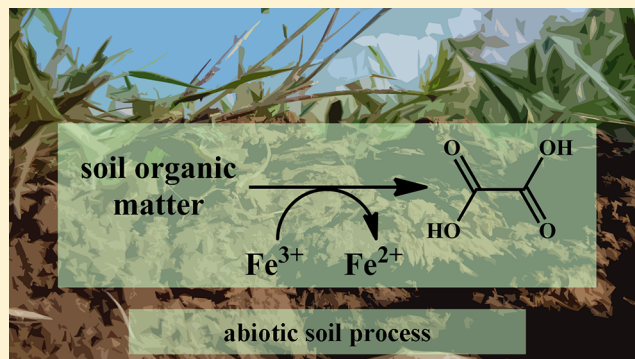


Natural Abiotic Formation of Oxalic Acid in Soils: Results from Aromatic Model Compounds and Soil Samples

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ABSTRACT: Oxalic acid is the smallest dicarboxylic acid and plays an important role in soil processes (e.g., mineral weathering and metal detoxification in plants). We have first proven its abiotic formation in soils and investigated natural abiotic degradation processes based on the oxidation of soil organic matter, enhanced by Fe^{3+} and H_2O_2 as hydroxyl radical suppliers. Experiments with the model compound catechol and further hydroxylated benzenes were performed to examine a common degradation pathway and to presume a general formation mechanism of oxalic acid. Two soil samples were tested for the release of oxalic acid and the potential effects of various soil parameters on oxalic acid formation. Additionally, the soil samples were treated with different soil sterilization methods to prove the oxalic acid formation under abiotic soil conditions. Different series of model experiments were conducted to determine a range of factors including Fe^{3+} , H_2O_2 , reaction time, pH, and chloride concentration on oxalic acid formation. Under certain conditions, catechol is degraded up to 65.6% to oxalic acid referring to carbon. In serial experiments with two soil samples, oxalic acid was produced, and the obtained results are suggestive of an abiotic degradation process. In conclusion, Fenton-like conditions with low Fe^{3+} concentrations and an excess of H_2O_2 as well as acidic conditions were required for an optimal oxalic acid formation. The presence of chloride reduced oxalic acid formation.



INTRODUCTION

Natural abiotic oxidation processes of soil organic matter (SOM) were studied intensely over the last years. Keppler et al. presented the abiotic formation of the volatile organic compound (VOC) methyl chloride.¹ They proposed that an abiotic reaction mechanism exists, in which SOM is oxidized, while the redox partner (e.g., Fe^{3+}) is reduced. The reaction mechanism was elucidated by using guaiacol as a model compound for humic substances, Fe^{3+} , or the mineral ferrihydrite and halides. The addition of H_2O_2 increased methyl halide yields.

Fe^{3+} was chosen as redox partner because it is the fourth most abundant element in the Earth's crust and is mostly present in the mineral form of oxyhydroxides. Thus, it plays an important role within redox systems in natural environments. H_2O_2 is present in the top layer of soils. O_2 can be reduced to H_2O_2 by organic carbon or is produced by fungi when depolymerizing lignin. In addition, rainwater can provide H_2O_2 to the soil.²

Further investigations already unravelled a variety of volatile organic compounds such as chloroform, chloroethyne, and carbon suboxide being produced in soils.^{3–11} Additionally, CO_2 ¹² and chloroacetic acids^{13–16} were found during abiotic degradation processes of SOM. Especially, the abiotic formation of chloroacetic acids in soils is of importance due to their phytotoxic effect possibly causing forest decline.¹⁷

Keppler et al.¹ and Huber et al.^{8,10,11} used aromatic organic model compounds such as catechol or guaiacol to facilitate the

elucidation of reaction mechanisms. By varying reaction parameters, for example, concentration of Fe^{3+} , reaction time, and H_2O_2 supplementation, they could determine the optimal reaction conditions for each product. They applied these parameters to further model compounds to establish tentative degradation pathways for natural aromatic constituents in soils.

