

## Emission of climate relevant volatile organochlorines by plants occurring in temperate forests

S.T. Forczek<sup>1</sup>, F. Laturnus<sup>2</sup>, J. Doležalová<sup>1,3</sup>, J. Holík<sup>1</sup>, Z. Wimmer<sup>1,4</sup>

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

<sup>2</sup>*Helmholtz Zentrum München GmbH Neuherberg, Research Unit Microbe-Plant Interactions, WG Plant Endophyte Physiology, Munich, Germany*

<sup>3</sup>*Department of Physical and Macromolecular Chemistry, Charles University in Prague, Prague, Czech Republic*

<sup>4</sup>*Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Prague, Czech Republic*

### ABSTRACT

Chlorine, one of the most abundant elements in nature, undergoes a complex biogeochemical cycle in the environment, involved in the formation of volatile organochlorines (VOCl), which in turn can contribute to environmental problems, contaminate natural ecosystems, and are of public health concern. Several industrial and natural sources of VOCl have already been identified; however, data – particularly on the natural sources – are still scarce. The aim of this study was to investigate the diversity of emission of VOCl from soil and several undergrowth plants collected in temperate spruce forest ecosystem and the effect of salting on the VOCl emission of plants. Undergrowth plants were found to emit chloroform ( $\text{CHCl}_3$ ) in the range of 2.2–201 pmol/day/g dry weight (DW), tetrachloromethane ( $\text{CCl}_4$ ) 0–23.5 pmol/day/g DW, and tetrachloroethene ( $\text{C}_2\text{Cl}_4$ ) 0–13.5 pmol/day/g DW; the average emission rates were about 10 times higher than that of soil (2.9–47.2; 0–5.8; 0–3.6 pmol/day/g DW of  $\text{CHCl}_3$ ;  $\text{CCl}_4$ ;  $\text{C}_2\text{Cl}_4$  emission, respectively). Addition of sodium chloride solution in most cases caused an increase in the emission of  $\text{CHCl}_3$  and caused a species specific – effect on the emissions of  $\text{CCl}_4$  and  $\text{C}_2\text{Cl}_4$ . The results suggest that the emission of VOCl from spruce forest contribute to the atmospheric input of reactive chlorine; however, on a global scale it is only a minor net source.

**Keywords:** volatile chlorinated hydrocarbons; biodegradation; Sphagnum moss; ozone-depleting substances; head-space cryofocusing method; boreal forests

Volatile organic compounds in the atmosphere come partway from plants (Penuelas and Llusia 2001). Volatile organochlorines (VOCl) participate in the complex biogeochemical cycle involving the formation, conversion and degradation of different inorganic and organic forms of chlorine. VOCl are of environmental importance as they can participate in climate relevant atmospheric reactions, such as reacting with stratospheric ozone (Millet et al. 2009). The calculated atmospheric budget, however, revealed large gaps pointing to still un-

known natural sources (Butler 2000). While the industrial input of VOCl is already well known, data on the natural part is still scarce. Anthropogenic sources can be important as compounds taken up by living organisms can be converted into different compounds during biodegradation (Forczek et al. 2011). Some of the converted compounds are more toxic than the original reactants, as seen for example at the formation of trichloroacetic acid from tetrachloroethylene (Forczek et al. 2008, Huang et al. 2014).

doi: 10.17221/900/2014-PSE

Natural sources, such as wild fires, volcanoes, and other geothermal processes account for a wide range of VOCIs and inorganic chlorine (Lobert et al. 1999). The emissions of chloroform, tetrachloromethane or chloromethane from some marine and terrestrial sources, such as marine macroalgae, coastal salt marshes and forest soil are documented (Laternus et al. 1998, Rhew et al. 2000, Hoekstra et al. 2001, Svensson et al. 2007). It has been suggested that chlorine contributes to the decay of soil organic matter (SOM) leading to the formation of large molecules of chlorohumus (Asplund 1995, Matucha et al. 2010). Degradation of chlorohumus leads to smaller intermediates, such as chlorinated acetic acids, anisol-, orcinol- and hydroquinone-based substances, which may be taken up by plants or fungi. Mineralization of chlorohumus and other intermediates may involve the production of VOCIs suggesting that the degradation of organohalogenes and SOM might be closely connected with VOCl emissions.

