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# Microbial Chlorination of Organic Matter in Forest Soil: Investigation Using $^{36}\text{Cl}$ -Chloride and Its Methodology

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Chloride, which comes into the forest ecosystem largely from the sea as aerosol (and has been in the past assumed to be inert), causes chlorination of soil organic matter. Studies of the chlorination showed that the content of organically bound chlorine in temperate forest soils is higher than that of chloride, and various chlorinated compounds are produced. Our study of chlorination of organic matter in the fermentation horizon of forest soil using radioisotope  $^{36}\text{Cl}$  and tracer techniques shows that microbial chlorination clearly prevails over abiotic, chlorination of soil organic matter being enzymatically mediated and proportional to chloride content and time. Long-term (> 100 days) chlorination leads to more stable chlorinated substances contained in the organic layer of forest soil (over time, chlorine is bound progressively more firmly in humic acids) and volatile organochlorines are formed. Penetration of chloride into microorganisms can be documented by the freezing/thawing technique. Chloride absorption in microorganisms in soil and in litter residues in the fermentation horizon complicates the analysis of  $^{36}\text{Cl}$ -chlorinated soil. The results show that the analytical procedure used should be tested for every soil type under study.

## Introduction

Organic matter in forest soil consists of various substances. Humic substances representing the major part of soil organic matter (SOM) constitute a resistant fraction with a long turnover time whose recycling proceeds mainly by oxidative degradation with participation of soil microbiota, in particular, of fungi. SOM degradation associated with the formation of chlorinated organic compounds was reported 20 years ago (1), and a recent report concerning chlorine cycling in a coniferous catchment has calculated the fluxes and budget of chloride ( $\text{Cl}_{\text{inorg}}$ ) and  $\text{Cl}_{\text{org}}$  (2, 3). Atmospheric

deposition of chloride depends on geographical and climatic conditions and ranges from 10 mg to 10 g  $\text{Cl}^-/\text{m}^2$  annually (4), with chloride being mainly of maritime origin. As shown in previous studies (1, 5), exoenzymes (chloroperoxidases, CPO, and CPO-like enzymes) produced by microorganisms (in particular in the fermentation horizon) are able to catalyze chlorination of organic matter in the soil environment, so that the biotic path of degradation of SOM—including humic substances—through chlorination is likely to constitute an important portion of the flux of organochlorines (OCls) in the soil.

Chlorination may also be understood as a decay process contributing to mineralization of SOM, i.e., to  $\text{CO}_2$  formation, a factor related to global climatic change. Its investigation should involve monitoring of chloride and total organic halogens (TOX) in soil and chloride, adsorbable organic halogens (AOX) and dissolved organic carbon (DOC) in runoff. Chlorination of humic substances (fulvic and humic acids) was observed in vitro by Niedan et al. (6) and Matucha et al. (7) in the presence of chloroperoxidase (CPO) (5). It seems, however, that chlorination of SOM in forest soil proceeds spontaneously, microbially under mediation of exoenzymes (5), and to a lesser degree, abiotically by the Fenton reaction (8). Among the chlorination intermediates in the forest soil are dichloroacetic (DCA) and trichloroacetic (TCA) acids, both accompanied by volatile chloroform (9, 10), chloroanisols, and other chlorinated substances (11). These compounds were reported also in earlier studies of water disinfection by chlorine (12): chlorination of humic substances at pH 7.2 afforded, apart from the main products, more than a hundred OCls, including  $\text{C}_4$ -dicarboxylic acids (suggested as chloroform precursors) and lower amounts of aromatic compounds (12). Of a similar character may be the products of pulp delignification, i.e., pulp bleaching using chlorine (13), the nature of which is nearest to chlorination of SOM. The formation of OCls present in only small amounts could not be confirmed in the forest soil because of their difficult isolation. They may form a major part of TOX, an analytical bulk parameter also including the other halogens, found regularly in coniferous forest soil in higher concentrations (more than 200 mg/kg (4)). The aromatic character of chlorinated substances coming from litter, i.e., from lignin, was shown earlier (14). Basically, chlorination in soil solution may differ from that of humic acids (HAs) in drinking water or pulp bleaching by chlorination mainly in the concentration of major substances and impurities; however, the main OCls produced in the process should be the same. Studies investigating the fate of  $^{36}\text{Cl}$  from nuclear weapon tests in soils after many years showed the binding of chlorine to soil organic matter of lower molecular weight (15). The common feature in these processes is presumably chlorination by chlorine radicals in aqueous solution. An abiotic chlorination of SOM, humic acids and phenolic moieties has also been described (8). Its basis is the Fenton reaction that leads, in the presence of iron (II) and hydrogen peroxide, to the formation of chlorine radicals from chloride ions.

