Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 437–439.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1180

JLCR

Short Research Article

A chlorine-36 and carbon-14 study of the role of chlorine in the forest ecosystem†

M. MATUCHA[1,*], **M. GRYNDLER**[2], **S. T. FORCZEK**[1], **P. SCHRÖDER**[3], **D. BASTVIKEN**[4], **J. ROHLENOVÁ**[1], **H. UHLÍŘOVÁ**[5] **and K. FUKSOVÁ**[6]

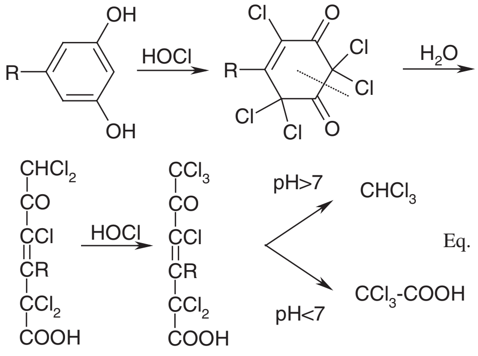
[1] Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2] Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[3] GSF-National Research Center for Environment and Health, Institute of Soil Ecology, Neuherberg, Germany
[4] Department of Geology and Geochemistry, Stockholm University, 106 91 Stockholm, Sweden
[5] Forestry and Game Management Research Institute, Prague, Czech Republic
[6] First Faculty of Medicine, Charles University, Prague, Czech Republic

Received 25 July 2006; Revised 7 November 2006; Accepted 21 November 2006

Keywords: chlorination; organochlorines; microbial degradation; chloroacetic acids; soil organic matter

Introduction

Chlorides were previously considered chemically inert substances in the environment, and chlorinated organic compounds were taken to be only of anthropogenic origin. The role of chlorine in the forest ecosystem is, however, much more active and complex than previously thought; adsorbable organic halogenes (AOX) in the forest soil indicated their active role,[1] whilst chloride is absorbed also by plants.[2] Our recent studies of the role of chloroacetic acids (CAA) in the forest ecosystem[3–9] showed that these compounds may affect coniferous trees and play a role as intermediates in the decomposition of soil organic matter (SOM). Uptake of [1,2–^{14}C]tetrachloroethylene and its biooxidation to [1,2–^{14}C]trichloroacetic acid (TCA) in spruce-needle chloroplasts was shown recently[10]. Enzymatic TCA and chloroform formation in the forest soil suggested earlier[11] (see Eq.1) was reported only for chloroform or for CAA in fulvic acid (FA) solution[12] and abiotic formation in humic acid (HA) suspensions later on.[13] Chlorination of SOM using chlorine-36 conducted for another purposes was reported by Silk *et al.*[14] and Lee *et al.*[15] Chlorinated aromatic structures were found in decaying plant material[16] as well as in chlorinated fulvic acid.[12] We show here microbial degradation of CAA in the forest soil using carbon-14 and their formation by natural spontaneous chlorination in the forest soil as well as chlorination of SOM using chlorine-36.



Eq. 1.

Results and discussion

Using [1,2–^{14}C]TCA and dichloroacetic acid (DCA) and radioindicator methods, CAA were found to be microbially degraded to carbon dioxide and chloride while chlorination of SOM was confirmed using chlorine 36. An important factor is the action of soil microorganisms but an abiotic chlorination is, however, not

---

*Correspondence to: M. Matucha, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic. E-mail: matucha@biomed.cas.cz

Contract/grant sponsor: Grant Agency of Czech Republic; contract/grant numbers: 522/20/0874 and 526/05/0636

†Proceedings of the Ninth International Symposium on the Synthesis and Application of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

Copyright © 2007 John Wiley & Sons, Ltd.