Above ground organisms like fungi and lichen produce a variety of organohalogenes, from simple molecules, such as chloroform and chloromethane, to exceedingly complex compounds. Many of these compounds are volatile, such as chloroform, tetrachloromethane and 1,1,1-trichloroethane, which are emitted for example from subtropical, tropical and temperate forest ferns and moss (Saito and Yokouchi 2006), however, their amounts are quite low in general except for chloromethane, which is emitted in considerable amounts (Butler 2000).

According to the current estimates, known VOCl losses from the atmosphere owing to oxidation by hydroxyl radicals, oceanic degradation, and consumption in soils exceed known inputs of VOCl emissions from oceanic sources, terrestrial plants and fungi, biomass burning and anthropogenic inputs. Since there are relatively constant concentrations of VOCl in the atmosphere, and the range of potential sources of VOCl emissions is not completely known, thus additional natural terrestrial sources may be important (Rhew et al. 2000). The VOCl emissions can be affected by some factors, for instance sodium chloride present in temperate spruce forest ecosystems due to extensive road salting during winter periods. NaCl acts as a stress factor and can contribute to VOCl production. In this study, we aimed to identify new sources of VOCIs by investigating soil and plants in these ecosystems in the absence and presence of sodium chloride.

## MATERIAL AND METHODS

Living plants and decaying plant material were collected in a pristine area currently used as a catchment for drinking water (close to Hamry water reservoir, Czech Republic, 49°43'50"N, 15°55'6"E) during the vegetation season. The samples investigated were liverwort *Marchantia polymorpha* L., mosses *Polytrichum commune* Hedw. and *Sphagnum* spp., clubmoss *Lycopodium clavatum* L., horsetail *Equisetum palustre* L., fern *Cystopteris fragilis* (L.) Bernh., lichen *Hypogymnia physodes* L., where around 200 g fresh plant material in the best visible health conditions was collected. Furthermore, decaying plant material consisting of *Sphagnum* spp. and soil from the organic horizon (humification and fermentation layers) of spruce forest was collected from 3 random spots per site and then mixed on site. After collection, the soil and plant samples were transported to the laboratory in airtight plastic containers. There the soil samples were homogenized using a 2 mm sieve and stored at 4°C in the dark. In case of salt applications, 100 mL of 0.1 mol/L sodium chloride solution was added by irrigation to the roots of the plants for the time of incubation overnight, to simulate high concentrations of NaCl which can occur in roadside runoff waters.

The emission of VOCIs was analyzed using the headspace-cryofocusing gas chromatography, which consisted of an incubation vessel, a dryer and a cryo-trap. For the incubation, around 10 g fresh weight samples were placed in 100 mL glass vessels, glass was precleaned by heating to 120°C for 1 h prior to the use and closed with plastic caps containing a polytetrafluoroethylene (PTFE) covered silicone septa. The incubation period was 20–24 h at laboratory temperature in the dark for soil samples and 22°C under artificial light (10 h, fluorescent neon tubes Arcadia L45/W15, Redhill, UK) for plant samples. After the incubation period, emitted compounds were purged from the incubation vessel by nitrogen (purity 4.0, Linde Gas, Prague, Czech Republic), passed through a Nafion tube (Perma Pure, Toms River, USA) to remove humidity and to prevent clogging of the cryo-trap, and entered the cryo-trap, which was dipped into liquid nitrogen to freeze and concentrate VOCIs. Sample introduction was performed by heating the cryo-trap with boiling water and therefore injecting the concentrated sample onto the gas chromatograph

with an capillary column (Rxi-624Sil MS, 30 m, ID 0.32 mm,  $d_f$  1.8  $\mu$ m, Restek, Bellefonte, USA) coupled to an electron capture detector (Varian 3400 ECD, Walnut Creek, USA). Identification of the compounds was done by internal standards diluted in methanol (all Sigma Aldrich, St. Louis, USA). The detection limits were 20.5 pg/L for chloroform (relative standard deviation/RSD/9%,  $n = 10$ ), 9.2 pg/L for tetrachloromethane (RSD 12%,  $n = 10$ ), and 1.6 pg/L for tetrachloroethene (RSD 8%,  $n = 10$ ). Dry weight (DW) of samples was determined by drying the plant and soil material at 90°C until constant weight. For further details about instrumentation and chromatographic details see Laturnus et al. (2000) and Haselmann et al. (2000). Blank tests of the sampling system were run daily to control background air quality, and subtracted from the result, then statistical analysis was performed in Microsoft Excel 2010 (Redmont, USA) to obtain means and standard deviation.