Because SOM chlorination has a microbial character and chloride did not exhibit any clear effect on soil respiration, it was concluded that chloride may affect microbiota (16). Microorganisms showed a certain role during the absorption and retention of salt solution by forest soil; they first took up a higher amount of chloride which was then slowly released, whereas SOM was slowly chlorinated after a lag phase of several weeks (17). Enzymatic formation of chloroacetic acids (CAAs) and chloroform in the forest soil was confirmed using stable isotope  $^{37}\text{Cl}$  (9), and chlorination of fulvic acids has

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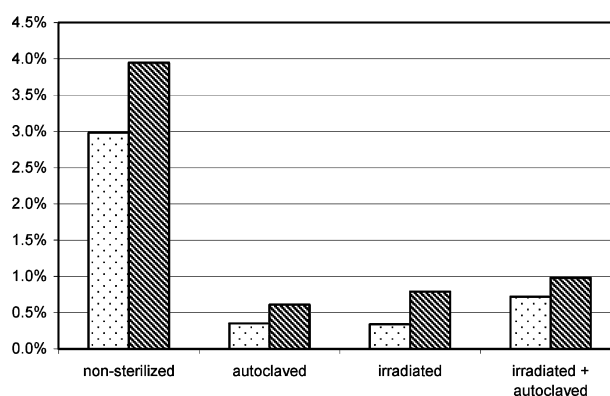
also been shown (6). Enzymatic formation of TCA in HA suspensions (7) and natural microbial chlorination in forest soil leading to DCA and TCA formation were reported later (10) using radioisotope  $^{36}\text{Cl}$ . This sufficiently hard  $\beta$ -emitter ( $E_{\text{max}} = 710 \text{ keV}$ ) with long half-life ( $3.01 \times 10^5$  years) appeared suitable for investigations of chloride reactions in the forest ecosystem. Chlorination of SOM using  $^{36}\text{Cl}$ -chloride, conducted for other purposes, was also reported by Lee et al. (15) who studied behavior of  $^{36}\text{Cl}$ -chloride as a component of radioactive waste in soil, and by Silk et al. (18), who investigated chlorination of peat. Chlorinated aromatic structures were found in decaying plant material (14) as well as in products of chlorinated fulvic acid (6).

Using  $^{36}\text{Cl}$ -chloride and the recently developed methodology used for the mineral horizon of the forest soil (17) we show here microbial degradation of SOM in the fermentation horizon of the forest soil, occurring via a natural spontaneous chlorination. Our study should elucidate, among other things, the influence of chloride concentration in soil and of the extraction procedures used for the humic substances that form the major part of organic matter in the fermentation horizon (FH) of the forest soil.

