

# Biogeochemical cycles of chlorine in the coniferous forest ecosystem: practical implications

M. Matucha<sup>1</sup>, N. Clarke<sup>2</sup>, Z. Lachmanová<sup>3</sup>, S.T. Forczek<sup>1</sup>, K. Fuksová<sup>4</sup>, M. Gryndler<sup>5</sup>

<sup>1</sup>*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

<sup>2</sup>*Norwegian Forest and Landscape Institute, Aas, Norway*

<sup>3</sup>*Forestry and Game Management Research Institute, Prague, Czech Republic*

<sup>4</sup>*First Faculty of Medicine, Charles University, Prague, Czech Republic*

<sup>5</sup>*Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

## ABSTRACT

Chlorine – one of the most widespread elements on the Earth – is present in the environment as chloride ion or bound to organic substances. The main source of chloride ions is the oceans while organically bound chlorine (OCl) comes from various sources, including anthropogenic ones. Chlorinated organic compounds were long considered to be only industrial products; nevertheless, organochlorines occur plentifully in natural ecosystems. However, recent investigations in temperate and boreal forest ecosystems have shown them to be products of biodegradation of soil organic matter under participation of chlorine. It is important to understand both the inorganic and organic biogeochemical cycling of chlorine in order to understand processes in the forest ecosystem and dangers as a result of human activities, i.e. emission and deposition of anthropogenic chlorinated compounds as well as those from natural processes. The minireview presented below provides a survey of contemporary knowledge of the state of the art and a basis for investigations of formation and degradation of organochlorines and monitoring of chloride and organochlorines in forest ecosystems, which has not been carried out in the Czech Republic yet.

**Keywords:** chlorine cycle; chlorination; enzymatic; abiotic; organochlorines; adsorbable organic halogenes

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 organic compounds of natural origin (Winterton 2000, Gribble 2003, Clarke et al. 2009). Chloride deposited in the forest ecosystem from the atmosphere reacts with soil organic matter (SOM) under the mediation of enzymes and/or microorganisms, forming chloroacetic acids (CAAs), chloromethane, chloroform, other aliphatic and aromatic compounds and chlorin-

ated humic substances. It is plausible that it also reacts with plant-tissue substances, and abiotic chlorination of SOM is known, too. Chloride is thus mostly bound in organochlorines. Chlorine is involved in SOM degradation, leading to smaller SOM decay products (e.g. to volatile compounds like chloromethane and chloroform) and finally to their mineralization. Closely related microbial processes were found in methodologically similar soil bioremediation studies conducted earlier. The aim of this survey is to provide the reader with

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results achieved in investigations of the uptake, effects and fate of phytotoxic trichloroacetic acid (TCA, previously considered to be only a secondary atmospheric pollutant, see Frank et al. 1994, Matucha et al. 2001) in the Norway spruce/soil-system, of the role of CAAs, and finally with considerations of the global role of chlorine in coniferous forest ecosystems and its implications.

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 before uptake by roots (Forczek et al. 2001, Matucha et al. 2001, Schröder et al. 2003). This microbial degradation depends on TCA concentration (it is slower at higher TCA levels), soil humidity (an optimal humidity depends on the soil characteristics) and temperature as well as on the soil composition and microorganisms (aerobic degradation preferably). The biodegradation of TCA took place also in the phyllosphere of the needles; TCA elimination in needles may be caused also by slow decarboxylation (Matucha et al. 2006). On the other hand, TCA may be formed by biooxidation of absorbed atmospheric tetrachloroethylene in needle chloroplasts (Weissflog et al. 2007) (Figure 1).