WILEY InterScience® DISCOVER SOMETHING GREAT

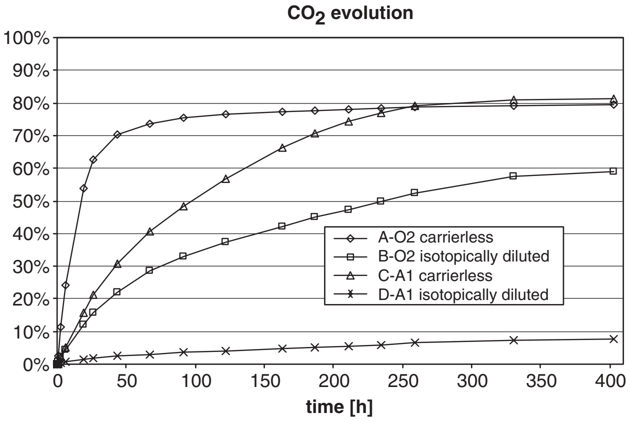


Figure 1 Microbial degradation of [1,2–^{14}C]TCA in spruce-forest soil (horizon A and O2) amended with 396 kBq of [1,2–^{14}C]TCA, i.e. with 17.5 (carrier-free) and 192 μg (11 times isotopically diluted) TCA, in 50 g soil at the beginning: the curves show influence of soil character on the released $^{14}CO_2$ in the course of degradation at room temperature and 30% soil moisture; residual soil radioactivity corresponds mostly to microorganisms biomass. At the end (after 17 days) the soil contained only 0.7 kBq [1,2–^{14}C]TCA from 76.4 kBq residual ^{14}C–activity determined by combustion method (or 81.2 kBq estimated by substraction of the released $^{14}CO_2$ from the sample). The major part of the residual ^{14}C–activity of experiments A and C belongs to radioactive microbial biomass, less than 0.5% radioactivity (about 220 Bq) was found as oxalic acid. The degradation rate is substantially influenced by TCA level and soil character.

Table 1 Radioactivity balance of the degradation experiments after 403 and 877 h of TCA biodegradation in soils, the given values (%) for 403 h correspond to Figure 1

Soil sample/degradation time	Radioactivity in soil (combustion)		Radioactivity of released $^{14}CO_2$		Radioactivity of [1,2-^{14}C]TCA (decarbox)		Total radioactivity (soil + $^{14}CO_2$)	
	403 h	877 h	403 h	877 h	403 h	877 h	403 h	877 h
O2–carrier-free TCA (A)	19.1	14.3	79.5	81.6	0.2	0.1	98.6	96.0
O2–isotopically diluted TCA (B)	38.9	13.1	58.9	78.3	22.0	0.6	97.8	91.4
A–carrier-free TCA (C)	15.4	12.8	81.1	82.8	0.6	0.2	96.5	95.6
A–isotopically diluted TCA (D)	96.6	n.d.	7.7	10.6	94.2	92.1	104.3	n.d.

excluded.[13,17] Differences in the degradation activity of microorganisms for TCA in two forest soil horizons under aerobic laboratory conditions is shown in Figure 1 and Table 1. Autoclaving sterilization suppressed the biodegradation to negligible values and the residual activity after TCA degradation belonged almost entirely to the mass of microorganisms grown on it.

The ability of extracellular enzymes of chloroperoxidase type (CPO) present in the forest soil to convert chloride to chlorine radical, which then chlorinates SOM, is shown in Figure 2. Chlorination of SOM may start at aromatic structures coming from lignin, and leads among others to TCA, DCA and chloroform, which are then decomposed, leached or released from the soil. The suggestions of Hoekstra[11] and Fahimi *et al.*[13] are thus confirmed, showing both chlorination possibilities of SOM, microbial as well as the abiotic one. The binding of chlorine to aromatic structures was shown by Flodin *et al.*[16] and Niedan *et al.*[12] The experiments showed that almost ten percent of chlorine added to soil O2 (depending on Cl^-,C_{ox}-content etc.) were bound to the humic acid fraction (in 1 M KOH extract) after a month; the remaining chlorine was found as chloride in the first aqueous extract. Gamma irradiation as well as autoclaving lowered chlorination while dehalogenation of ^{36}Cl-chlorinated HA added to the forest soil was indicated; isolated [^{36}Cl]HA lost its radioactivity which then appeared in the aqueous chloride extract. Thus both, microbial as well as abiotic chlorination, were indicated together with dehalogenation, which will be reported in more detail elsewhere (Bastviken D, Thomsen F, Svensson T, Karlsson S, Sandén P, Shaw G, Matucha M, Öberg G. *Geochim Cosmochim Acta*, submitted for publication).

Chlorination of SOM may start at resorcinolic structures coming from lignin, and leads among others to TCA, DCA and chloroform, which are then decomposed or released from the soil. Isotope exchange of