## Experimental Section

$^{36}\text{Cl}$ -chloride (GE Healthcare Amersham, U.K.,  $3 \mu\text{Ci}/\text{mg Cl}$ ) and forest soil (20 g FH soil Baně; soil B–O2 pH( $\text{H}_2\text{O}$ )/(KCl) 4.17/3.37  $\text{C}_{\text{ox}}$  (% dw) 15.4 Cl (ppm) 31.8), homogenized by a 2 mm sieve, amended by sprinkling of the corresponding volume of  $^{36}\text{Cl}$ -chloride solution on the soil and its thorough mixing in a plastic bag (10), and put in a 250 mL Erlenmeyer flask with moistened air flow (30 mL/min) were used for the 1–3 month-long study. The Baně spruce forest stand, approximately 70 years old, is situated 20 km south of Prague. The extraction procedures used followed roughly the method described by Bastviken et al. (17). Extraction of chloride from soil was conducted twice with 50 mL water and twice with 50 mL 0.1 M  $\text{KNO}_3$  per 20 g FH soil followed by centrifugation at 4000g. AOX in the aqueous extract obtained were not determined; however, the content of chlorinated organic substances in the aqueous chloride extract was occasionally checked by chromatography on a PVPP column (19). To fractionate chlorinated compounds, the extracts were subjected to ultrafiltration (Nucleopore 100 mL, Millipore, membrane exclusion limit 1 kDa) or separation by gel filtration (Sephadex G25, Pharmacia Uppsala, Sweden). To determine the content of chloride absorbed in microorganisms, the soil after extraction of chloride was frozen at  $-20^\circ\text{C}$  overnight, and the previous extraction of chloride was repeated after free thawing (i.e., with ruptures of cell walls and membranes) (17). The extracted soil (without removed chloride) was then extracted with 0.1 M NaOH (2x with 50 mL) and 1x washed with 50 mL water; all three extracts were pooled and the radioactivity of humic substances measured by liquid scintillation (LS) spectrometry (LS Rotiscint eco plus Carl Roth GmbH, Karlsruhe, Germany, and LS spectrometer Beckman 8500, Fullerton, CA). Each experiment with complete analyses was conducted twice.

To determine residual  $^{36}\text{Cl}$ -content in extracted soil after extraction of chloride and of humic substances by the AOX method, extracted soil was dried and a 100–200 mg sample was combusted in oxygen atmosphere in a Euroglas Chlorine Analyzer ECS 1200 (NL) instrument at  $1000^\circ\text{C}$ . The oxygen flow was then introduced into 10 mL of 1 M KOH and the absorbed  $^{36}\text{Cl}$ -radioactivity of an aliquot was measured by LS counting. Soil sterilization was done with a  $^{60}\text{Co}$ -source (radiation dose 25 kGy, used currently for sanitary purposes) (Artim Prague, Czech Republic) and/or by 20 min autoclaving at  $121^\circ\text{C}$ . To test radiation stability of CPO in aqueous solution, 224 U CPO (Fluka) in 5 mL  $\text{H}_2\text{O}$  in a closed ampule



**FIGURE 1. Microbial chlorination and influence of soil sterilization. Proportion of  $^{36}\text{Cl}$ -chlorinated HA found in FH soil (in % of  $^{36}\text{Cl}$  radioactivity) compared with sterilized soils chlorinated under the same conditions (50 g soil,  $7.40 \mu\text{Ci } ^{36}\text{Cl}$ -chloride, chlorination time 80 days at room temperature). Results obtained in two independent experiments are presented as column pairs.**

**TABLE 1. Chlorination as a Function of Chloride Concentration<sup>a</sup>**

added $^{36}\text{Cl}$ amount	$^{36}\text{Cl}$ in aqueous extract	$^{36}\text{Cl}$ in alkaline extract
5.668	5.235	0.143
5.668	5.164	0.167
11.336	10.237	0.250
11.336	10.267	0.267
17.004	15.660	0.316
17.004	15.940	0.323

<sup>a</sup>  $^{36}\text{Cl}$  amounts in  $\mu\text{Ci}$ ,  $3 \mu\text{Ci} = 1 \text{ mg Cl}$ , added to 20 g FH soil Baně (first column), chlorination time 40 days, total amount of chloride extracted 2x with water and 2x with 1 M  $\text{KNO}_3$  (second column), extraction of chlorinated humic substances 3x with 0.1 M NaOH after extraction of microbial  $^{36}\text{Cl}$ -chloride (third column); double precipitation of  $^{36}\text{Cl}$ -HA led to loss of  $^{36}\text{Cl}$ -radioactivity bound to HA (not shown here).

were irradiated with a 10 or 50 kGy dose. The activity of the irradiated enzyme was shown by the method used for HA chlorination earlier (7) and compared with that of the nonirradiated enzyme in the same way.