## RESULTS

Several forest plants and forest soil were investigated for their emission of volatile organochlorines (Table 1). Common liverwort emitted  $\text{CHCl}_3$ ,  $\text{CCl}_4$  and  $\text{C}_2\text{Cl}_4$  in relatively high amounts similar to peat moss and to fragile fern ( $\text{CHCl}_3$  54.3–201.5 pmol/day/g DW,  $\text{CCl}_4$  6.9–23.5 pmol/day/g DW,  $\text{C}_2\text{Cl}_4$  1.4–13.5 pmol/day/g DW). The emissions of VOCs from this group was unlike to the one found of marsh horsetail and wolf's-foot clubmoss, which is characterized by high emissions of chloroform and low emissions of tetrachloromethane and tetrachloroethene ( $\text{CHCl}_3$  18.8–137.4 pmol/day/g DW,  $\text{CCl}_4$  0.3 pmol/day/g DW,  $\text{C}_2\text{Cl}_4$  0–2.1 pmol/day/g DW). In decaying plant material of *Sphagnum* spp., the emission of

VOCs decreased, while it was still similar to the results from untreated and healthy plants ( $\text{CHCl}_3$  32.0 pmol/day/g DW,  $\text{CCl}_4$  4.9 pmol/day/g DW,  $\text{C}_2\text{Cl}_4$  2.7 pmol/day/g DW). Common haircap moss naturally emitted only negligible amounts of  $\text{CHCl}_3$  (2.2 pmol/day/g DW) and no  $\text{CCl}_4$  or  $\text{C}_2\text{Cl}_4$ . Soil from the organic layer of spruce forest invariably emitted all studied VOCs (Table 1). The release rates, however, were lower than those found of most plant species ( $\text{CHCl}_3$  10.2 pmol/day/g DW,  $\text{CCl}_4$  1.8 pmol/day/g DW,  $\text{C}_2\text{Cl}_4$  1.5 pmol/day/g DW).

Decaying peat moss showed a highly increased emission of all VOCs when exposing the plants to sodium chloride solution (Table 2). The emission rates of  $\text{CHCl}_3$  determined for common haircap moss increased 20 fold after the application of NaCl solution, moreover  $\text{CCl}_4$  and  $\text{C}_2\text{Cl}_4$  also appeared. The addition of a NaCl solution to fragile ferns triggered a large increase in the emission of chloroform and rendered the emission of tetrachloromethane and tetrachloroethene emissions almost to zero.

## DISCUSSION

To assess the environmental input of VOCs, marine and terrestrial sources must be taken into account. The VOC emissions of marine algae are in the same range as of terrestrial plants (2.8–259 pmol/day/g DW  $\text{CHCl}_3$ , 8.5–447 pmol/day/g DW  $\text{CCl}_4$  and 4.5–14 100 pmol/day/g DW  $\text{C}_2\text{Cl}_4$  (Abrahamsson et al. 1995)), and also produce volatile brominated and iodinated compounds. The main natural terrestrial sources of VOCs so far identified are soil, salt marshes, rice fields, tropical forests, peatlands, temperate forest soil, litter, plants and fungi (Khalil et al. 1998, Haselmann et

Table 1. The diversity of emission of volatile organochlorine compounds (pmol/day/g dry weight,  $n = 9$ , mean  $\pm$  SD) from plants and soil