The proposed degradation mechanism of the model compound catechol implies a variety of further reaction channels (e.g., to polar organic compounds such as carboxylic acids). This assumption is supported by the results of advanced oxidation processes (AOP) from wastewater treatment by Sun and Pignatello.¹⁸ They found oxalic acid as intermediate during the mineralization of 2,4-dichlorophenoxyacetic acid with Fe^{3+} and H_2O_2 in the dark with a yield of 60% after 1.5 h. Furthermore, in catalytic wet air oxidation (CWAO) of phenol with an iron/activated carbon catalyst and molecular oxygen a wide range of different short-chain organic acids such as oxalic and maleic acid were identified as intermediate oxidation products.¹⁹ Further oxidation experiments of phenols with and without catalyst also lead to different dicarboxylic acids such as oxalic, maleic and malonic acid presuming oxalic acid as the end product of degradation schemes.^{20–25}

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In this paper, we focus on oxalic acid, the smallest dicarboxylic acid, whose pK_a values are 1.23 and 4.19,²⁶ reflecting its strong acidity. Oxalic acid is present in sediments, soils, and the rhizosphere. A small amount of oxalic acid enters soils by atmospheric deposition and throughfall. The main part originates from root exudation and plant residue decomposition and is also produced from fungi or soil bacteria.^{27–32} Oxalic acid plays an important role in many soil processes. It exhibits a diverse range of functions in the rhizosphere, such as mineral weathering and nutrient acquisition by mobilizing the poorly soluble nutrients (Mn and Fe) for organisms, and therefore improves their bioavailability.^{33–38} It also has the ability to release phosphate and K from soils and minerals. In detoxification processes, oxalic acid is also used by plants as a chelator for metal cations. Furthermore, oxalic acid serves as plant protection against herbivores and insects and also regulates pH and osmotic pressure.³⁹

First, we investigated the oxalic acid formation starting with the model compound catechol since it was often used as representative for structural elements of SOM.⁴⁰ To enable Fenton-like conditions a soluble Fe^{3+} salt was chosen as redox-sensitive element and H_2O_2 as a strong oxidant. During the reaction course hydroxyl radicals are formed, which play an important role during these redox processes.^{41,42} Different reaction parameters were varied to define the optimal range of reaction condition for oxalic acid formation. Optimized conditions were applied to further polyhydroxylated benzenes to examine whether a general degradation pathway to oxalic acid can be proposed for aromatic compounds with similar structures.

Finally, the abiotic oxalic acid formation in soil samples was investigated using two freeze-dried and sterilized soil samples supplemented with Fe^{3+} and H_2O_2 . The H_2O_2 application to soils might form calcium oxalate, as Martin revealed.⁴³ Sterilization of the soil samples was carried out by three different methods that eliminate the microbial activity and enzymes of soil sample.⁴⁴ The experiments with the sterilized soil samples were conducted to confirm the oxalic acid formation under abiotic conditions. The model reactions and the soil sample experiments were conducted to present a further route for a natural abiotic oxalic acid formation from SOM.

EXPERIMENTAL SECTION

Chemicals. The following chemicals were used: catechol (99%, Sigma-Aldrich); 2,5-dihydroxy-1,4-benzoquinone (98%, Aldrich); fumaric acid (100%, Sigma-Aldrich); H_2O_2 (30%, Appli Chem); hydroquinone (99%, Fluka); iron(III)-sulfate ($Fe^{21-23\%}$, Riedel-de-Haën); maleic acid (99%, Sigma-Aldrich); malonic acid (98%, Fluka); oxalic acid (99%, Sigma-Aldrich); phloroglucin (99%, Aldrich); potassium chloride (99.5%, Merck); pyrogallol (99%, Aldrich); resorcinol (99%, Riedel-de-Haën); sodium carbonate (99.5%, Grüssing); NaOH (1M, Grüssing); H_2SO_4 (96%, Riedel-de-Haën); and 1,2,4-trihydroxybenzene (99%, Aldrich). Doubly deionized water (18 M Ω cm, ELGASTAT UHQ PS) was used in all experiments.

Soil Samples. Two soil samples were investigated to verify the natural abiotic formation process of oxalic acid. Both soil samples were collected from a peatland soil from the nature reserve Rotwasser near Olfen in the Hessian Odenwald/Germany. The watershed of this area comprises around 16 ha predominantly afforested with spruce, pine, birch, and buck-

thorn trees.⁴⁵ It is assumed that the atmospheric impact in this area equates to the background concentration without direct industrial or agricultural emissions to the reserve. The chemical characterization of the soils is shown in Table 1.

Table 1. Soil Properties

	RW 1	RW 2
location	Rotwasser/Germany	Rotwasser/Germany
sample type	peaty soil	peaty soil
geographic position	49°36'39"N 8°53'11"E	49°36'22.3"N 8°53'26.11"E
organic carbon (C_{org}) ^a	1.6%	15.3%
inorganic carbon ^b	<0.05%	<0.05%
Cl [−] concn. ^c	3.1 ppm	33.5 ppm
Fe concn. ^d	1.4%	1.4%
pH value	4.4	4.2

^aCS measurement. ^bVia a carbonate bomb.⁴⁶ ^cIC measurement. ^d μ -XRF-measurement (EMMA)⁴⁷

Instrumentation. Ion chromatography analysis was performed on a Dionex DX120 complete system, which includes pump, self-regenerating suppressor ASRS ULTRA II, and conductivity detector, coupled to an autosampler Dionex AS90. The chromatographic separation was performed using an AS9-HC (4 × 250 mm, Dionex) analytical column with an AG9-HC guard column (Dionex) connected upstream. The eluent consisted of an 11 mM sodium carbonate solution, which was pumped with a flow rate of 1 mL/min through the system.

