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FINDINGS SUPPORTING THE NATURAL FORMATION OF TRICHLOROACETIC ACID IN SOIL

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ABSTRACT

The concentrations of trichloroacetic acid (TCAA) in soil and of chlorinated solvents in soil air from simultaneously taken samples from four sites are presented. The data show that there is a significant positive correlation between the concentrations of TCAA and chloroform - but not between TCAA and other chlorinated solvents - in soil. The calculated half-life of TCAA shows that the contribution of decarboxylation to the chloroform concentration in soil is negligible. Since TCAA and chloroform were found to be formed from humic acids in the chloroperoxidase (CPO)-mediated reaction and CPO activity has been detected in soil, the observed correlation between the concentrations of TCAA and chloroform provides novel support for the hypothesis that TCAA is formed naturally in soil. © 1999 Elsevier Science Ltd. All rights reserved

INTRODUCTION

There is one known pathway which can be important for the natural formation of trichloroacetic acid (TCAA) in soil. It involves the natural formation of reactive chlorine species such as hypochlorous acid, from chloride and hydrogen peroxide by a peroxidase-mediated reaction [1]. Next, the reactive chlorine species chlorinate the organic material in soil in a non-specific way. Peroxidases are known to be ubiquitous in organisms and plants. Although not all of them are able to produce reactive chlorine species, the so-called chloroperoxidases (CPO) do. Their catalytic chlorination activity depends on the individual species and pH. In general, optimum chlorination yields occur at pH 3-6, which is the typical pH range of soil with a humic top layer.

Walter and Ballschmiter [2] reported the CPO-mediated formation of chloroform from simple organic compounds such as acetone, propionic acid and citric acid, and Hoekstra and de Leer [3] demonstrated the CPO-mediated formation of chloroform and TCAA from humic acids. CPO activity has been observed in several soil extracts [4,5]. Hoekstra et al. [6] showed that chloroform enriched with ³⁷Cl is formed in Douglas

fir soil which was spiked in situ with an aqueous solution of Na³⁷Cl.

Field et al. [7] reviewed the *de novo* production of specific chlorinated aromatic compounds by basidiomycetous fungi, while Hoekstra et al. [8] recently found that basidiomycetous fungi also form chloroform *de novo*. The CPO-producing deuteromycete *Caldariomyces fumago* produced considerably more chloroform but it could not be concluded from the experiments that the chloroform formation was due to CPO. It is still unknown which enzyme(s) is (are) responsible for the specific chlorination reactions involved.

The natural biosynthesis of chlorinated metabolites and the natural formation of reactive chlorine species seem to be based on a different mechanism. Some chlorinating enzymes may not be able to form reactive chlorine species but may produce an activated enzyme-containing complex instead, which can accelerate specific chlorination reactions inside or outside the cell [1].

From studies on the anthropogenic use of hypochlorous acid in disinfection and bleaching processes, chloroform and TCAA were found to be the main chlorinated products of low molecular weight [9]. They are formed by chlorination of the aromatic rings in the humic structures rather than by chlorination of aliphatic chains via the haloform reaction. Figure 1 shows the formation of chloroform and TCAA from a resorcinolic structure, which is a common structural element of humic material.



Figure 1 The formation of chloroform and TCAA from a resorcinolic structure. * means ¹³C.

The final ratio of the amounts of chloroform and TCAA produced during chlorination of humic material by hypochlorous acid depends on the structural elements of the humic material [9]. All di- and trihydroxy-substituted aromatic structures give rise to the formation of chloroform and TCAA. Despite large differences observed for individual substructures, the overall result of chlorination of humic material from different sources at pH 7 or lower, is a higher yield of TCAA than chloroform. At pH>8 the formation of chloroform is favoured. At constant pH and chlorine dose, chlorination of water samples containing humic material results

in a linear correlation between the concentration of TCAA and chloroform [10].