Species name	NaCl	$\text{CHCl}_3$	$\text{CCl}_4$	$\text{C}_2\text{Cl}_4$
<i>Marchantia polymorpha</i> L. (common liverwort)	–	54.3 $\pm$ 58.0	9.5 $\pm$ 8.1	2.7 $\pm$ 15.8
<i>Lycopodium clavatum</i> L. (wolf's-foot clubmoss)	–	18.8 $\pm$ 26.5	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0
<i>Equisetum palustre</i> L. (marsh horsetail)	–	137.4 $\pm$ 68.3	0.3 $\pm$ 4.5	2.1 $\pm$ 30.3
<i>Sphagnum</i> spp. (peat moss)	–	201.5 $\pm$ 81.6	23.5 $\pm$ 5.8	13.5 $\pm$ 13.1
Soil from spruce forest, F + H horizon, no plants	–	10.2 $\pm$ 16.5	1.8 $\pm$ 1.9	1.5 $\pm$ 1.1

doi: 10.17221/900/2014-PSE

Table 2. Effect of sodium chloride on the emission of volatile organochlorine compounds (pmol/day/g dry weight,  $n = 9$ , mean  $\pm$  SD) from plants

Species name and treatment	NaCl (mol/L)	CHCl <sub>3</sub>	CCl <sub>4</sub>	C <sub>2</sub> Cl <sub>4</sub>
<i>Sphagnum</i> sp. decaying plant material	–	32.0 $\pm$ 135.7	4.9 $\pm$ 12.3	2.7 $\pm$ 5.8
	0.1	67.5 $\pm$ 19.3	5.1 $\pm$ 5.8	5.3 $\pm$ 2.8
<i>Polytrichum commune</i> Hedw (common haircap moss)	–	2.2 $\pm$ 0.7	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	0.1	43.0 $\pm$ 12.5	1.0 $\pm$ 0.2	5.0 $\pm$ 4.3
<i>Cystopteris fragilis</i> (L.) Bernh. (fragile fern)	–	70.1 $\pm$ 106.0	6.9 $\pm$ 42.5	1.4 $\pm$ 26.7
	0.1	178.7 $\pm$ 43.4	1.7 $\pm$ 2.7	0.9 $\pm$ 1.2

al. 2000, Hoekstra et al. 2001, Scheeren et al. 2003, Rhew et al. 2008). In our measurements all plants and soil samples emitted CHCl<sub>3</sub> in the range of 2.2–201 pmol/day/g DW, while CCl<sub>4</sub> and C<sub>2</sub>Cl<sub>4</sub> were also found on average at 0–23.5 pmol/day/g DW and 0–13.5 pmol/day/g DW, respectively. High diversity of emissions can be found among different species (Table 1) and the inhomogeneity of soil is also well known (Matucha et al. 2010). Living plants form VOCl for various purposes; defense mechanism against bacterial and fungal infection, deterrence of grazers, allelopathic interactions and accidental formation of biological by-products are mentioned most often (Mtolera et al. 1996). VOCl production in plants is connected with overproduction of H<sub>2</sub>O<sub>2</sub> as a result of oxidative stress, where hydrogen peroxide is scavenged by chloroperoxidase enzyme (CPO). In the presence of reactive oxygen species CPO forms hypohalous acids, which may react with nucleophilic acceptors such as ketones to produce halocarbons. Formation of brominated (VOBr) and iodinated (VOI) halocarbons in marine algae is not affected by H<sub>2</sub>O<sub>2</sub> as they utilize S-adenosyl methionine transferase instead of CPO; this enzyme has a preference of halide ions in the order iodide > bromide > chloride (Mtolera et al. 1996). Subtropical ferns and algae emitting chlorinated volatile compounds show a strong diurnal variation (Saito and Yokouchi 2006), which could cause a high variation in our samples as well. Biogenic volatile organic compounds are emitted at their highest rates during the day, and increasing from the morning towards the evening. Therefore due to the presence or absence of purpose and due to triggering environmental factors the VOCl emission can vary significantly, and the wide range of detected emissions in our study is not surprising.

Similarly to our study (Table 1), high variance was found also in other studies with higher number of replicates (Rhew et al. 2008).

Decaying plant material, present in the litter, emitted mainly decreased amounts of VOCl in comparison with living plants, but higher amounts than those of soil. Further studies emphasize the importance of decaying plant material in the soil degraded by microorganisms, but other processes like abiotic redox alkylation of organic matter can also be important (Keppler et al. 2000).