Based on dried and powdered soil material, total carbon content was determined applying a carbon/sulfur-analyzer (Leco, SC-144DR).

Inorganic carbon was determined using a carbonate bomb.⁴⁶

Organic carbon content was calculated by subtracting the inorganic carbon content from the total carbon content.

An energy-dispersive miniprobe multielement analyzer (EMMA) was employed to analyze the total iron content.⁴⁷

All pH measurements were made using a Knick pH-Meter 761 with a SenTix 41 (WTW), calibrated on the free hydrogen scale with 4.0 and 7.0 buffer solutions.

Soil Preparation and Sterilization. Soil samples were freeze-dried (Steris LYOVAC GT2) and milled (Planetary mill, Fritsch Pulverisette 5) to a fine powder (<315 μ m). Soil parameter analysis was conducted with the freeze-dried soil samples.

As freeze-drying is not ensuring that all microbial activity and enzymes are eliminated, the soil samples were additionally sterilized by 3 different methods.⁴⁴ The following sterilization methods were performed: (1) Dry heat: the soil samples were heated at 105 °C overnight in a drying oven. (2) Moist heat: an autoclave from Holzner was used to sterilize the soil samples with moist heat at 121 °C and 2.2 atm for 20 min. (3) Irradiation: the soil samples were irradiated in a Gammabeam-650 (MDS Nordion). The soil samples were given a dose of 25 kGy. The Curie load of the ⁶⁰Co source was approximately 2900 Curies, which corresponds to a dosing time of 14.5 h. The experiments with the sterilized soil samples are further evidence for the abiotic formation of oxalic acid.

Experimental Procedures. The experiments were carried out in airtight 20 mL headspace glass vials each filled with 10 mL of the respective aqueous solution of reactants. Prior to analysis, the vials were shaken for a certain time on a rotary