To examine the possibility of hydrolysis of the C–Cl bond in  $^{36}\text{Cl}$ -HA, the HA fraction was precipitated again carefully by 1 M  $\text{H}_2\text{SO}_4$ , dissolved in 1 M KOH and the  $^{36}\text{Cl}$  radioactivity of the supernatant above the precipitated HA or of the alkaline HA solution was measured again by LSC. Each result represents a mean of two measurements.

## Results and Discussion

Chlorination of SOM in forest ecosystems under the influence of chloride deposition leads to formation of chlorinated substances, the content of which in forest soil is usually several times higher than that of chloride (4). Here we confirm that chloride is not conservative and that its reactivity with SOM of FH soil is mediated mainly by enzymes produced by soil microorganisms, in agreement with previous findings (2). As a result of these reactions, chlorinated substances (TOX), a small part of which are soluble in water, are formed in the forest soil and may, in principle, penetrate as AOX into ground- and runoff water.

The greater part of SOM chlorination proceeds microbially, as confirmed by comparison of long-term chlorination (80 days) of nontreated coniferous forest soil (FH horizon) with that of the same soil samples sterilized by autoclaving

TABLE 2. Chlorination of SOM as a function of time<sup>a</sup>

no.	experiment time (days)	<sup>36</sup> Cl [ $\mu$ Ci] found in:					deficit <sup>36</sup> Cl [ $\mu$ Ci/%]
		aqueous extract	Cl <sup>-</sup> in micro-organisms	Cl-HA	residue in soil	$\Sigma$	
1	53	12.020	0.521	0.109	0.306	12.956	0.699/5.1
2	53	11.941	0.570	0.121	0.317	12.949	0.706/5.2
3	106	11.331	0.490	0.220	0.548	12.589	1.066/7.8
4	106	11.284	0.542	0.202	0.552	12.580	1.075/7.9

<sup>a</sup> 20 g FH soil Baně were amended with 13.655  $\mu$ Ci <sup>36</sup>Cl, extraction procedures are described in the Experimental section, <sup>36</sup>Cl deficit is calculated as difference between the amount of <sup>36</sup>Cl applied and the sum of <sup>36</sup>Cl amount found in fractions ( $\Sigma$ ).

and/or radiation (using amendment of <sup>36</sup>Cl-chloride). The content of <sup>36</sup>Cl-HAs formed abiotically in the treated soil corresponded to 1/4 of that found in the nonsterilized soil samples, this greater content being due mainly to enzymatic/microbial activity (Figure 1). Interestingly, the CPO enzyme in water solution was destroyed already by the low radiation dose used (see the Experimental Section), so that it is suggested that the chlorination was mediated enzymatically.

To compare the previous results on chlorination of SOM in mineral horizon soil (17), we examined the chlorination of SOM in coniferous forest FH soil. The FH contains more microbial biomass and is thus more microbially active; however, in spite of homogenization by 2 mm sieving, it contained litter residues (10–20% dry weight of old needles and branch wood residues) which absorbed more than 30% of the applied salt solution. This shows great inhomogeneity in the FH soil samples, and a possible influence of litter residues on the chlorination process.