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

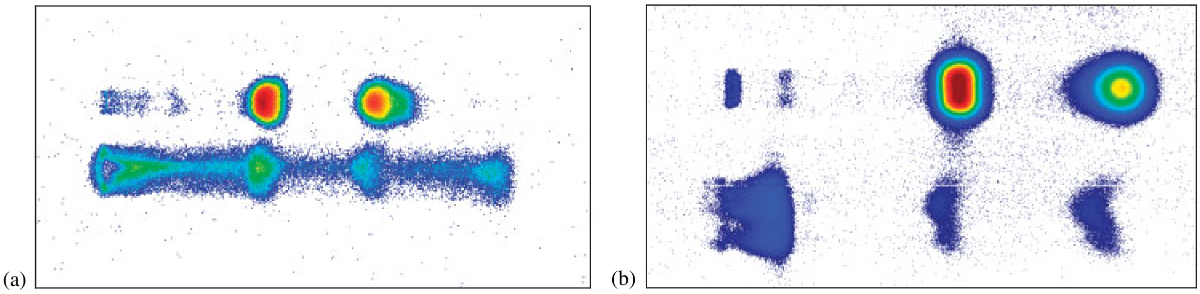


Figure 2 Chlorination of HA isolated from forest soil (spruce plot Baně): radio-TLC of extracted ^{36}Cl-products (lower trace belongs to the ether extract of ^{36}Cl-CAA formed) and of the standards of [1,2-^{14}C]TCA (R_f=ca. 0.65) and [1,2-^{14}C]DCA (R_f=ca. 0. 45) (upper traces) developed in n-butanol-ammonia-water (85:1:14 v/v). Visible yellowish spots (probably of chlorinated FA) remained at the start on the left, DCA bound higher ^{36}Cl-radioactivity than TCA. Chromatogram A – CAA formed by chlorination of HA suspension (forest soil from the spruce plot Baně), B – traces of DCA and TCA spontaneously formed in the JHA soil (soil horizon A from Iser Mountains). Figure available in colour online at www.interscience.wiley.com

^{36}Cl-chloride with organochlorines (TCA, chloroform and o-chlorophenol were tested at pH 4.6) has not been ascertained, while CPO mediated substitution of hydroxyl group by chlorine (in veratrylalcohol) and dechlorination of chlorinated humic acids were indicated. The more or less preliminary examination of the formation and degradation of organochlorines in the forest ecosystem showed thus a complexity of the processes.

Acknowledgements

This work was supported by grants no. 522/02/0874 and 526/05/0636 of the Grant Agency of the Czech Republic.

REFERENCES

1. Öberg GM. In *The Handbook of Environmental Chemistry*, vol. 3, G. Gribble (ed.). Part P. Springer: Berlin, 2003; 43–62.
2. Shaw G, Wadey P, Bell JNB. *J Environ Qual* 2004; **33**: 2272–2280.
3. Matucha M, Uhlířová H, Bubner M. *Chemosphere* 2001; **44**: 217–222.
4. Matucha M, Forczek ST, Gryndler M, Uhlířová H, Fuksová K, Schröder P. *Chemosphere* 2003; **50**: 303–309.
5. Matucha M, Gryndler M, Forczek ST, Uhlířová H, Fuksová K, Schröder P. *Environ Chem Lett* 2003; **1**: 127–130.
6. Matucha M, Rohlenová J, Forczek ST, Uhlířová H, Gryndler M, Fuksová K, Schröder P. *Chemosphere* 2006; **63**: 1924–1932.
7. Forczek ST, Matucha M, Uhlířová H, Albrechtová J, Fuksová K, Schröder P. *Biol Plant* 2001; **44**: 317–320.
8. Forczek ST, Uhlířová H, Gryndler M, Albrechtová J, Fuksová K, Vágner M, Schröder P, Matucha M. *Chemosphere* 2004; **56**: 327–333.
9. Schröder P, Matucha M, Forczek ST, Uhlířová H, Fuksová K, Albrechtová J. *Chemosphere* 2003; **52**: 437–442.
10. Weissflog L, Krueger G, Forczek ST, Lange CA, Kotte K, Pfennigsdorff A, Rohlenová J, Fuksová K, Uhlířová H, Matucha M, Schröder P. *South African J. Bot* (in press). DOI: 10.1016/j.sajb
11. Hoekstra EJ, de Leer EWB, Brinkman UAT. *Chemosphere* 1999; **38**: 2875–2883.
12. Niedan V, Pavasars I. Öberg G. *Chemosphere* 2000; **41**: 779–785.
13. Fahimi IJ, Keppler F, Schöler HF. *Chemosphere* 2003; **52**: 513–520.
14. Silk PJ, Lonergan GC, Arsenault TL, Boyle CL. *Chemosphere* 1997; **35**: 2865–2880.
15. Lee RT, Shaw G, Wadey P, Wang X. *Chemosphere* 2001; **43**: 1063–1070.
16. Flodin C, Johansson E, Boren H, Grimvall A, Dahlman O, Mörck R. *Environ Sci Technol* 1997; **31**: 2464–2468.
17. Keppler F, Eider R, Niedan V, Pracht J, Schöler HF. *Nature* 2000; **403**: 298–301.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr