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Volatile Organochlorine Compounds Emitted by Temperate Coniferous Forests

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***Abstract*—**Chlorine is one of the most abundant elements in nature, which undergoes a complex biogeochemical cycle. Chlorine bound in some substances is partly responsible for atmospheric ozone depletion and contamination of some ecosystems. As due to international regulations anthropogenic burden of volatile organochlorines (VOCls) in atmosphere decreases, natural sources (plants, soil, abiotic formation) are expected to dominate VOCl production in the near future. Examples of plant VOCl production are methyl chloride, and bromide emission from (sub)tropical ferns, chloroform, 1,1,1-trichloroethane and tetrachloromethane emission from temperate forest fern and moss. Temperate forests are found to emit in addition to the previous compounds tetrachloroethene, and brominated volatile compounds. VOCls can be taken up and further metabolized in plants. The aim of this work is to identify and quantitatively analyze the formed VOCls in temperate forest ecosystems by a cryofocusing/GC-ECD detection method, hence filling a gap of knowledge in the biogeochemical cycle of chlorine.

***Keywords*—**chloroform, cryofocusing-GC-ECD, ozone depletion, volatile organochlorines

I. Introduction

VOLATILE chlorinated hydrocarbons (VOCls) participate in the complex biogeochemical cycle of chlorine involving their formation, conversion and degradation, where chlorine is present in inorganic and organic form.Organohalogens in general originate from various sources, due to anthropogenic and natural activities (biotic and abiotic way), in the atmosphere, soil or vegetation.The occurrence of organohalogens in nature is generally ascribed formerly only to anthropogenic activities, as a result of application of chlorine in the pulp- and paper-, water purification-, and sewage treatment industries. The large chlorine-containing molecules are originating from the reaction of chlorine chemicals with lignin and from the reaction of chlorophenols with humus. By the action of chlorine, the lignin is chlorinated, oxidized, and, to some degree, depolymerized, resulting in a partial water solubilization. Examples include various chlorinated aliphatic acids, chlorinated phenolic compounds, and a number of neutral compounds such as various chlorinated alkanes, alkenes, furan, pyrone, thiophene, and cymene derivatives [1]. About 30% of the organically bound chlorine in spent chlorination liquor is of low relative molecular mass. Waste water treatment plants and the spent liquor from a kraft pulp chlorination has been reported to emit C_1- and C_2-chlorocarbons and TCA into the environment [1].Organohalogens in general and also VOCls are produced in the nature. Natural combustion sources such as biomass fires, volcanoes, and other geothermal processes account for a wide range of organohalogens. The emissions of methyl chloride, methyl bromide, chloroform and tetrachloromethane from biomass burning and other natural sources, such as coastal salt marshes are well documented [2] [3]. It has been suggested that chlorine contributes to the decay of soil organic matter (SOM) [4], and proved later, that the breakdown processes of SOM proceed with the participation of chlorine and microorganisms [5]. The formation of large molecules of chlorohumus have been confirmed [6] showing the importance of abiotic formation is to a lesser extent, than previously expected [7]. Degradation of chlorohumus leads to smaller intermediates which can be possibly also taken up by plants or fungi. The known intermediates are chlorinated acetic acids, anisyl-, orcinol- and hydroquinone substances which are produced during degradation of SOM. Mineralization of chlorohumus and other intermediates can lead also to production of VOCl, and this way, degradation of organohalogens and SOM is closely connected with VOCl emissions. Chloroform, 1,1,1-trichloroethane, tetrachloromethane, tetrachloroethene, bromoform and bromodichloromethane are emitted by temperate forest soils that contain a humic top layer [2].Natural formation of VOCls is known not only by soil and its biota, but higher organisms as well. Currently more then three thousands halogenated compounds have already been reported to be produced naturally in soil, in marine organisms and also in terrestrial plants and animals [8]. For example, fungi and lichen produce a variety of organohalogens, from the simple methyl chloride and chloroform to exceedingly complex compounds. Many of these compounds are volatile, such as methyl chloride and methyl bromide, chloroform, tetrachloromethane and 1,1,1-trichloroethane which are emitted from (sub)tropical and temperate forest ferns and moss [9] [10]. The concentrations

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of VOCl produced by plants and fungi are low except for some massively produced compounds such as methyl chloride and methyl bromide [11]. Some studies have suggested that natural sources may be significant for atmospheric CCl_4 [12].Many of VOCl can reach the stratosphere where their halogen atoms, released through photolysis, catalytically destroy ozone. The calculated budget, however, has large gaps, pointing to unknown sources. On the other hand, VOCl in the biosphere have also adverse effects. VOCl can be produced, or taken up by living organisms, and can be converted into different compounds during physiological processes or biodegradation [13]. Some of the newly formed compounds (even though present only in much lower concentrations) are more toxic than the original reactants, such as confirmed in the case of trichloroacetic acid formation from tetrachloroethylene [14].Current estimates of VOCl emissions from oceanic sources, terrestrial plants and fungi, biomass burning and anthropogenic inputs do not balance their losses owing to oxidation by hydroxyl radicals, oceanic degradation, and consumption in soils, suggesting that additional natural terrestrial sources may be important [15]. In this study, we wanted to determine the formation of known VOCl and to find unknown VOCl produced by soil and common plants in spruce forest ecosystems. For this aim, we assembled and optimized a cryofocusing system and tested several plants and soil samples.

II. Materials and Methods

Living plants and decaying plant material were collected in a very clean natural area used currently as a catchment for drinking water, near to Hamry water reservoir, Czech Republic (GPS: 49°43'50" N, 15°55'6"E). The samples were transported in airtight plastic containers into the lab, where plants were cultivated for a short period of time preceding analysis. The cultures were illuminated with fluorescent neon tubes (Arcadia L45/W15, UK) promoting plant growth. Lichen *Hypogymnia physodes* (L.); horsetail *Equisetum palustre* (L.); liverwort *Marchantia polymorpha* (L.); fern *Cystopteris fragilis* (Bernh.); mosses *Polytrichum commune* (Hedw.) and *Sphagnum spp*.; Norway spruce (*Picea abies* (/L./ Karst.)), decaying plant material consisting of *Sphagnum spp*. and soil, and soil alone from F and H horizons of spruce forest were stored or cultivated at 20 °C with occasional irrigation by ultrapure water (Milli-Q, Millipore) to avoid possible contamination by water.

Natural samples were placed into glass vessels for the incubation period (20 hours). The vessels were pre-cleaned with n-hexane/acetone and heated to 120 °C prior to use, had a volume of 100 ml, and were sealed with polytetrafluoroethylene (PTFE) covered silicone septa. The natural samples are listed in Table 1.

The headspace of glass vessels was purged by pure helium current. The flow rate of purging gas, total purging volume and desorption temperature of the cryotrap were to be optimized in this study. The preconcentration trap consisted of a 20 cm stainless steel tube with an inner diameter of 1 mm. 2 cm of the tube were filled with dimethyldichlorosilane (DMS) treated glass balls (25 µm) and sealed with small plugs of DMS treated glass wool. Water was removed from the gas current by a Nafion dryer (model DM with molecular sieve 4A, Perma Pure, USA), which did not affect the VOCl. This procedure prevented the input of moisture into the cold trap and avoided a rapid freeze-up of the trap. For further details see Laturnus [16]. The cryofocusing apparatus was attached to a gas chromatograph by a thermostat controlled (40 ºC) 6-way valve.The separation of the different VOCl was performed with a gas chromatograph (GC Varian 3400) equipped with electron capture detector (ECD), and an Rxi-624Sil MS capillary column (Restek, USA, 30 m, 1.8 µm df, 0.32 mm ID). Sample injection proceeded at an inlet pressure of 140 kPa He (corresponding to a linear velocity of 25 cm/s) and a temperature program 40 °C 7 min, 11°C/min to 60 °C, then finally 20 °C/min to 220°C.

III. Results And Discussion

Various optimization procedures have been performed on the cryotrap apparatus on a selected set of main compounds ($CHCl_3$, CCl_4, C_2Cl_4, $CHBr_3$). Optimizing purging volume on sample recovery has been performed on 100 ml sampling flasks. For C_2Cl_4 and $CHBr_3$ the longer purging obliged, while $CHCl_3$ and CCl_4, has an optimum around 300 ml. For that reason, a compromise was chosen with a purging volume of 450 ml/sample. Optimizing purging flow rate on sample recovery has been done similarly to the previous measurements. $CHCl_3$ and CCl_4 showed decreasing recoveries with higher flow rate, while C_2Cl_4 and $CHBr_3$ showed increasing recoveries with higher flow rate. Therefore a compromise was chosen again with flow rate of 45 ml/min.

Optimizing desorption temperature of the cryotrap has been performed in the range of 20-100 ºC. It can be deduced, that for $CHCl_3$ and CCl_4 the higher temperature is optimal, while for C_2Cl_4 and $CHBr_3$ the change does not matter, therefore the highest temperature was set as standard. Temperature for the thermostat around the 6-way valve was set to 40 ºC as it is one step before the injection of the sample onto the GC column. The temperature of the sample vial was found to be optimal at higher temperatures (~100 ºC) for directly injected standards, but as additional volatile organic compounds of natural samples can be emitted into the atmosphere, disturbing the measurements, it must be left at temperature of incubation.

On the new capillary column, the calibration curves were made for the three main compounds. Limit of detections were calculated (n=10): chloroform 2.05 ng / 100 ml (RSD 9-20%), tetrachloromethane 0.92 ng / 100 ml (RSD 12-20%) and tetrachloroethene 0.16 ng / 100 ml (RSD 8-25%). Based on the calibration curves, quantitative analysis was performed several natural samples. The emission rates of *Sphagnum* moss (living and decaying), *Cystopteris* fern, soil and lichens were mostly in the same magnitude. All of them emitted chloroform, in the range of 0.7-35.9 ng/g dry weight (DW) and CCl_4 (0.2-4.4 ng/g DW) and some emitted C_2Cl_4 (0.04-2.1 ng/g DW).

Semiquantitative analyses were performed on the cryotrap–GC-ECD system. The mixture of standards contained 17 VOCl compounds, where two brominated compounds co-eluted with two chlorinated compounds. In spite of this fact, we can tell the presence of these 17 with high probability. The background air

usually contained CCl_4 and C_2Cl_4, and $CHCl_3$ only in lower concentrations. Unknown compounds were present in the background air and also in the natural samples. Only one unknown compound was recorded in the background air with retention time (r_t) 1.1 min, which due to the conditions of chromatography anticipates a highly volatile compound, probably methyl chloride, commonly found in GC-MS measurements. The other two compounds are less volatile, but also halogenated, with retention times 8.8 min and 11.3 min, which anticipates boiling points approximately 90-100 and 110-120 ºC. Semiquantitative analyses were performed on several natural samples (Table 1.) VOCl compounds present in the background air were deduced.Horsetail was studied without soil, emission of higher amounts of chloroform and lower amounts of tetrachloroethene has been found. High emissions of two unknown halogenated compounds were also registered (r_t 8.8 and 11.3 min). A preliminary experiment showed salting causing formation of a further unknown halogenated compound (r_t 1.1 min), while salting also caused to stop the emission of less volatile compounds (tetrachloroethene and unknowns r_t 8.8 and 11.3 min). The living, unsalted plants therefore emit VOCl very similar to soil, even though no soil or any roots were put in the measuring compartment. The emission of *Sphagnum* mosses is also similar to the emission of Equisetum, varying only in one unknown compound (r_t 11.3 min).The effect of salting, decay and soil was studied on *Sphagnum* moss. In living form *Sphagnum* emitted tetrachloromethane, tetrachloroethene, and the unknown compound (r_t 8.8 min) while chloroform and the unknown compound (r_t 11.3 min) was recorded only in lower concentrations. Salted living *Sphagnum* moss did not emit any VOCl compounds, which can be dedicated to experimental error, or that the plant was exposed only to a short period of elevated concentration of NaCl.In the decaying plant material, consisting of *Sphagnum* and soil, different compounds were emitted. The formation of chloroform and the unknown compound (r_t 1.1 min) highly increased, while CCl_4 emission decreased. The other 3 compounds previously found were not recorded at all. When decaying plant material did not contain soil, the emitted compounds were the same as the living, with the loss of the unknown compound (r_t 8.8). In case of salting the decaying plant material, the emission of the unknown halogenated compound (r_t 1.1 min) was recorded.Liverwort emitted $CHCl_3$, CCl_4 and C_2Cl_4 in great amounts, and also two unknown chlorinated compounds (r_t 8.8 and 11.3 min), when determined in living form. The emission of $CHCl_3$ and CCl_4 was determined in dead plants, too, while lost the emission of C_2Cl_4 and the two unknowns (r_t 8.8 and 11.3 min). Liverwort moss contains much soil in its samples, and so its emissions are similar. On the other hand, loss of emission of the above mentioned 3 compounds can point to the chlorination activity of the plants.Ferns were found to emit $CHCl_3$, CCl_4 and C_2Cl_4 also in great amounts, and also two unknown chlorinated compounds (r_t 8.8 and 11.3 min) in smaller quantities. These results are similar to that of liverwort and of soil; however, the roots of the plants were washed before placing into the measuring compartment, proving plant VOCl production.Most of the measurements of *Polytrichum* moss were lost due to experimental errors. The remaining results point to the fact, that *Polytrichum* exposed to elevated levels of NaCl emit great amounts of the unknown chlorinated compound (r_t 1.1 min) and low amounts of $CHCl_3$, CCl_4 and C_2Cl_4. These findings must be completed with replications and with the measurements of non-salted material.Preliminary experiments on Norway spruce seedlings showed only the emission of tetrachloroethene in small amounts. Soil showed different emissions of VOCl. Soil close to the roots of the sampled ferns emitted compounds similar to decaying plant material: $CHCl_3$, CCl_4 and trace amounts of C_2Cl_4, and one unknown chlorinated compound (r_t 1.1 min). Soil samples not close to plants emitted mainly $CHCl_3$, C_2Cl_4 and the other two unknown chlorinated compounds (r_t 8.8 and 11.3 min), while formation of CCl_4 cannot be confirmed. It is well known, that soil has a great variability even in very small distances, and it is caused by its inhomogeneity of soil microorganisms. The rhizosphere is such a compartment, where microorganisms present are dissimilar to that of soil. Further aimed studies can help to understand, whether the difference is due to fungi and/or bacteria.The problem of measurements in the case of spruce and lichens were evoked by the same experimental problem; the steel capillary has been clogged and therefore the measurements could not be finished. As the water vapor is actively removed from the sample by the Nafion dryer, the clogging material is surely not water, and unfortunately nothing else is known about its nature. As lichens and preliminary fungi measurements also blocked the capillary of the cryotrap, the clogging material may be somehow related to fungi, and they are possibly volatile fatty acids or terpenoid compounds.