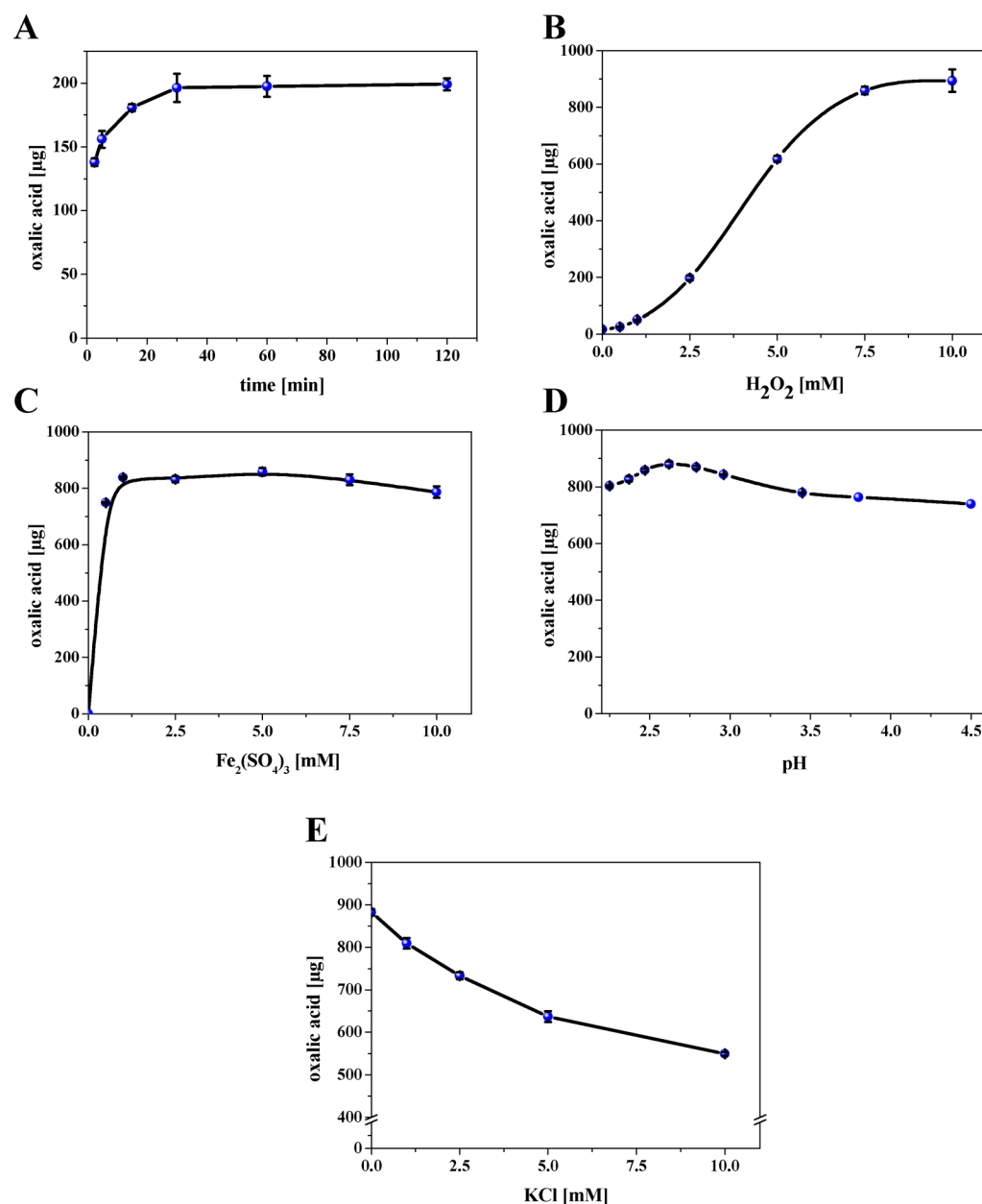


Figure 1. Effects of different parameters on oxalic acid formation. (A) Time dependence: reaction of 0.5 mM catechol, 5.0 mM $Fe_2(SO_4)_3$, and 2.5 mM H_2O_2 at 40 °C. (B) Variation of H_2O_2 : reaction of 0.5 mM catechol, 5.0 mM $Fe_2(SO_4)_3$, and 0–10.0 mM H_2O_2 at 40 °C after 1 h. (C) Variation of Fe^{3+} : reaction of 0.5 mM catechol, 0–10.0 mM $Fe_2(SO_4)_3$, and 7.5 mM H_2O_2 at 40 °C after 1 h. (D) Variation of the pH value from 2.2 to 3.2: reaction of 0.5 mM catechol, 1.0 mM $Fe_2(SO_4)_3$, and 7.5 mM H_2O_2 at 40 °C after 1 h. (E) Variation of chloride: reaction of 0.5 mM catechol, 1.0 mM $Fe_2(SO_4)_3$, 7.5 mM H_2O_2 , and 0–10.0 mM KCl at 40 °C after 1 h. Symbols are average data from experiments; error bars indicate standard deviations from triplicate measurements. The concentration unit [μg] corresponds to the oxalic acid amount obtained from an initial concentration of 0.5 mM catechol.

board (IKA MTS 2) at 500 rpm at 40 °C in the dark. These incubation conditions were applied to all model and soil reactions.

Model Compounds. After shaking the solutions of the model compound experiments, NaOH (1 M) was added to quench the reaction and to avoid the precipitation of iron during IC analysis. The solutions were centrifuged at 5000 rpm for 3 min, and the supernatant was measured subsequently. Preliminary experiments showed that the addition of sodium hydroxide precipitates Fe^{3+} from the reaction solution but has no negative influence on the oxalic acid concentration.

Soil Samples. Aqueous solutions (10 mL) with 1.0 g soil with and without supplementation of the reactants Fe^{3+} and H_2O_2 were used for each soil experiment. The pH value of 4.4 (RW 1) and 4.2 (RW 2) were not adjusted and represent the natural soil buffer system. After shaking, the soil samples were centrifuged at 5000 rpm for 10 min and filtered through a 0.45 μm cartridge prior to analysis.

Reactions with Catechol. To find suitable parameters for an optimal oxalic acid formation systematic sequential variation of the following reaction parameters was conducted. Each optimal measured value was used for the subsequent optimization steps.

Time Dependence. The first optimization step was to determine the time dependence of the oxalic acid formation. Solutions of 10 mL water with initial concentrations of 0.5 mM catechol, 5.0 mM $\text{Fe}_2(\text{SO}_4)_3$, and 2.5 mM H_2O_2 were prepared and shaken for different durations.

H_2O_2 . In the next step, solutions of 10 mL water with initial concentrations of 0.5 mM catechol and 5.0 mM $\text{Fe}_2(\text{SO}_4)_3$ were prepared together with different concentrations of H_2O_2 and were shaken for 1 h.