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 (Matucha et al. 2007a). 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 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 in the forest ecosystem was indicated and strongly suggested: products of chlorination of SOM are further mineralized and contribute to the litter decay and a part of chlorine is released as volatile organochlorines from the forest ecosystem again (Öberg et al. 2005a,b). The previous chlorine level is thus restored. Moreover, the monitoring of chloride, AOX and other substances in forest soil, needles, precipitation and soil solution in forest stands, especially near salted roads, shows an adverse effect of these substances on conifer-

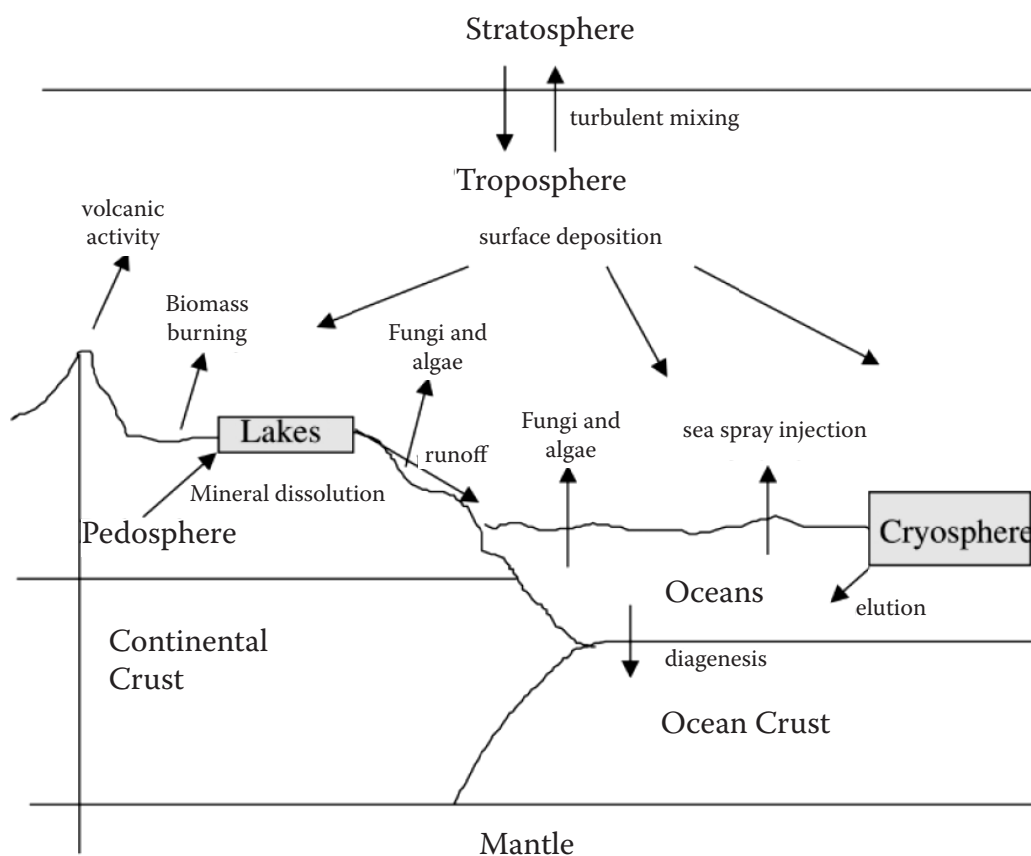


Figure 1. The earth's major reservoirs of chlorine and natural processes which transfer chlorine between reservoirs (Graedel and Keene 1996, with kind permission of Pure and Applied Chemistry)

ous trees, especially spruce. These new, otherwise hardly attainable results can be obtained mostly using radiotracer methods.

### Biogeochemical cycling of chlorine and chlorination in the forest ecosystem

Chlorine is one of the most abundant elements on the surface of the Earth (Graedel and Keene 1996, Winterton 2000) and the level of its inclusion in natural organic matter, and its role in nature, e.g. in the photosystem II, Popelkova and Yocum (2007), is compared with that of phosphorus (Öberg 2003) (Figure 2). In the forest ecosystems – especially in coniferous forests (Johansson et al. 2003) – it is present in both the soil and the biosphere, as chloride ion and bound to organic matter ( $Cl_{org}$ ). The major source of chloride ions is the oceans, whereas the  $Cl_{org}$  originates from various sources. Chloride level in the environment depends largely on the geographic situation (i.e. the distance to the coast or to local combustion sources), while chlorinated organic compounds were previously considered to be only xenobiotics of anthropogenic origin (Asplund and Grimvall 1991, Öberg 1998, 2002, 2003). Precipitation washes out chloride and AOX from the atmosphere and leaves (as dry aerosols are caught by the canopy), and their deposition to the forest differs in magnitude between several grams and hundreds of

kg/ha and year, depending on geographical and climatic conditions, on the distance from the sea or other sources, sea water salinity, wind direction, altitude etc. (Aamlid and Horntvedt 2002, Clarke et al. 2009). Chloride content in soil and its effects in the forest environment were mostly assumed to be negligible. However, it contributes to degradation of soil organic matter (SOM) and some woody plants like Norway spruce might be sensitive to its excessive concentration.

