM is chlorinated abiotically by the Fenton reaction (Matucha et al. 2007a,b) as shown by chlorination of sterile and non-sterilized soils. An important factor is participation of enzymes (Matucha et al. 2003) reported earlier (Asplund et al. 1993, Laturus et al. 1995, Schröder and Wolf 1996, Schröder et al. 1997, Niedan et al. 2000), however, abiotic chlorination was also reported (Fahimi et al. 2003). This chlorination mechanism resembles the mechanism of chlorination of dissolved humic substances in water, affording the same products (e.g. during water disinfection); however, it was proved for the first time in forest soil under natural conditions. The combination of both processes – of formation and degradation - means that DCA and TCA represent intermediates in the degradation of SOM (Matucha et al. 2007a) and that their level in the forest soil may correspond to an equilibrium or show fast degradation when this is absent (Peters 2003).

A preliminary examination of the behavior of chloride after its uptake in lower forest plants showed also a possibility of formation of volatile organochlorines there (Laturus and Matucha 2007). Chloride absorption by microorganisms and formation of chlorinated humic acids in forest soil was reported recently (Bastviken et al. 2007). The author, often cooperating on an international scale, has participated in three review articles from this field (Laturus et al. 2005, Cape et al. 2006, Clarke et al. 2007).

The processes studied - concerning among other things chlorine and carbon cycling in the coniferous forest ecosystem - showed that chloride and chloroacetic acids are not inert at all and that they are rapidly translocated into plants, that they are there microbially converted and function as stressors and that their reactions in soil in the forest ecosystem lead (with respect to the chloride content in soil and litter) always towards an equilibrium, a state often found in natural ecosystems. These studies also lead to useful applications, showing the formation in soil of chloroform that may be translocated from soil into groundwater (Svensson et al. 2007) and evaporated to some degree into the atmosphere, and documenting that road salting causes direct damage to conifers (Kayama et al. 2003).

Finally, the results of the studies explain the presence of  $Cl_{org}$ , including TOX, in the forest soil and show that chlorine (and especially TCA and chloride) in temperate and boreal forest ecosystems may not be presented as an anthropogenic stressor. It was further shown

that chlorine also takes part in natural SOM decay (and the carbon cycle) and some of the metabolic and microbiological mechanisms of this degradation process were elucidated (Matucha et al. 2003, Matucha et al. 2004, Matucha et al. 2007a,b, Rohlenová et al. 2007, Gryndler et al. 2007). Radiotracer methods facilitated the (otherwise not always easy) investigations of the above problems and pointed out the main processes regarding chlorine participation in forest ecosystems, representing a substantial part of the chlorine and carbon biogeochemical cycling there. The study also indicated some practical questions connected with analysis (thermal decarboxylation of TCA, TCA's reaction with acetone - see Cape et al. 2006, TCA instability during drying of soil samples), with road salting and with handling of chlorinated solvents.

It is evident that this research field is a developing one: less has so far been done than for example in more common investigations on toxic PAHs (polycyclic aromatic hydrocarbons) (Vyas et al., 1994b, 52 citations). Nevertheless, its environmental importance is undoubted.

## **Conclusions**

The research carried out revealed that:

1. Chloride deposited in the coniferous forest ecosystems is not conservative; on the opposite, it is reactive and contributes to the degradation of SOM, i.e. of litter, which was mediated mostly through microbial processes. Chlorine thus takes part in the biogeochemical cycling in the forest ecosystem.
2. Application of radioactively labelled compounds makes it possible to follow the processes taking place in the ecological systems studied (TCA in the plant/soil-system, SOM chlorination in the forest ecosystem as well as mycoremediation of PCB contaminated soils) and to check their completeness by means of a radioactivity budget.
3. Several analytical methods were elaborated (elimination of TCA biodegradation in forest soil samples by their freezing, determination of TCA in environmental studies using  $^{14}\text{C}$  and  $^{36}\text{Cl}$ , extraction of chlorine and  $\text{Cl}_{\text{org}}$  from forest soil, determination of chloroform in soil samples) and some reactions in soil and needles (TCA decarboxylation, reaction of TCA with acetone, substitution of the OH-group in veratryl alcohol by chlorine) were explained.