Although the difficulties described above complicated the study of chlorination, we then tested the influence of chloride concentration on SOM chlorination. In the range of 100–300 mg Cl<sup>-</sup> kg<sup>-1</sup> soil the chlorination was found to be proportional to the chloride content, in agreement with previous results (e.g., ref 20, see Table 1), and also to time (Table 2). As found by the extraction procedures used, <sup>36</sup>Cl-chlorohumus formation is apparently proportional to time also for FH soil. Prolonged chlorination led to more stable substances (<sup>36</sup>Cl-chlorohumus HA extracted by alkaline solution was precipitated but only partially hydrolyzed, as confirmed by repeated HA isolation then showing <sup>36</sup>Cl-HA of constant radioactivity) in contrast to chlorination shorter than six weeks, which formed only hydrolyzable <sup>36</sup>Cl-HA (see Figure 2). As shown by ultrafiltration <1 kDa and analysis on a PVPP column, shorter chlorination (<6 weeks) and repeated HA precipitation afforded supernatant which did not contain organically bound chlorine, i.e., it showed very similar results. Obviously, these humic substances, which contained organically bound <sup>36</sup>Cl (as indicated by gel filtration on Sephadex 25 after (15)), released chlorine during dissolution of precipitated <sup>36</sup>Cl-HA in alkaline solution, again by hydrolysis (see Figure 1). A similar substitution, which is probably not only enzymatically mediated, has been indicated for veratryl alcohol, a compound with a substituted aliphatic chain, and chloride (21). Such substances, which are not firmly chlorinated (most probably in aliphatic chains like that of benzyl chloride), may be expected to arise during a short SOM chlorination while long-term chlorination affords firmly bound (i.e., nonhydrolyzable) chlorine, presumably on an aromatic ring. A similar result, formation of alkaline-labile organochlorines, was found when analyzing AOX in aqueous precipitation samples (22) and chlorinated HA in a chlorination study (23). In experiments simulating long-term chlorination of SOM by <sup>36</sup>Cl-chloride as a nuclear fission product in lysimeters and gel filtration of soil extracts (15), variably chlorinated substances were also found. In this way, lignin biodegradation in forest ecosystems with chlorine participation leads presumably to a greater production of

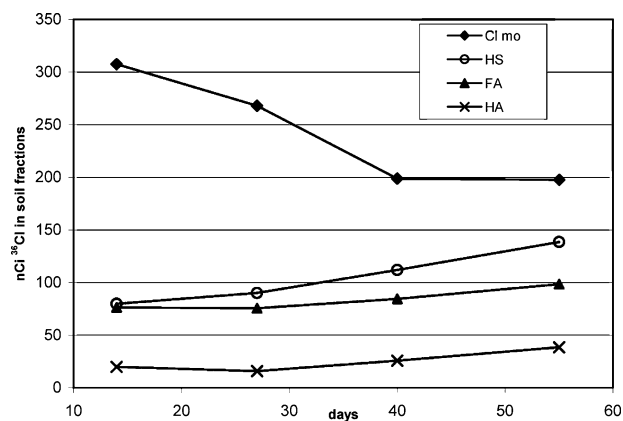


FIGURE 2. Chlorination of FH forest soil as a function of time, showing decreasing chlorine content in microorganisms and increasing content of chlorination products. One precipitation of HA from HS led to the release of FA (chloride); repeated dissolution and precipitation of <sup>36</sup>Cl-HA led to a total loss of bound <sup>36</sup>Cl. HS-humic substances (alkaline soil extract), HA-humic acids (precipitated extracted HS redissolved in 0.1 M NaOH), FA-fulvic acids (supernatant after precipitation of HA at pH 1), Cl mo-chloride obtained by freezing/thawing from microorganisms.

TABLE 3. Extraction procedures and influence of freezing/thawing<sup>a</sup>

	radioactivity found [ $\mu$ Ci]	
	With freeze/thaw	Without freeze/thaw
aqueous extract	12.157	12.015
chloride in microorganisms	0.366	n. d.
second extraction of chloride	n. d.	0.197
chlorinated HA	0.135	0.248
residual <sup>36</sup> Cl-HA	0.008	0.006

<sup>a</sup> 15 g Baně FH soil, 13.6  $\mu$ Ci <sup>36</sup>Cl, one month chlorination; extraction procedure—see the Experimental Section; nd, not determined; 2nd extraction of chloride, repeated fourfold aqueous extraction; residual <sup>36</sup>Cl-HA, additional fourth HA extraction.

chlorinated substances with chlorine bound in aromatic rings and to greater litter decay; deficiency in the radioactivity balance may indicate also formation of volatile chlorocarbons like chloromethane and chloroform (Table 2). However, as demonstrated recently, a higher chloride concentration (500 mg/kg soil) in its turn changes the composition of the microbial community (16).