Chloride was earlier considered a chemically inert substance in the environment (e.g. Ogard et al. 1988, Neal and Rosier 1990). Currently the common assumption that chloride is conservative in soils and can be used as a groundwater tracer is being questioned, and an increasing number of studies indicate that chloride can be retained in soils. In past it was not understood that the processes of decomposition of organic matter in the forest environment (Eriksson et al. 1990) proceed with the participation of chlorine and microorganisms (Asplund et al. 1989). However, rather than being inert, it seems that chloride participates in complex biogeochemical cycles involving the formation, leaching, degradation and volatilization of  $Cl_{org}$ . Presently, more than three thousand halogenated compounds have been reported in nature (Gribble 2003), among them tens of biodegradation products of SOM by basidiomycetes (de Jong and Field 1997). It has lately been suggested that chlorine contributes to the decay of forest organic matter and SOM in

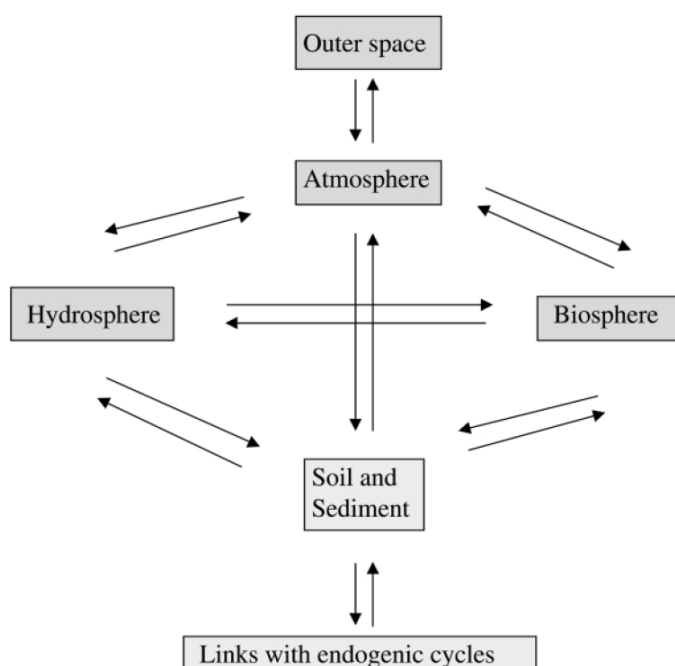


Figure 2. The main exogenic cycles and associated reservoirs at the earth's surface (Winterton 2000, with permission of the author)