When reactive chlorine species are produced naturally, the formation of both chloroform and TCAA from humic material can be expected, and there should be an essentially positive linear correlation between the concentrations of chloroform and TCAA. This paper presents the concentrations of chloroform, other chlorinated solvents and TCAA determined in simultaneously taken soil (TCAA analysis) and soil air (chloroform and chlorinated solvents analysis) samples. Part of the experimental data were already reported some years ago [11], but their interpretation is attempted here. Experimental data reported by Plümacher [12] were also included in the evaluation.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical quality. Trichloroacetic acid isopropyl ester (TCAA-iPr) was not commercially available and was supplied by Dr. R.J.B. Peters (TNO-MEP). TCAA-iPr was prepared by heating TCAA with an excess of isopropanol in the presence of concentrated sulphuric acid. After refluxing for several hours, the reaction mixture was washed with a NaHCO₃ solution till the evolution of CO₂ ceased. The resulting mixture was extracted twice with diethylether and the combined ether fractions were dried. After the removal of the solvent, the residue was distilled to give pure TCAA-iPr (bp 172 °C).

Sampling

Rural sampling sites in The Netherlands were selected on the basis of their vegetation and relatively undisturbed history. The Speulderbos near Apeldoorn has been a forest for more than 150 years. Two areas were selected: a Douglas fir forest which was replanted in 1959 without using fertilisers and a beech forest which was replanted in 1835. The peat moor near Fochteloo originates from 3000-4000 years ago. It is relatively undisturbed, although in the 19th century the slash-and-burn culture was used. Wet deposition is the only source of water and heather and grass are the main plants today. De Wieden is a peat bog area which has known different soil usages during the centuries; it is an active peat formation area. A blue grass area was selected.

TCAA

Leaves and needles were removed from the soil before sampling. 10-15 cm of the soil top layer was sampled with a stainless-steel tube (length = 30 cm; i.d. = 8 cm) and put into a 800-ml glass jar with Teflon[®]-lined caps by a plunger with a Teflon[®] head. The soil samples were stored at -20 °C.

10-50 g of field wet soil were shaken with 100 ml of Milli-Q water for 16 h. The extract was filtered over a glass fibre filter and saturated with sodium chloride. After extraction with 10 ml of methyl-*tert*-butylether (MTBE), which was discarded, the aqueous extract was acidified with sulphuric acid to pH 0.5 and extracted again with 10 ml of MTBE. The MBTE extraction solvent contained 100 ng/l of TCAA-iPr as an internal standard. The extract was stored below -20 °C in order to freeze out the water. Ice crystals were removed with

a glass fibre filter. TCAA was derivatised to its methyl ester with diazomethane. Diazomethane was generated from 500 mg diazald (N-methyl-N-nitroso-*p*-toluene sulfonamide) by adding 200 μ l of a methanolic solution of KOH (KOH/water/MeOH = 10 g/3 ml/15 ml) in a washbottle. A stream of nitrogen saturated with MTBE was passed over the diazald reaction mixture and through 1 ml of the extract. The derivatisation was terminated when the extract coloured yellow.

1 μ l of extract was analysed with GC-ECD (HP 5880A) with autoinjector (HP7673A) using a split/splitless injector and a capillary column (HP-1; length = 50 m; i.d. = 0.32 mm; 0.52 μ m film). The column temperature was held at 40 °C for 1 min, than programmed to 80 °C at 1 °C/min and held at that temperature for 1 min. After each analysis the column was heated to 250 °C. The carrier gas was helium which had a precolumn pressure of 1.1 Pa. The injector and detector temperatures were 250 °C and 350 °C, respectively.

TCAA was determined in its methylated form (TCAA-Me) and quantified using TCAA-iPr as the internal standard. The calibration curve of TCAA-Me was linear ($R^2 = 0.9996$) in the concentration range of 0-100 ng/l at a spiking concentration of 100 ng/l of TCAA-iPr.

Chloroform

Chloroform and other volatile chlorinated compounds were sampled in soil air by a stainless-steel enclosure (length = 30 cm; i.d. = 10 cm). The enclosure consists of a tube and a disk that fits on one end of the tube. The tube was hammered carefully into the soil in such way that the soil structure was disturbed as little as possible and no headspace was present between the soil surface and the disk that was fixed airtight on the tube. An adsorbent tube filled with 250 mg of Tenax GR^{\bullet} was fixed on the disk and one litre of soil air (100 ml/min) was pumped through.