Fe^{3+} . The third series of measurements concerned the concentration of Fe^{3+} . Solutions of 10 mL water with initial concentrations of 0.5 mM catechol and 7.5 mM H_2O_2 were prepared with different concentrations of $\text{Fe}_2(\text{SO}_4)_3$. The pH value was kept at a constant level of 2.5, which was adjusted by adding appropriate amounts of NaOH or H_2SO_4 . The solutions were shaken for 1 h.

pH Value. To investigate the pH dependent formation of oxalic acid, 10 mL aqueous solutions with initial concentrations of 0.5 mM catechol, 1.0 mM $\text{Fe}_2(\text{SO}_4)_3$, and 7.5 mM H_2O_2 were prepared. The different pH values were adjusted by adding NaOH or H_2SO_4 (pH range between 2.2 and 4.5) and were shaken for 1 h.

Chloride. Finally, the influence of chloride on the oxalic acid yield was examined. For this purpose, solutions of 10 mL water were prepared with initial concentrations of 0.5 mM catechol, 1.0 mM $\text{Fe}_2(\text{SO}_4)_3$, 7.5 mM H_2O_2 and 1.5 mM H_2SO_4 (pH 2.6) together with different concentrations of KCl and shaken for 1 h.

Reactions with Different Hydroxylated Benzenes. The next investigation of oxalic acid formation was to replace catechol as model compound by different hydroxylated benzenes (e.g., the constitutional isomers of catechol such as resorcinol and hydroquinone, the isomers 1,2,4-trihydroxybenzene, phloroglucin and pyrogallol, and in addition 2,5-dihydroxy-1,4-benzoquinone). The optimized conditions of catechol experiments were applied to these model compounds. Solutions of 10 mL water with 0.5 mM of the respective organic compound, 1.0 mM $\text{Fe}_2(\text{SO}_4)_3$, 7.5 mM H_2O_2 , and 1.5 mM H_2SO_4 (pH 2.6) were prepared and shaken for 1 h.

Time Dependence of Soil Sample RW 1. In a preliminary study, the time dependent oxalic acid formation was tested. 1.0 g of the freeze-dried soil sample RW 1 was shaken in 10 mL water solutions for different durations between 5 min and 48 h and analyzed for oxalic acid formation.

Sterilized Soil Samples. To ensure that the oxalic acid formation process originated from an abiotic pathway, both soil samples RW 1 and RW 2 were sterilized by 3 different methods (dry heat, moist heat and pressure, and γ -irradiation) after freeze-drying and milling. Subsequently, solutions of 10 mL water with 1.0 g of the sterilized soil sample were prepared and shaken for 5 min and 24 h. Further on, the optimal Fe^{3+} and H_2O_2 concentrations derived from catechol optimization were applied to the sterilized soil sample RW 1. Solutions of 10 mL water with initial concentrations of 1.0 mM $\text{Fe}_2(\text{SO}_4)_3$, 7.5 mM H_2O_2 , and 1.0 g of soil were prepared and shaken for 5 min and 24 h. The oxalic acid yields after 5 min are assumed to be residue or product formed during the sterilization process. Thus, the obtained oxalic acid yields after 5 min were subtracted from the oxalic acid yields formed after 24 h.

Identification and Quantification. The oxalic acid was identified as oxalate by its retention time and quantified via a multipoint calibration curve. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Reactions with Catechol. The time dependent formation of oxalic acid is shown in Figure 1A. The fast formation of oxalic acid within the first minutes ends in a plateau after approximately 1 h. For the next optimization steps, the reaction time of 1 h was chosen.

In the second optimization step, the effect of H_2O_2 on the oxalic acid formation was investigated. By increasing the H_2O_2 concentration, the formation of oxalic acid behaved similar to an S-shaped curve depicted in Figure 1B. The maximum yield is achieved when H_2O_2 is used in a molar ratio of 15:1 compared to catechol. Furthermore, in the absence of H_2O_2 , oxalic acid is still produced in small amounts, pointing to O_2 present in the reaction solution. H_2O_2 accelerated and increased oxalic acid formation. At low H_2O_2 concentrations between 0.5 to 2.5 mM, a series of dicarboxylic acids are formed. Three of them are identified as malonic, fumaric, and maleic acid. When increasing the H_2O_2 concentration, only oxalic acid is formed, indicating that these dicarboxylic acids might be intermediate structures within the reaction pathway to oxalic acid. A H_2O_2 concentration of 7.5 mM was chosen for the following optimization experiments.