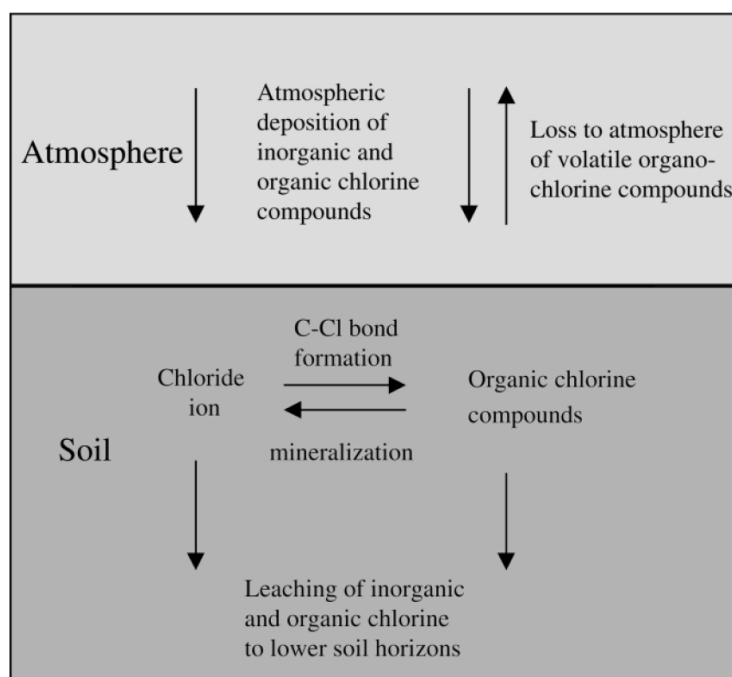


Figure 3. Transport, transformation and storage of chloride and chloroorganic compounds in soil (Winterton 2000, with permission of the author)

general. These processes were intensively studied and described many times, especially for coniferous forest (Asplund et al. 1993, 1995, Hjelm et al. 1995, 1996, 1999, Hoekstra et al. 1998, Öberg 1998, 2002, 2003, Öberg et al. 1998a,b). An anthropogenic source of chloride in the environment is winter road salting in industrial countries; its environmental influence was treated for example by Greenway and Munns (1980), Kozłowski (1997), Orcutt and Nilsen (2000), Fostad and Pedersen (2000), and Norrström and Bergstedt (2001). Knowledge of these processes is, however, still not satisfactory (e.g. Laternus et al. 2005) (Figure 3).

It has long been known that microorganisms are able to convert chloride to chlorinated organic matter and that chloroperoxidases are the enzymes responsible for this (Shaw and Hager 1959, Asplund et al. 1993, Laternus et al. 1995). Keppler et al. (2000) reported abiotic chlorination of SOM mediated by iron(III) leading to aliphatic haloderivatives; Fahimi et al. (2003) demonstrated another kind of abiotic formation of chloroacetates (this process was successfully explained by the Fenton reaction); and Biester et al. (2004) found halogen retention in peat bogs. It seems that formation of hydroxyl radicals is the basis of either microbial or abiotic chlorination of organic matter by hypochlorous acid (HOCl) formed from chloride present in the soil (Laternus et al. 2005, Matucha et al. 2007a). Formation of methyl chloride by fungal methyl-

tion of chloride was reported by Harper (1985), and reaction of chloride with pectin (Hamilton et al. 2003) leads to the same VOCls as those that cause damage to the ozone layer. Myneni (2002) and Leri et al. (2006, 2007) showed also chlorination of plant litter material.

Under the mediation of enzymes (Asplund et al. 1993, Laternus et al. 1995) chloride reacts with SOM and thus participates in the chlorine cycle, i.e. it is not conservative at all and becomes bound in SOM relatively soon.  $\text{Cl}_{\text{org}}$  concentration in soil (ca. 5–364 mg/ $\text{Cl}_{\text{org}}$ /kg soil DW) is usually higher than that of chloride (ca. 1–357 mg  $\text{Cl}_{\text{inorg}}$ /kg, Öberg 2005b) (Johansson 2003,  $\text{Cl}_{\text{inorg}}$  13–410 mg/kg,  $\text{Cl}_{\text{org}}$  32–2100 mg/kg) as has been shown several times for coniferous forest soil (Johansson et al. 2003, Öberg et al. 1998, 2005a,b), while deciduous forest displays lower values (Johansson et al. 2003). The role of fungi was recognized by this group from Linköping in the nineties (Hjelm et al. 1996), degradation and transformation of humic substances by saprotrophic fungi was found to be an important process but far from being understood (Grinhut et al. 2007); however, involvement of chlorine was not included.

Our recent studies of the role of chloroacetic acids (CAAs) in environmental processes confirmed that these compounds may affect coniferous trees and play an important role as intermediates in the decomposition of SOM (which represents the

major carbon sink in the forest ecosystem). Using  $^{14}\text{C}$ -labelled TCA and DCA, it was found that CAAs are microbially degraded to carbon dioxide relatively quickly (Matucha et al. 2003a,b, Schröder et al. 2003). While chlorination of organic matter yielded the same CAAs, formation of TCA and DCA was confirmed using radio-chlorine  $^{36}\text{Cl}$  as well (Matucha et al. 2003b, 2006, 2007a,b). The influence of TCA was noticed also in the needle ultrastructure, where the chloroplasts were affected by TCA and diminished (Sutinen et al. 1995); later, chlorosis, necrosis and needle loss – visible damage symptoms – were observed. Significant damage of the photosynthetic apparatus may also be observed after perchloroethylene uptake over the cuticle into the needle cell with its following biooxidation in chloroplasts to phytotoxic TCA (Forczek et al. 2007, Weissflog et al. 2007).