The extraction procedures together with the effect of the freezing/thawing step on chlorine extraction were examined as shown in Table 3. As originally suggested for forest soil from the mineral horizon (17), by destroying cell walls and membranes this procedure leads to the release of chloride from microorganisms, showing its presence there. However,



the destruction of the microorganisms need not be complete, whereas a residue of previously nonextracted chloride may occur in this extract (see Table 3 showing the influence of freezing/thawing and extraction of chloride from microorganisms on the whole procedure). Freezing/thawing caused an additional extraction of chloride from soil microorganisms (366 nCi, two times extracted with water, two times extracted with 1 M KNO<sub>3</sub>) while the same second extraction with the same solvent amounts but without freezing/thawing showed a still appreciable chloride amount (197 nCi). This might be due to diffusion of chloride from microorganisms and to residual chloride absorbed in the litter residues and dissolved in the pore water of the FH soil (about 0.5–1% of chloride was extracted by the additional fifth aqueous extraction). This result shows that, in chlorination studies of FH soil, content of residual chloride and chloride in microorganisms (17) might be underestimated, especially in cases of high contents of chloride and old litter residues; the second extraction series should have shown chloride diffusion from microorganisms during the time between both extractions rather than residual chloride. On the other hand, the four extractions in the first aqueous extraction series removed practically all the amended <sup>36</sup>Cl-chloride together with <sup>36</sup>Cl-AOX (sorbed on PVPP) and the HA fraction may have contained some chloride from microorganisms. Residual chlorine extracted as HA by an alkaline solution (like in the fourth extract) was negligible.

The results of our investigations lead to the conclusion that analysis of the resulting chlorinated soil containing added <sup>36</sup>Cl-chloride must be conducted very carefully, especially in the case of FH soils. It is obvious (see Figure 2 and Table 2), that, in agreement with previous findings (17), soil microorganisms absorb chloride. An important process that may occur is the hydrolysis of organically bound (alkaline-labile) chlorine after a shorter chlorination time, whereas the first aqueous extracts of chloride contain AOX as well. It is therefore recommended to check extraction recoveries and the character of extracted substances, for example by XAD-8 or PVPP column chromatography after Řezáčová and Gryndler (19). Formation of organochlorines (<sup>36</sup>Cl-TOX, i.e., <sup>36</sup>Cl-HA and <sup>36</sup>Cl-AOX) with different stabilities of the carbon-chlorine bond against alkaline or even enzymatic hydrolysis in the forest soil should be investigated in more detail. This process might cause the imbalances found by Öberg et al. (2, 24), and our results give an explanation of their findings. The use of chlorine 36 for the in vitro investigations described above may elucidate only the starting phase of SOM chlorination, which may proceed for a long time in the forest ecosystem as a part of SOM degradation processes.