The Fe^{3+} dependent reaction is shown in Figure 1C. These measurements revealed that Fe^{3+} is essential for the formation of oxalic acid, as no oxalic acid was detected in the reaction without Fe^{3+} supplementation. While H_2O_2 has to be present in excess for an optimal reaction, only low concentrations of Fe^{3+} are required. A plateau is observed between concentrations of 1.0 mM and 5.0 mM Fe^{3+} . By further increasing the Fe^{3+} concentration oxalic acid yields decreased. As the pH value of the reaction solution is mainly conditioned by the added $\text{Fe}_2(\text{SO}_4)_3$ -solution, H_2SO_4 or NaOH was added to adjust the pH value to 2.5. When adding NaOH, part of the Fe^{3+} precipitated as oxyhydroxides, and the availability of Fe^{3+} for the reaction is reduced. A $\text{Fe}_2(\text{SO}_4)_3$ concentration of 1.0 mM was chosen for the following experiments.

The pH dependence of oxalic acid formation is displayed in Figure 1D. The measurements were performed in a pH range from 2.2 to 4.5 with maximum yields at 2.6. Above a pH of 2.9, oxalic acid formation decreased slowly. This might be related to the addition of NaOH, used to adjust higher pH values. Fe^{3+} ions precipitated as oxyhydroxides and thus become unavailable for the reaction, resulting in lower oxalic acid yields. The pH value of 2.6 was chosen as optimal reaction condition for further experiments.

Finally, the influence of chloride on the oxalic acid formation was investigated as depicted in Figure 1E. An increasing chloride concentration constrained the oxalic acid formation. At a concentration of 10 mM KCl, oxalic acid yield decreased by almost 40%. In the presence of chloride, side reactions forming chlorinated byproducts might occur, which would explain the decrease of oxalic acid yields. However, no polar chlorinated byproducts (e.g., chloromalonic or chloromaleic acid) were detected in the reaction solution. These findings are in agreement with the results of advanced oxidation processes (AOP) in saline waters where only small amounts of halogenated byproducts were found.⁴⁸ Huber et al.⁸ investigated the decomposition of catechol to trihalomethanes under similar conditions and found a maximum CHCl_3 yield of 0.003%. Side reactions to chlorinated compounds seem to play a secondary role here.

A more convincing explanation could be related to the fact that chloride ions are known to be significant hydroxyl radical scavengers.⁴⁸ While hydroxyl radicals formed through Fenton-like conditions from Fe^{3+} and H_2O_2 are required in excess to form large amounts of oxalic acid, the addition of chloride will scavenge them and consequently reduce oxalic acid yields.

In summary, oxalic acid is produced relatively fast, with a catechol/ Fe^{3+} ratio of 1:2, a catechol/ H_2O_2 ratio of 1:15, and an optimal pH at 2.6. The presence of chloride impeded oxalic acid formation.

Reactions with Different Hydroxylated Benzenes. The experiments with the hydroxylated benzenes resorcinol and hydroquinone, as well as with 1,2,4-trihydroxybenzene, pyrogallol, phloroglucin, and 2,5-dihydroxy-1,4-benzoquinone provided similar oxalic acid yields under the applied reaction conditions as with the model compound catechol (Figure 2).

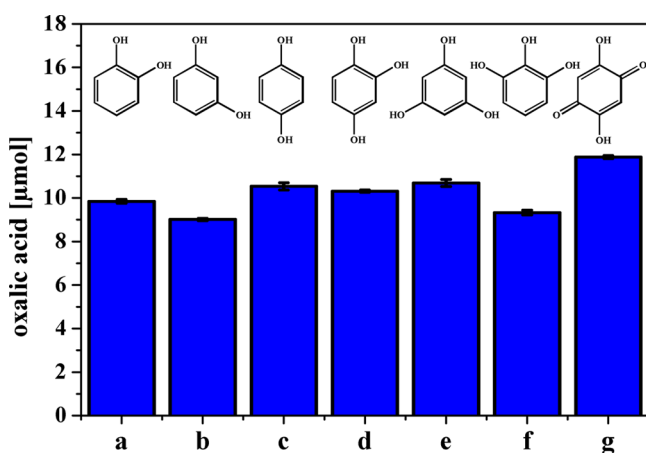
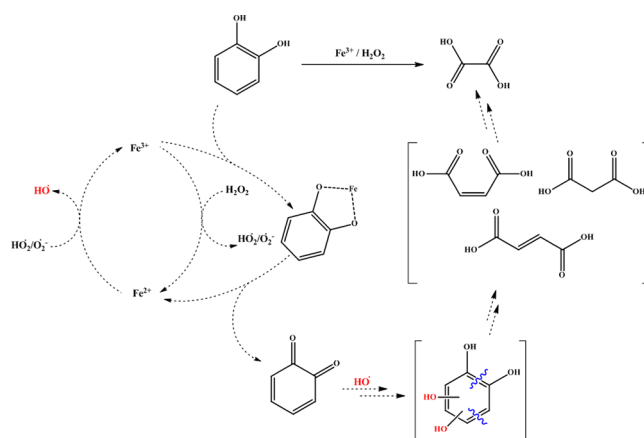


Figure 2. Reactions of different hydroxylated benzenes (0.5 mM), 1.0 mM $\text{Fe}_2(\text{SO}_4)_3$, and 7.5 mM H_2O_2 at 40 °C after 1 h: a = catechol, b = resorcinol, c = hydroquinone, d = 1,2,4-trihydroxybenzene, e = phloroglucin, f = pyrogallol, g = 2,5-dihydroxy-1,4-benzoquinone. Symbols are average data from experiments and error bars indicate standard deviations from triplicate measurements.

Therefore, a common degradation pathway can be hypothesized for these structurally related aromatic compounds. The highest oxalic acid yield was achieved with 2,5-dihydroxy-1,4-benzoquinone, the oxidation product of 1,2,4,5-tetrahydroxybenzene, indicating that a polyhydroxylated intermediate might favor higher oxalic acid yields. Thus, as already proposed by Li et al.,²⁰ the hydroxylated benzenes are partly oxidized to a further polyhydroxylated intermediate before ring cleavage. After ring-opening, the formation of several dicarboxylic acids are assumed as possible intermediates during degradation of aromatic compounds to oxalic acid, which is already well described.^{20–25} Maleic, fumaric, and malonic acid are proposed as intermediates as they were identified within the catechol degradation process at low H_2O_2 concentrations.

Scheme 1 presents the proposed degradation pathway from catechol to oxalic acid under Fenton-like conditions. In the redox cycling of iron, H_2O_2 reacts with Fe^{3+} to a hydroperoxyl radical and superoxide, and in a subsequent step, hydroxyl radicals are formed.^{42,49} Additionally, Fe^{3+} oxidizes catechol to 1,2-benzoquinone, while Fe^{3+} is reduced to Fe^{2+} . 1,2-benzoquinone is then hydroxylated to a polyhydroxylated intermediate before ring cleavage into several dicarboxylic acids

Scheme 1. Presumed Degradation Pathway of Catechol to Oxalic Acid^{20–25,42,49}



(e.g., maleic and fumaric acid), and after further oxidation and degradation steps, finally, oxalic acid is formed.

Soil Sample RW 1. The time dependent oxalic acid formation starting with the freeze-dried soil sample RW 1 is shown in Figure 3A. After 5 min, a small amount of oxalic acid was already detected, which is assumed to be already present in soil. Within the first hours, a considerable increase of oxalic acid was observed. Between 18 and 24 h, the maximum oxalic acid yield was achieved ending in a plateau. The shape of this graph is similar to the results of the time dependent catechol optimization depicted in Figure 1A. The extended time scale for the natural soil sample might be related to the complex structure of soil organic matter. For the following experiments, a reaction time of 24 h was chosen.

Sterilized Soil Samples RW 1 and RW 2. The formation of oxalic acid was observed for all sterilized soil samples, which confirmed that oxalic acid is produced under abiotic conditions (Figure 3B). Comparing all sterilized soil samples, the freeze-dried soil samples produced lowest oxalic acid yields. The different preparation and sterilization methods modify and change the physicochemical soil properties. While freeze-drying is a relatively gentle method, sterilization with dry and moist heat can transform the soil structures and might hydrolyze and oxidize natural organic compounds, which would explain the higher oxalic acid release, as oxidized SOM favors oxalic acid formation. In general, the soil sample RW 2 produced higher oxalic acid yields which is expected due to its elevated organic carbon content.

Supplementation of Fe^{3+} and H_2O_2 to the freeze-dried and sterilized soil sample RW 1 increased oxalic acid yields (Figure 3C). Highest oxalic acid yields were delivered by the experiments of the freeze-dried and irradiated soil samples, with 30 to 40 times higher oxalic acid yields than without Fe^{3+} and H_2O_2 addition. As the soil sterilization should eliminate any microbial activity and enzymes, the sterilized soil samples provided abiotic conditions. Thus, the experiments with supplementation showed that SOM reacts with Fe^{3+} and H_2O_2 and produced oxalic acid, which made an abiotic formation of oxalic acid in natural soil systems plausible. The results revealed that Fe^{3+} and H_2O_2 addition accelerate and enhance the oxalic acid formation, even if its formation in soil is already possible without supplementation.