An important factor is the action of soil microorganisms (Gryndler et al. 2008, Rohlenová et al. 2009), especially of fungi. They are obviously able to convert chloride to hypochlorous acid (or chlorine radicals), which then chlorinate SOM (Matucha et al. 2007a,b). Using molecular methods, we succeeded in detecting a specific organism or group of organisms present exclusively in soil samples artificially enriched by chloride (Gryndler et al. 2008). On the basis of the nucleotide sequence of T-RFLP (terminal restriction fragment length polymorphism) fragments found in these chloride-enriched soil samples, we designed an oligonucleotide primer selectively amplifying rDNA of the said group of organisms. We sequenced the amplified ITS region and a part of the LSU rRNA gene and received an exotic sequence which did not show a similarity sufficient to determine the identity of its bearer precisely, although it seems probable that it is an unknown fungus or a group of taxonomically related fungi. Recently, we have been trying to identify these organisms on the basis of the SSU rRNA gene sequence. It is extremely interesting that the presence of this organism or group of organisms correlates with the humic matter chlorination rate. This suggests that this

microbial group is involved in the chlorination process.

Apart from the known chlorinated compounds such as CAA and chloroform (Hoekstra et al. 1999a,b, 2001, Niedan et al. 2000, Laturnus et al. 2005, Matucha et al. 2007b, Figure 4), other chlorinated compounds are formed by fungi in soil, e.g. volatile chloromethane (Harper 1985) or water soluble chlorinated humic substances (Lee et al. 2001), which might be contained in the run-off of forest catchments. Chlorinated aromatic structures which might originate from lignin are also expected (de Jong and Field 1997, Flodin et al. 1997, Niedan et al. 2000); however, the exact structures of soil  $\text{Cl}_{\text{org}}$ , especially of adsorbable organohalogens (AOX), have not been sufficiently reported. AOX are formed in the forest soil; their formation is proportional to the TOC and chloride contents and influenced by pH (Johansson et al. 2003). Large molecules of chlorohumus were anticipated and also confirmed in our preliminary investigations (Bastviken et al. 2007). We found a plausible reason for the difference between the products of short and long chlorination times: after longer chlorination, chlorine was bound more firmly, probably to aromatic rings (Rohlenová et al. 2009). Formation of chlorinated compounds in forest soil is undoubted and was sufficiently proved in a spruce forest ecosystem (Öberg 2003, Öberg et al. 2005a,b, Bastviken et al. 2007). Chlorination leads to compounds with structures more easily degradable and water soluble, partially even to volatile chlorinated hydrocarbons. It proceeds microbially, enzymatically and also abiotically and all these processes contribute in the end to the degradation of organic matter and mineralization of carbon, i.e. to its loss from the whole forest ecosystem (e.g. Schlesinger 1999, Piccolo 2001, Piccolo et al. 2004). Volatile chloromethane and chloroform can reach the atmospheric ozone layer and the estimated production of chloroform in forest soil of about  $100 \text{ ng/m}^2/\text{h}$  (Hoekstra et al. 2001) indicates possible contamination of ground water (Laturnus et al. 2002). Chlorine thus par-

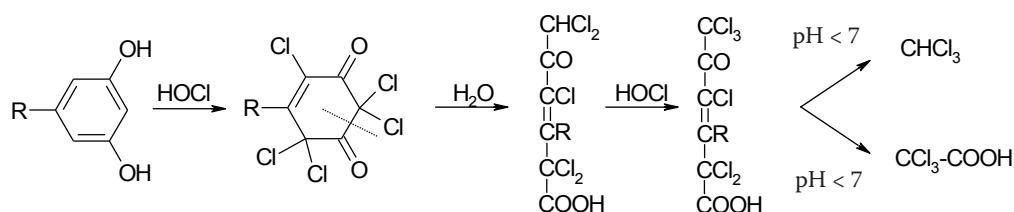


Figure 4. Formation of chloroacetic acids and/or of chloroform from resorcinolic structures in humic acids or lignin, respectively (Matucha et al. 2007b)

ticipates in the carbon cycle and its connection with the release of CO<sub>2</sub> into the atmosphere is more and more obvious (e.g. Adams and Piovesan 2005). CAAs, involved in the degradation of SOM (Matucha et al. 2003a, 2007a,b), are good examples of mineralization intermediates, while similar behavior was reported for 3,5-dichloroanisylalcohol (Verhagen et al. 1998), and further compounds of the same character may be anticipated.

These chlorination processes were indicated using chlorine 36 (Silk et al. 1997, Lee et al. 2001, Shaw et al. 2004, Matucha et al. 2003b, 2006 and 2007a,b, Bastviken et al. 2007, Rohlenová et al. 2009). Microbial dehalogenation might also be involved. It should be stressed that the carbon content in the pedosphere is higher than in the biosphere (Krull et al. 2003). Decomposition of SOM produces not only CO<sub>2</sub> but also dissolved organic matter (Marschner and Kalbitz 2003, Kalbitz et al. 2000, 2003), sometimes in concentrations of more than 100 mg/l. A chemically very similar process is the disinfection of water (Rook 1980, Christman et al. 1983, Miller and Uden 1983, de Leer et al. 1985, Reckhow et al. 1990) involving chlorination of remaining humic substances (HS) in diluted aqueous solutions. The difference is due to differences in HS concentration, rate of diffusion in soil solution, pH value, concentration of HOCl and of chlorinated products, etc. De Leer et al. (1985) found more than one hundred chlorinated compounds produced by chlorination of an aqueous solution of humic acid (HA) with hypochlorite at pH 7.2. Apart from CAA and chloroform, they also found chlorinated acetones, pyruvic and C<sub>4</sub>-diacids as principal chlorination products, some of them considered precursors of chloroform (Hoekstra et al. 1985). Another similar process is pulp bleaching, leading to the same chlorination products. Qualitative results concerning chlorination in soil indicated by Hoekstra et al. (1999a,b) and by us (Matucha et al. 2007a,b) confirmed the same chlorination mechanism. Aromatic compounds containing phenolic and benzoic acid structures indicated the HS origin and basidiomycetes were considered the most important decomposers of forest litter (de Jong and Field 1997).

It is evident that we meet chlorination of SOM, of lignin (Johansson et al. 2000), humic and of fulvic acids (FA) (Niedan et al. 2000), and its products – chlorocarbons, CAAs, chlorinated aromatic compounds (Harper 1985, Haiber et al. 1996, de Jong and Field 1997, Pracht et al. 2001, Fahimi et al. 2003, Matucha et al. 2003b, 2004, 2007a,b, Laternus et al. 2005) etc. – are expected or present

in the forest ecosystems, where haloperoxidases are present at the same time (Asplund et al. 1993, Laternus et al. 1995). It can be presumed that these chlorinated substances represent intermediates in the degradation of organic matter in the forest soil coming from litter. The recent results from this research field show the important role of microorganisms. Bastviken et al. (2007) showed retention of chloride by microorganisms with subsequent chlorination of SOM, Gryndler et al. (2008) the influence of chloride concentration on microbial communities, Rohlenová et al. (2009) the chlorination of SOM in FH soil caused by microorganisms (only a small part being chlorinated abiotically) and Laternus and Matucha (2008) chloride as a precursor of volatile Cl<sub>org</sub> (VOCl<sub>s</sub>) in lower forest plants – in ferns and moss. Formation of volatile chlorocarbons was indicated indirectly, by chlorine deficit also during chlorination of SOM using <sup>36</sup>Cl-chloride (Rohlenová et al. 2009).