Emissions of VOCl were already studied in a variety of ecosystems. Coastal shrublands of southern California emitted 6.6  $\pm$  7.7 nmol/m<sup>2</sup>/day CHCl<sub>3</sub> and 0.5  $\pm$  1.2 nmol/m<sup>2</sup>/day CCl<sub>4</sub>, while salt marsh showed emissions of 14.6  $\pm$  18.7 nmol/m<sup>2</sup>/day CHCl<sub>3</sub> and 1.2  $\pm$  2.9 nmol/m<sup>2</sup>/day CCl<sub>4</sub> (Rhew et al. 2008). For forest soils an average emission rate of 6.7 nmol/m<sup>2</sup>/day CHCl<sub>3</sub> was detected in field studies, while laboratory studies indicated even higher chloroform emission rates in the range of 67–159 nmol/m<sup>2</sup>/day (Haselmann et al. 2000). Scheeren et al. (2003) reported the emission of VOCl from a Surinam tropical rainforest ecosystem (223  $\pm$  43 nmol/m<sup>2</sup>/day CHCl<sub>3</sub> and 53  $\pm$  9.6 nmol/m<sup>2</sup>/day C<sub>2</sub>Cl<sub>4</sub>). When extrapolating our results obtained from spruce forest soil (Table 1), we verified that soil can be a solid source of chloroform, and other VOCl also were found to be consistently emitted. By our rough estimation assuming a depth of the organic-rich top soil layer of 0.1 m, and a density of the soil of 1 kg/L similarly to Haselmann et al. (2000), the following VOCl fluxes can be calculated from the initial release rates: approximately 300 nmol/m<sup>2</sup>/day CHCl<sub>3</sub>, 50 nmol/m<sup>2</sup>/day CCl<sub>4</sub> and 45 nmol/m<sup>2</sup>/day C<sub>2</sub>Cl<sub>4</sub>. Therefore according to our measurements, in some cases VOCl emissions



of spruce forests are among the highest between terrestrial ecosystems. Some ecosystems in contrast can also act as a net sink for VOCIs, e.g. salt marshes in growing season, where scarcely known abiotic and biotic processes can occur (Wang et al. 2007). The properties of the soil (sorption to soil organic matter, pH, temperature, humidity, redox potential, porosity, aeration etc.) and rhizosphere of plants can influence the emission of VOCl, while the emission of previously adsorbed VOCl is also possible.

The results of this study revealed that plants in temperate forest ecosystems contribute to the environmental input of chloroform, tetrachloromethane and tetrachloroethene, and, thus, are in line with other natural terrestrial sources identified so far.

In this study, two different types of samples can be distinguished according to their VOCl emission: living plants and soil. The emission of chloroform was confirmed in varying rates in both sample types. In living plants an occasional formation of tetrachloromethane and small amounts of tetrachloroethene were recorded, similarly to other studies (Abrahamsson et al. 1995). Decaying plant material in the rhizosphere invariably emitted tetrachloroethene, and a lower amount of chloroform and resembled therefore to soil. Our preliminary results of the addition of sodium chloride to simulate road salting showed that NaCl affected living plants and their emission of chloroform became similar to the results found in soil samples with relatively high concentrations of chloroform and lower concentrations of tetrachloromethane and tetrachloroethene (Table 2). As plants live in symbiosis with microorganisms and our experiments were conducted in non-sterile environment, part of the observed VOCl emission can be dedicated to plants, and part to microorganisms. It is possible that the addition of sodium chloride suppresses the plant metabolism, thus only the VOCl emission by microbial chlorination activity remains detectable in the rhizosphere and in the phyllosphere. On the other hand, salt stress (NaCl) changes the regulation of plant metabolism, which can play a major role in the change of VOCl emission.

The exact mechanisms behind the formation of VOCIs in plants are not known in all details, yet, and tend to be highly species specific. However, forest plants have to be considered as natural contributors of VOCIs to the environmental input

although their contribution is so far a minor one. Our results suggest that the emission of VOCIs from spruce forest ecosystem contribute to the atmospheric input of reactive chlorine; however, only as a minor net source on a global scale.

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doi: 10.17221/900/2014-PSE

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Received on November 18, 2014

Accepted on February 16, 2015

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*Corresponding author:*

Dr. Sándor T. Forczek, Ph.D., Ústav experimentální botaniky AV ČR, v.v.i., Izotopová laboratoř,  
Václavská 1083, 142 20 Praha, Česká republika; e-mail: alex067@biomed.cas.cz

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