In summary, in catechol optimizations reactions, we obtained 9.84 μmol of oxalic acid (886 μg), which corresponds to a

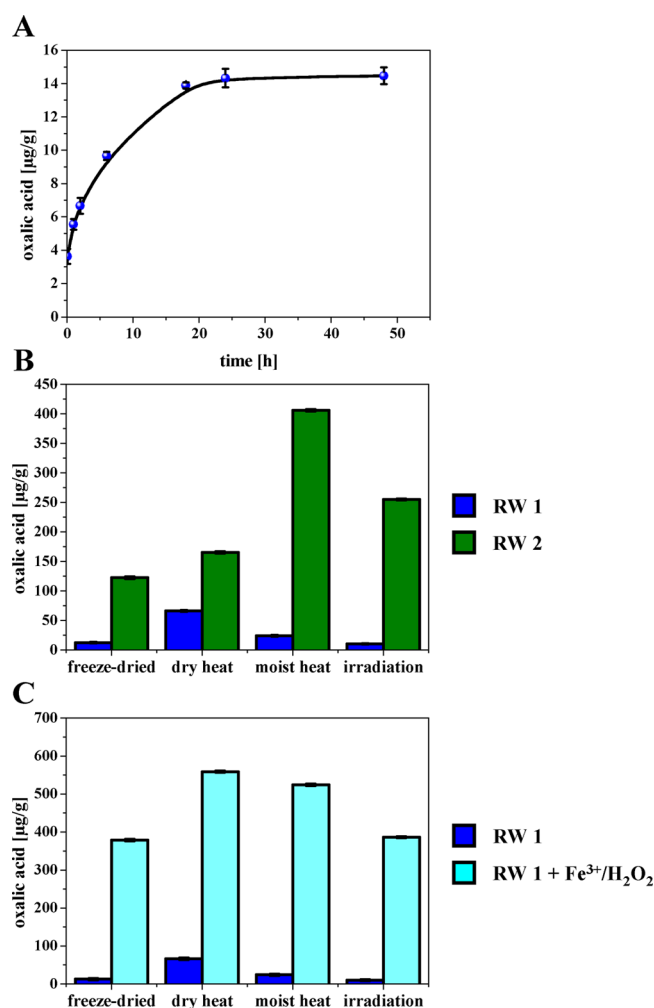


Figure 3. Time dependence and effects of $\text{Fe}_2(\text{SO}_4)_3$ and H_2O_2 on oxalic acid formation from freeze-dried and sterilized soils. (A) Time dependent oxalic acid formation from freeze-dried soil sample RW 1 at 40 °C. (B) Oxalic acid formation from freeze-dried and sterilized soil samples RW 1 and RW 2 at 40 °C after 24 h. (C) Reaction of freeze-dried and sterilized soil sample RW 1 with and without supplementation of 1.0 mM $\text{Fe}_2(\text{SO}_4)_3$ and 7.5 mM H_2O_2 at 40 °C after 24 h. Symbols are average data from experiments, and error bars indicate standard deviations from triplicate measurements.

turnover of 65.6% carbon. Fenton-like conditions with low Fe^{3+} concentrations and an excess of H_2O_2 , as well as acidic conditions, were required for optimal oxalic acid formation. The presence of chloride as radical scavenger reduced oxalic acid formation. A series with different hydroxylated benzenes, which are representatives for some structural elements of humic substances in soil, formed similar oxalic acid yields indicating a common degradation pathway of aromatic compounds to oxalic acid. Here, a polyhydroxylated benzene is assumed to be the intermediate before ring cleavage. In experiments with catechol and low H_2O_2 concentrations, a number of dicarboxylic acids (e.g., maleic acid, malonic acid) were detected. At higher H_2O_2 concentrations, only oxalic acid was formed pointing to dicarboxylic acids as further intermediates. In view of several proposed degradation pathways^{20–25,42,49} and considering our results a global degradation pathway from catechol to oxalic acid under Fenton-like conditions is presumed.

Two soil samples from the nature reserve Rotwasser were used to determine the abiotic formation of oxalic acid under

laboratory constraints. The preliminary experiments of freeze-dried soil samples including a time dependent study already pointed to an abiotic formation process. Experiments with sterilized soil samples and addition of $\text{Fe}_2(\text{SO}_4)_3$ and H_2O_2 confirmed this hypothesis and led to the conclusion that oxalic acid can be produced under abiotic reaction conditions in natural soil systems. This paper presents new insights and information to the natural abiotic degradation of soil organic matter.

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Notes

The authors declare no competing financial interest.

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