The adsorbent tubes were analysed by an on-line automated thermal desorption unit (Perkin Elmer ATD-400) and GC-ECD (Varian 3700). The compounds were desorbed with helium gas at 300 °C for 10 min, trapped at -100 °C in a cold trap containing Tenax-GR and injected splitless by quickly heating the cold trap to 300 °C for 5 min. The temperatures of the valve and the transfer line to the gas chromatograph were 180 °C and 200 °C, respectively. A capillary column (DB-1; length = 30 m; i.d. = 0.55 mm; 0.5 µm film) was temperature programmed from 40 °C to 100 °C at 4 °C/min and, after the run, heated to 250 °C. The carrier gas was helium which had a precolumn pressure of 0.5 Pa. The detector temperature was 300 °C.

The analytes were identified on the basis of their retention time. The amount of analyte which had been adsorbed on the adsorbent tube, was calculated from standard tubes which were analysed together with each series of samples. The standard tubes contained tri- and tetrachloromethane, tri- and tetrachloroethene, 1,1,1-trichloroethane and were prepared in the gas generation laboratory of NMi. For all compounds the calibration curves were linear ($R^2 \ge 0.99$) in the range of 0.5-25 ng.

Adsorbent tubes used for sampling or standards were conditioned by a nitrogen flow at 300 °C overnight. One of every series of twenty conditioned adsorbent tubes was tested for blank levels. None of the compounds was ever detected in the blanks; travel blanks were also clean.

RESULTS AND DISCUSSION

Portions of one soil sample were spiked with 5, 10 and 20 ng TCAA/g in order to determine the recovery. The non-spiked soil contained 0.48 ng TCAA/g. The recoveries of TCAA in the spiked samples were 104 (n=1), $90\pm10\%$ (n=6) and 84% (n=1), respectively.

In the four selected areas TCAA and chloroform were found to be present in the range of 0.2-4.6 μ g/kg dry weight (dw) soil and 0.22-6.0 ng/l soil air, respectively, as reported in ref. [11]. The pH of the soil was in the range of 2.5-4.5. The concentration of chloroform in soil air was 2-60 times higher than the average concentration we measured in atmospheric air [11]. However, the concentrations of the other chlorinated solvents in soil air (see Table 1) were essentially the same as their concentrations in atmospheric air. Table 1 shows that part of the results of ref. [11] which were obtained from simultaneously taken soil (TCAA) and soil air (chlorinated solvents) samples. In order to compare the TCAA concentrations were recalculated on the basis of the organic matter (om) content determined by loss of ignition. The average TCAA concentration in the soil of the Douglas fir forest (2.6±0.4 µg/kg om) was significantly higher than the average concentration in the soil of the beech forest (1.6±0.5 µg/kg om). The average concentration of TCAA in the peat bog (4±1 µg/kg om) was higher than that in the peat moor (2±1 µg/kg om).

Sampling site		TCAA		Chloroform	111T3CE	T4CM	T3CE	T4CE	
	no.	µg/kg dw	µg/kg om	ng/l	ng/l	ng/l	ng/l	ng/l	
Douglas fir	a	0.2	2.7	0.83 ± 0.04	1.83 ± 0.05	0.94 ± 0.06	0.47 ± 0.02	0.71 ± 0.03	
	b	0.3	2.1	0.9 ± 0.2	2.0 ± 0.8	0.84 ± 0.07	$0.12\pm\ 0.02$	0.24 ± 0.08	
Beech	a	0.2	1.4	0.7 ± 0.1	1.7 ± 0.4	0.7 ± 0.1	0.10 ± 0.02	0.24 ± 0.02	
	b	0.2	1.2	0.58 ± 0.07	2.4 ± 0.4	1.01 ± 0.05	0.36 ± 0.03	0.55 ± 0.04	
Peat bog	a	4.6	5.2	6 ± 3	2 ± 1	0.6 ± 0.2	0.5 ± 0.2	0.9 ± 0.3	
	b	2.9	3.1	1.9 ± 0.8	1.1 ± 0.6	0.4 ± 0.2	$0.21~\pm~0.04$	0.18 ± 0.07	
Peat moor		1.0	1.1	0.22 ± 0.07	1.2 ± 0.4	0.6 ± 0.2	0.11 ± 0.06	0.12 ± 0.02	

 Table 1 Concentrations of TCAA in soil (n=1) and average concentrations of volatile chlorinated compounds (n=6) in soil air using simultaneously taken samples.