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## Literature Cited

- Asplund, G.; Grimvall, A. Organohalogenes in nature. *Environ. Sci. Technol.* **1991**, *25*, 1346–1350.
- Öberg, G.; Sandén, P. Retention of chlorine in soil and cycling of organic matter-bound chlorine. *Hydrol. Processes*. **2005**, *19*, 2123–2136.
- Öberg, G.; Holm, M.; Sandén, P.; Svensson, T.; Parikka, M. The role of organic-matter-bound chlorine in the chlorine cycle: A case study of the Stubbetorp catchment, Sweden. *Biogeochemistry*. **2005**, *75*, 241–269.
- Öberg, G. In *The Handbook of Environmental Chemistry Vol. 3, Part P. The Natural Production of Organohalogen Compounds*; Gribble, G. W. Ed.; Springer-Verlag: Berlin, Heidelberg, 2003.
- Asplund, G.; Christiansen, J. V.; Grimvall, A. B. A chloroperoxidase-like catalyst in soil detection and characterization of some properties. *Soil Biol. Biochem.* **1993**, *25*, 41–46.
- Niedan, V.; Pavasars, I.; Öberg, G. Chloroperoxidase-mediated chlorination of aromatic groups in fulvic acid. *Chemosphere*. **2000**, *41*, 779–785.
- Matucha, M.; Gryndler, M.; Forczek, S. T.; Uhlířová, H.; Fuksová, K.; Schröder, P. Chloroacetic acids in environmental processes. *Environ. Chem. Lett.* **2003**, *1*, 127–130.
- Fahimi, I. J.; Keppler, F.; Schöler, H. F. Formation of chloroacetic acids from soil, humic acid and phenolic moieties. *Chemosphere*. **2003**, *52*, 513–520.
- Hoekstra, E. J.; de Leer, E. W. B.; Brinkman, U. A. T. Findings supporting the natural formation of trichloroacetic acid in soil. *Chemosphere*. **1999**, *38*, 2875–2883.
- Matucha, M.; Gryndler, M.; Schröder, P.; Forczek, S. T.; Uhlířová, H.; Fuksová, K.; Rohlenová, J. Chloroacetic acids--Degradation intermediates of organic matter in forest soil. *Soil Biol. Biochem.* **2007**, *39*, 382–385.
- de Jong, E.; Field, J. A. Sulfur tuft and turkey tail: Biosynthesis and biodegradation of organohalogenes by basidiomycetes. *Annu. Rev. Microbiol.* **1997**, *51*, 375–414.
- de Leer, E. W. B.; Damste, J. S. S.; Erkelens, C.; de Galan, L. Identification of intermediates leading to chloroform and C-4 diacids in the chlorination of humic acid. *Environ. Sci. Technol.* **1985**, *19*, 512–522.
- Juuti, S.; Vartiainen, T.; Ruuskanen, J. Formation of organochlorine compounds in kraft pulp bleaching processes. *Chemosphere*. **1996**, *33*, 2431–2440.
- Flodin, C.; Johansson, E.; Boren, H. B.; Grimvall, A. B.; Dahlman, O.; Mörc, R. Chlorinated structures in high molecular weight organic matter isolated from fresh and decaying plant material and soil. *Environ. Sci. Technol.* **1997**, *31*, 2464–2468.
- Lee, R. T.; Shaw, G.; Wadey, P.; Wang, X. Specific association of <sup>36</sup>Cl with low molecular weight humic substances in soils. *Chemosphere*. **2001**, *43*, 1063–1070.
- Gryndler, M.; Rohlenová, J.; Kopecký, J.; Matucha, M. Chloride concentration affects soil microbial community. *Chemosphere*. **2008**, *71*, 1401–1408.
- Bastviken, D.; Thomsen, F.; Svensson, T.; Karlsson, S.; Sandén, P.; Shaw, G.; Matucha, M.; Öberg, G. Chloride retention in forest soil by microbial uptake and by natural chlorination of organic matter. *Geochim. Cosmochim. Acta* **2007**, *71*, 3182–3192.
- Silk, P. J.; Loneragan, G. C.; Arsenault, T. L.; Boyle, C. D. Evidence of natural organochlorine formation in peat bogs. *Chemosphere*. **1997**, *35*, 2865–2880.
- Řezáčová, V.; Gryndler, M. Fluorescence spectroscopy: A tool to characterize humic substances in soil colonized by microorganisms. *Folia Microbiol.* **2006**, *51*, 215–221.
- Johansson, E.; Sandén, P.; Öberg, G. Organic chlorine in deciduous and coniferous forest soils in southern Sweden. *Soil Science*. **2003**, *168*, 347–355.
- Matucha, M.; Gryndler, M.; Forczek, S. T.; Schröder, P.; Bastviken, D.; Rohlenová, J.; Uhlířová, H.; Fuksová, K. A chlorine-36 and carbon-14 study of the role of chlorine in the forest ecosystem. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 437–439.
- Laniewsky, K.; Dahlén, J.; Boren, H. B.; Grimvall, A. B. Determination of group parameters for organically bound chlorine, bromine and iodine in precipitation. *Chemosphere*. **1999**, *38*, 771–782.
- Carlsen, L.; Lassen, P. Enzymatically mediated formation of chlorinated humic acids. *Org. Geochem.* **1992**, *18*, 477–480.
- Rodsteth, M.; Stahlberg, C.; Sandén, P.; Öberg, G. Chloride imbalances in soil lysimeters. *Chemosphere*. **2003**, *52*, 381–389.

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