## Methodology

From methodological point of view, chlorine 36 and carbon 14, radioisotopes with suitable properties for long-term radioanalytical studies of the role of chlorine in the complex forest ecosystem, may be used as radionuclides with suitable properties: long half-life (3.01 × 10<sup>5</sup> and 5760 years, resp.) and sufficiently hard β-emitters (E<sub>max</sub> = 710 and 156 keV). Carbon 14 is a well-known tracer, while chlorine 36 was used first as a conservative tracer as chloride for translocation studies in plants by Gage and Aronoff (1960) and then for hydrological investigations (e.g. Ogard et al. 1988) presuming inert character of chloride. One of the first papers on the use of chlorine 36 for soil studies is that of Silk et al. (1997). Holst et al. (2000) used chlorine 36 for similar purposes. Hardly measurable isotope effects for both nuclides were reported (Huang et al. 1999, Holmstrand et al. 2006), and no isotopic exchange was found (Matucha et al. 2007b).

Several suitable analytical methods were already described in reported investigations (Matucha et al. 2004, 2006, 2008, Bastviken et al. 2007); these may serve as prerequisites for anticipated further research. Extractions with aqueous solutions or organic solvents combined with size exclusion chromatography (gel filtration), ultrafiltration and GC or HPLC combined with MS may be used for separation and identification of chlorination products and a SPME technique for chloroform determination in soil and water. An important

issue is the analysis of the treated soil, especially the separation or isolation of  $^{36}\text{Cl}$ -chlorinated SOM (Lee et al. 2001) and  $\text{Cl}_{\text{org}}$ . We found that an extraction procedure used for fulvic acids (FA) (e.g. Bastviken et al. 2007) could be improved by chromatographic separation of fulvic acids (Řezáčová and Gryndler 2006). The procedure of Bastviken et al. (2007) afforded high FA values in the supernatant after HA precipitation, which was, however, not confirmed by the chromatographic procedure of Řezáčová and Gryndler (2006) and showed different chlorine binding to HA, probably into aliphatic chains and after longer chlorination to aromatic rings (Rohlenová et al. 2009). VOCs will be determined by capillary GC-ECD/MS after thermodesorption from adsorption tubes. Ultrafiltration as a separation method might be used for characterisation of the molecular size in spite of some methodological problems (Lin et al. 1999, Jones and O'Melia 2001). Further need of a development, or at least a check of analytical methods is obvious.

As indicated above, soil microbiota are an essential factor mediating and affecting natural chlorine cycling. Most intensive microbial activity in soil is located at the soil/plant interface, the rhizosphere, which is extremely rich in microorganisms (mainly bacteria); its role in the chlorination/biodegradation of chlorinated compounds has not, however, been stressed. The other compartment containing a lot of microorganisms is the fermentation horizon  $\text{O}_2$ . The development of rhizosphere microflora at different levels of soil chloride can be followed using two methods: a conventional dilution plate cultivation technique as well as a molecular approach using TRFLP (terminal restriction fragment length polymorphism, Gryndler et al. 2008); microbial activity might be shown by chlorination. Occurrence of microorganisms (eubacteria, eukaryota, fungi) is correlated with rhizosphere chloride concentration to conclude whether chloride affects selectively specific groups of organisms or the rhizosphere as a whole. Particular attention should be paid to mycorrhizal fungi, their detection in ectomycorrhizae, and their potential ecological role in soils with increased chloride input. Interesting in this context is the finding of the chlorination of dissolved humic substances inside of microorganisms reported lately (Steinberg et al. 2006).

To be able to predict concentrations and fluxes of organochlorines in forest soils, a modelling approach is likely to be helpful. A dynamic model of DOC formation and transport (DyDOC, Michalzik

et al. 2003) can be adapted to include DOC chlorination and thus model the formation and transport of organochlorines.

To provide values for chlorine input and output in the forest ecosystem and subsequent precise calculation of the budget, monitoring should be done in the field. In order to put the chlorine cycle in a more concrete form, the components of the chlorine cycle of a small forested catchment was constructed by Öberg (2002). Chloride and organic chlorine were monitored in a small forested catchment in southern Sweden (Svensson et al. 2006). Field observations include storage in soil, input and output by deposition and leachate, and output through volatilisation. A simultaneous monitoring of chloride and organochlorines in the forest ecosystems on the catchment scale has not been carried out in the Czech Republic yet.

Only a pilot study of chloride,  $\text{Cl}_{\text{org}}$  and carbon in the soil, and of the chlorine concentration in the needles, was recently carried out at the plots of Jarosov and Nezarkou and Plhov near Jičín affected by chlorine salt application, and at the control locality of Černý Důl in the Novohradské Mountains (Matucha et al. submitted).

The experimental catchment plot of the FGMRI, near the Zelivka water reservoir (dam) was chosen for intensive monitoring of chloride and organic chlorine in the Czech Republic. This catchment has been studied since the 1960's and a long data series is at our disposal. In 1995 the research plot of Zelivka was included in the ICP Forests network as a Level II plot. At Zelivka, a detailed monitoring of chlorine will be carried out. Besides the parameters permanently measured within the ICP Forests monitoring programme, AOX in the soil, precipitation water, soil water and runoff is currently being measured. To compare the chlorine amount in disturbed and undisturbed ecosystems, monitoring of chlorine and other parameters in the soil, needles and runoff water should be done.

## Conclusions

Based on this survey, it is suggested, to deepen the basic knowledge of the biogeochemical cycling of chlorine, to investigate chlorination processes and evaluate their role as a little elucidated contribution to the mineralization of soil carbon in the forest ecosystem. For detailed investigations of the formation and degradation processes, it is presumed to use common analytical and radiotracer techniques in laboratory model experiments (ton

gether with radioactively  $^{14}\text{C}$ -labelled compounds for degradation and  $^{36}\text{Cl}$ -chloride for formation studies). Some of these were successfully used or modified in previous or recent studies used for this purpose. The research should involve monitoring of chloride, AOX and VOCs in the forest soil, in the soil solution and in the runoff. These data are presently missing in the Czech Republic. Special attention should be paid to forest stands near roads salted in winter with appropriate analyses of soil solution and runoff with respect to their contamination and effects on conifers. A special aim is to investigate whether forest soil and common forest plants produce atmospherically reactive VOCs and low molecular substances penetrating into ground water.

The role of chlorine may be seen as that of a degradation agent in the decomposition of SOM in forest soil, a big terrestrial reservoir of carbon, and chlorination may be understood as one of the decomposition processes leading to a steady state in the spruce forest ecosystem with regard to chlorine as well as carbon, i.e. by contributing to SOM mineralization and elimination or blocking of chloride at the same time. The natural AOX content in soil is higher than that of chloride, and only a small part of  $\text{Cl}_{\text{org}}$  is released into the atmosphere (Öberg et al. 2005b, Rohlenová et al. 2009, Matucha et al. 2008).

Further research will increase and deepen the basic knowledge on biogeochemical cycling of chlorine in the coniferous forest ecosystem under significant participation of microorganisms, will advance our recent research on chlorinated substances (directed towards volatile and aromatic ones), and from a practical point of view will elucidate the possibility of penetration of chloroform and other chlorinated substances formed into surface and ground water and the influence of road salting on adjacent spruce stands. Microbiological investigations will further elucidate relationships between microorganisms and soil chlorination activity and radiotracer methods will trace the studied processes in the soil.

To summarize, the following should be investigated:

(1) The mechanism of SOM (or HA) chlorination together with the formation and decay of low-molecular aromatic intermediates using radiotracer methods.

(2) Molecular genetic analysis of putative microorganisms participating in SOM chlorination and the influence of chloride on the microbial communities.

(3) Verification of the formation of VOCs in spruce forest ecosystems including plants.

(4) Monitoring of chloride and AOX in several forest stands (including salted ones) and analysis of soil for chloroform and its penetration into ground and drinking water.

(5) Modelling of the chlorination process using an adaptation of the DyDOC model.

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*Corresponding author:*

Ing. Miroslav Matucha, CSc., Ústav experimentální botaniky AV ČR, v.v.i., Izotopová laboratoř, Vídeňská 1083, 142 20 Praha 4, Česká republika  
e-mail: matucha@biomed.cas.cz

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