dw, dry weight; om, organic matter; 111T3CE, 1,1,1-trichloroethane; T4CM, tetrachloromethane; T3CE, trichloroethene; T4CE, tetrachloroethene

The concentrations of TCAA we found in the four different types of soil are rather low compared to the concentrations of 20-380 μ g/kg found by Frank [13] in soil of German spruce forests, and by Plümacher [12] in soil of German pine forests (1.4-120 μ g/kg dw) and agricultural land (0.08-7.9 μ g/kg dw). Frank sampled 20-cm cores but it is not clear from his paper whether the calculated concentrations were based on dry or wet weight, and whether the leaf litter layer had been removed from the soil. Our data from the Douglas fir forest

and pine forest data of Plümacher can be compared since the soil structure of both forests are known: the O horizont in our Douglas fir forest (5 cm) was found to be of comparable thickness as that of Plümacher's pine forests (4 cm). The TCAA concentration of a 10-15 core in pine forests was recalculated to be $30\pm20 \ \mu g/kg$ om from its concentration in the O (9-120 $\mu g/kg$ dw) and A horizont (2.4-14 $\mu g/kg$ dw). The corrected TCAA concentration in the pine forests is still significantly higher than that in the Douglas fir forest. One can only speculate about the observed differences being caused by biotic or abiotic factors.

The presence of TCAA in soil was extensively discussed in another recent paper [14]. The mass balance calculations discussed in that study provide tentative evidence on a source of TCAA in soil because the calculated production fluxes of TCAA in different soil types generally were much higher than the calculated deposition fluxes. However, no definitive conclusion could be drawn whether TCAA is produced naturally in soil or is formed from chlorinated solvents such as tri- and tetrachloroethene, which are present in soil as a result of anthropogenic activity. Actually, natural formation of TCAA is very likely because of (i) the CPO-mediated formation of TCAA and chloroform from humic acids [3], (ii) the demonstration of CPO activity in soil [4,5] and (iii) the formation of chloroform enriched with ³⁷Cl at *in situ* Na³⁷Cl spiked soil [6].

Because the CPO-mediated reaction seems to form reactive chlorine species, one may expect that a correlation between TCAA and chloroform concentrations in soil will exist as was found for chlorination of surface water [10]. However, such a correlation may also arise if TCAA is decarboxylated to chloroform in soil. TCAA is decarboxylated only if it is present as trichloroacetate and the presence of light increases the degradation rate [15]. From decarboxylation studies of TCAA in water, one can calculate that TCAA has a half-life of about 35-350 years at a temperature of 5-15°C in the presence of light [16-18] and of 200-2000 years in darkness [19]. At 26 °C and under UV exposure only about 10% of the degraded TCAA was converted into chloroform [20]. Therefore, it can be stated safely that the contribution of chemical degradation of TCAA to the concentration of chloroform in soil is negligible.

Actually, the real half-life of TCAA in soil is 15-90 days [21]. This is due to biodegradation of TCAA by several bacteria and fungi [22]. TCAA is biodegraded to CO_2 and chloride only and the formation of chloroform has never been reported [23-25]. In other words, if a correlation between TCAA and chloroform concentrations in soil is found, this provides additional support on the natural formation of TCAA in soil. If, on the other hand, a correlation with the concentrations of (an)other chlorinated solvent(s) is observed, TCAA is mainly formed from this(these) compound(s).

As regards the data of Table 1, a positive linear relationship with $R^2=0.82$ (P<0.01) was found for the concentration of TCAA in soil (μ g/kg dw) and chloroform in soil air (ng/l). The correlation improved when the concentrations of TCAA were expressed in μ g/g om ($R^2=0.88$ (P<0.01)). As Table 2 clearly illustrates, no positive linear correlation at the P<0.01 level was found between the concentration of TCAA in soil and that of any of the other chlorinated solvents studied in soil air, and only two weak linear correlations at a significance level of P<0.1.

The correlation observed for our data is confirmed by data of Plümacher who determined concentrations of TCAA, chloroform and other chlorinated solvents in 72 soil samples from pine forests and agricultural areas

[14] (see Table 3). In each sample, chloroform and the other chlorinated compounds were determined first. Subsequently, TCAA was determined after its decarboxylation to chloroform [26]. Using Plümacher's data, we found a strong positive linear correlation ($R^2=0.97$ (P<<0.001)) between the concentrations of TCAA and chloroform in soil. On the other hand, the linear correlations between the concentrations of TCAA and those of the other chlorinated solvents in soil were very weak at a high significance level as is evident from the data included in Table 2.

Table 2	Squared correlation	coefficients (R ²) of	f the correlation	between the co	ncentrations of	TCAA in soil
	and the chlorinated	solvents in soil air (Table 1) or soil [12].*		

Compound	TC	TCAA		
	n=7 [7	n=72 [12]		
	dry weight	organic matter		
Chloroform	0.82 (P<0.01)	0.88 (P<0.01)	0.97 (P<<0.001)	
1,1,1-Trichloroethane	0.00	0.05	0.25 (P<0.02)	
Tetrachloromethane	0.52 (P<0.1)	0.20	0.19 (P<0.05)	
Trichloroethene	0.12	0.36	0.20 (P<0.05)	
Tetrachloroethene	0.16	0.45 (P<0.1)	0.29 (P<0.01)	

* P, the level of significance.

Table 3 Averaged concentrations of TCAA and volatile chlorinated compounds in soil ($\mu g/kg dw$) from [14].

Н		TC	ĀA	Ch	loroform	111	T3CE	Т	'4CM	7	T3CE	T	'4CE
0	40	±	40	7	± 8	0.20	± 0.05	1.0	± 0.3	0.2	± 0.1	0.8	± 0.2
Α	7	±	4	2	± 2	0.12	± 0.03	0.06	± 0.03	0.2	± 0.1	0.5	± 0.2
В	3	±	4	0.4	± 0.6	0.02 :	± 0.008	<	0.01	0.01	± 0.02	0.08	± 0.04
0	8	±	2	1.1	± 0.2	0.04	± 0.02	0.04	± 0.006	0.03	± 0.004	0.06	± 0.01
Α	0.9	2 ±	0.05	0.11	t ± 0.01	0.03 :	± 0.003	0.01	± 0.001	<	< 0.01	0.04	± 0.004
В	0.0	8 ±	0.004	0.01	± 0.001	<	0.01	<	0.01	<	< 0.01	<	0.01
	H O A B O A B	H O 40 A 7 B 3 O 8 A 0.9 B 0.0	$\begin{array}{c ccc} H & TC \\ \hline O & 40 & \pm \\ A & 7 & \pm \\ B & 3 & \pm \\ O & 8 & \pm \\ A & 0.92 \pm \\ B & 0.08 \pm \end{array}$	H TCAA O 40 ± 40 A 7 ± 4 B 3 ± 4 O 8 ± 2 A 0.92 ± 0.05 B 0.08 ± 0.004	H TCAA Ch O 40 \pm 40 7 A 7 \pm 4 2 B 3 \pm 4 0.4 O 8 \pm 2 1.1 A 0.92 \pm 0.05 0.11 B 0.08 \pm 0.004 0.01	HTCAAChloroformO 40 ± 40 7 ± 8 A7 ± 4 2 ± 2 B3 ± 4 0.4 ± 0.6 O 8 ± 2 1.1 ± 0.2 A 0.92 ± 0.05 0.11 ± 0.01 B 0.08 ± 0.004 0.01 ± 0.001	H TCAA Chloroform 111 O 40 \pm 40 7 \pm 8 0.20 A 7 \pm 4 2 \pm 2 0.12 B 3 \pm 4 0.4 \pm 0.6 0.02 O 8 \pm 2 1.1 \pm 0.2 0.04 A 0.92 \pm 0.05 0.11 \pm 0.01 0.03 B 0.08 \pm 0.004 0.01 \pm 0.001 $<$	HTCAAChloroform111T3CEO40 \pm 407 \pm 8 0.20 ± 0.05 A7 \pm 42 \pm 2 0.12 ± 0.03 B3 \pm 4 0.4 ± 0.6 0.02 ± 0.008 O8 \pm 2 1.1 ± 0.2 0.04 ± 0.02 A 0.92 ± 0.05 0.11 ± 0.01 0.03 ± 0.003 B 0.08 ± 0.004 0.01 ± 0.001 < 0.01	HTCAAChloroform111T3CETO40 \pm 407 \pm 8 0.20 ± 0.05 1.0A7 \pm 42 \pm 2 0.12 ± 0.03 0.06B3 \pm 4 0.4 ± 0.6 0.02 ± 0.008 <	HTCAAChloroform111T3CET4CMO40 \pm 407 \pm 8 0.20 ± 0.05 1.0 ± 0.3 A7 \pm 42 \pm 2 0.12 ± 0.03 0.06 ± 0.03 B3 \pm 4 0.4 ± 0.6 0.02 ± 0.008 < 0.01 O8 \pm 2 1.1 ± 0.2 0.04 ± 0.02 0.04 ± 0.006 A $0.92 \pm$ 0.05 0.11 ± 0.01 0.03 ± 0.003 0.01 ± 0.001 B $0.08 \pm$ 0.004 0.01 ± 0.001 < 0.01 < 0.01	HTCAAChloroform111T3CET4CMT4CMO40 \pm 407 \pm 8 0.20 ± 0.05 1.0 ± 0.3 0.2 A7 \pm 42 \pm 2 0.12 ± 0.03 0.06 ± 0.03 0.2 B3 \pm 4 0.4 ± 0.6 0.02 ± 0.008 < 0.01 0.01 O8 \pm 2 1.1 ± 0.2 0.04 ± 0.02 0.04 ± 0.006 0.03 A 0.92 ± 0.05 0.11 ± 0.01 0.03 ± 0.003 0.01 ± 0.001 $<$ B 0.08 ± 0.004 0.01 ± 0.001 < 0.01 < 0.01 < 0.01	HTCAAChloroform111T3CET4CMT3CEO40 \pm 407 \pm 8 0.20 ± 0.05 1.0 ± 0.3 0.2 ± 0.1 A7 \pm 42 \pm 2 0.12 ± 0.03 0.06 ± 0.03 0.2 ± 0.1 B3 \pm 4 0.4 ± 0.6 0.02 ± 0.008 < 0.01 0.01 ± 0.02 O8 \pm 2 1.1 ± 0.2 0.04 ± 0.02 0.04 ± 0.006 0.03 ± 0.004 A 0.92 ± 0.05 0.11 ± 0.01 0.03 ± 0.003 0.01 ± 0.001 < 0.01 B 0.08 ± 0.004 0.01 ± 0.001 < 0.01 < 0.01 < 0.01	HTCAAChloroform111T3CET4CMT3CETO40 \pm 407 \pm 8 0.20 ± 0.05 1.0 ± 0.3 0.2 ± 0.1 0.8 A7 \pm 42 \pm 2 0.12 ± 0.03 0.06 ± 0.03 0.2 ± 0.1 0.5 B3 \pm 4 0.4 ± 0.6 0.02 ± 0.008 < 0.01 0.01 ± 0.02 0.08 O8 \pm 2 1.1 ± 0.2 0.04 ± 0.02 0.04 ± 0.006 0.03 ± 0.004 0.06 A 0.92 ± 0.05 0.11 ± 0.01 0.03 ± 0.003 0.01 ± 0.001 < 0.01 0.04 B 0.08 ± 0.004 0.01 ± 0.001 < 0.01 < 0.01 < 0.01 < 0.01

<, below detection limit; H, horizont; Agr., agricultural land; 111T3CE, 1,1,1-trichloroethane; T4CM, tetrachloromethane; T3CE, trichloroethene; T4CE, tetrachloroethene</p>

CONCLUSIONS

Experimental data on the presence of TCAA, chloroform and several other volatile chlorinated solvents in soil and/or soil air show that there is a significant positive linear correlation between the concentrations of TCAA and chloroform in soil, but not between the concentrations of TCAA and those of any other chlorinated solvents studied. This strongly suggests that TCAA is not formed from 1,1,1-trichloroethane,

tetrachloromethane, tri- or tetrachloroethene in these soils. The correlation of the concentrations of TCAA and chloroform in soil (air) cannot be attributed to decarboxylation of TCAA to chloroform because of the chemical half-life of TCAA in soil of 200-2000 years, while biodegradation of TCAA apparently does not yield chloroform at all. Since TCAA and chloroform are formed from humic acids in the CPO-mediated reaction and CPO-like activity is detected in soil, the observed correlation is a novel indication for the natural formation of TCAA in soil.

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