IV. Conclusion

It can be concluded from the measurements, that three different type sample can be distinguished according to their VOCl emissions: living plants, decaying plant material and rhizosphere, and “open” soil. Emission of chloroform can be confirmed in varying degrees in every type of samples. In living plants occasional formation of tetrachloromethane, and small amounts of tetrachloroethene was recorded, additionally to fair amounts of two unknown chlorinated compounds (r_t 8.8 and 11.3 min). These two unknown chlorinated compounds and tetrachloroethene were also present in “open” soil samples, whereas their chloroform emission was quite low. Decaying plant material similarly to that from the rhizosphere emitted invariably tetrachloromethane and occasionally the unknown chlorinated compound (r_t 1.1 min). Preliminary results of salting show that NaCl affects living plants in such a way that their emission becomes similar to rhizospheric and soil samples, losing the formation of higher compounds (tetrachloromethane, tetrachloroethene, and unknowns – r_t 8.8 and 11.3 min). It can be assumed, that in those samples after changing the plant metabolism due to NaCl, only microbial chlorination activity remains, or the plants retain only Cl-metabolism similar to microorganisms. Salting did not effect considerably the formation of VOCl in decaying plant material.

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TABLE I
SEMIQUANTITATIVE EVALUATION OF VOCL EMISSION FROM NATURAL SAMPLES

Sample name and treatment	unknown r_t 1.1	$CHCl_3$ r_t 5.0	CCl_4 r_t 5.5	unknown r_t 8.8	C_2Cl_4 r_t 11.0	unknown r_t 11.3
Cystopteris	-	+++	+++	+++	+++	+
Cystopteris with washed roots	+	++	++	+++	+	+
Cystopteris with washed roots	+	+	+	++	+	+
Cystopteris with washed roots	+	++	+	+	+	-
Equisetum	-	+	++	+++	+++	+++
Equisetum	-	+++	-	+++	+	+++
Equisetum	-	+++	-	+++	+	+++
Equisetum	+	+++	-	-	-	-
Equisetum, watered	-	+++	-	-	-	-
Equisetum salted, 0.5 M NaCl	+++	+++	-	-	-	-
Hypogymnia	++	+++	++	-	+	-
Marchantia and soil	-	+++	-	+++	-	+
Marchantia and soil	-	+++	+++	+++	+++	+++
decaying *Marchantia* and soil	-	+++	+++	-	-	-
Polytrichum salted, 0.1M NaCl	+++	+	+	-	+	-
Sphagnum	-	++	++	+++	+++	-
Sphagnum	-	++	+++	+++	+++	+
decaying *Sphagnum* and soil	+++	+++	++	-	-	-
decaying *Sphagnum* and soil	+++	++	+	-	-	-
decaying *Sphagnum*	-	+++	+++	-	+++	-
decaying *Sphagnum*	-	+	+	-	+	-
decaying *Sphagnum*	-	+++	++	-	+	-
decaying *Sphagnum*	-	++	+++	-	+	-
decaying *Sphagnum*, salted 0.1M NaCl	+	++	++	-	+	-
decaying *Sphagnum*, salted 0.1M NaCl	+	+++	+++	-	+	-
soil under the *Cystopteris* fern	+++	++	++	-	+	-
soil under the *Cystopteris* fern	+++	++	++	-	+	-
forest soil, F+H horizon	-	+++	-	+++	++	+++
forest soil, F+H horizon	-	+++	-	+++	-	++
forest soil, F+H horizon	+	+++	+++	+++	+++	++

r_t – retention times, - not detected, + detected in trace amounts (<150 mVsec), ++ (150-400 mVsec), +++ (>400 mVsec)