4. TCA and DCA as phytotoxic substances are formed in coniferous forest soil mainly by enzymatic or microbial chlorination of soil organic matter; in addition, TCA is produced in needle chloroplasts by biooxidation of penetrating tetrachloroethylene.
5. TCA uptake from soil by roots is caused by the transpiration stream and is highest in the current year needles and slower in older needles. It can be slowly retransported from needles through phloem back into the soil.
6. TCA in soil is microbially degraded to carbon dioxide and chloride and used as the source of carbon and energy. The biodegradation rate depends on TCA concentration, soil character and humidity, temperature and microbial activity. The degradation at low environmental concentrations proceeds much more rapidly than was shown by investigations of TCA used as a herbicide in the past.
7. The chlorination process explains the formation of chlorinated compounds, including TOX, and - because of their biodegradation – it may be considered as one of the processes in SOM (litter) decomposition.
8. Chlorination of soil organic matter is a slow process mediated by microorganisms, above all basidiomycetes, which first take up chloride from the soil. Chlorination (proceeding also abiotically) leads to formation of chlorinated humic substances and to smaller aliphatic and aromatic substances (including chloromethane, chloroform, chloroacetic acids and chlorinated phenols). Some of these are volatile (e.g. chloromethane, chloroform).
9. It is obvious that some environmental processes may be very slow and take place also at low concentrations (e.g. TCA decarboxylation in needles). The results achieved show again the main ecological principle of nature – the principle of equilibrium conservation.
10. A practical result was also achieved: knowledge of the behavior of chloride in soil is important for understanding of road salting and its impact on afflicted vegetation.

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### **Publications forming the basis of the dissertation**

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## Chlor v lesním ekosystému: biogeochemické cykly (radiochemická studie)

### Resumé

V přírodě se chlor nevyskytuje pouze jako chlorid nebo látky antropogenního původu, ale také jako sloučeniny původu přírodního. Chlorid deponovaný v lesním ekosystému z atmosféry reaguje s půdními organickými látkami (SOM) prostřednictvím enzymů a/nebo mikroorganismů (do jisté míry také abioticky) za tvorby chloroctových kyselin, methylchloridu, chloroformu, dalších alifatických i aromatických sloučenin a chlorovaných huminových látek. Chlorid, na nějž je smrk ztepilý citlivý, je tak z větší části v organických chlorovaných látkách, které nad ním v lesní půdě převládají, blokovan. Chlor je tak zapojen do degradace SOM vedoucí přes menší chlorované organické produkty rozkladu až k jejich konečné mineralizaci. Prakticky stejné procesy založené na schopnosti mikroorganismů rozkládat aromatické struktury ligninu byly zkoumány v našich metodicky blízkých bioremediačních studiích. Cílem předkládaných prací z oblasti lesního ekosystému bylo nejprve (i) studovat příjem, účinek a osud fyto toxické kyseliny trichloroctové (TCA, považované dříve pouze za sekundární atmosférický polutant) v systému smrk ztepilý/půda, (ii) objasnit roli chloroctových kyselin, a nakonec (iii) celkovou úlohu chloru v ekosystému jehličnatého lesa.

Při aplikacích uhlíkem 14 značených látek bylo zjištěno, že k nejrychlejšímu příjmu TCA docházelo v nejmladších výhoncích jehličí smrku díky vyššímu transpiračnímu proudu a v jehličí k pomalé následné degradaci; TCA je však rychle mikrobiálně degradována v lesní půdě. Tato biodegradace je závislá na koncentraci TCA, vlhkosti a teplotě půdy, jejím složení i přítomných mikroorganismech. K biodegradaci TCA dochází také ve fylosféře jehličí; eliminace TCA může být způsobena také pomalou dekarboxylací. Naopak bylo ale nalezeno, že TCA vzniká v chloroplastech jehličí biooxidací absorbovaného tetrachlorethylenu.

Za užití chloru 36 byla námi poprvé prokázána dříve předpokládaná tvorba TCA chlorací SOM a rovněž kyseliny dichloroctové (DCA), která je v půdě mikrobiálně degradována dokonce rychleji než TCA. Bylo zjištěno, že chlorace probíhá převážně mikrobiálně, abioticky jen do malé míry; jako odpovědné mikroorganismy byly navrženy basidiomycety. Není zcela jasné, zdali je chlorace zprostředkována extracelulárními enzymy nebo také intracelulárně, zdali dochází k dehalogenaci a jaké další vlivy se uplatňují. Byla jasně prokázána souvislost mezi uhlíkovým cyklem a cyklem chloru: produkty chlorace jsou

dále mineralizovány, což přispívá k rozkladu opadu, a dochází i k opětovnému uvolnění chloru z lesního ekosystému i jako těkavých chlorovaných látek. Obnovuje se tak předchozí stav. Navíc, monitoring chloridu, TCA, AOX (adsorbable organic halogens) a dalších látek v lesní půdě, jehličí a půdním roztoku na lesních plochách, zejména poblíž solených komunikací, ukázal na jejich nepříznivý vliv (především chloridu) na jehličnany, zejména na smrk ztepilý. Tyto nové, jinak obtížně dostupné výsledky byly dosaženy aplikacemi převážně radiochemických metod.