

# Abiotic catalysis of the Maillard reaction and polyphenol-Maillard humification pathways by Al, Fe and Mn oxides

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## Abstract

The Maillard reaction and integrated polyphenol-Maillard reaction are regarded as important pathways in natural humification. Little is known on the abiotic catalysis of these pathways by poorly crystalline Al and Fe(III) oxides. Therefore, the objective of this study was to compare catalysis of these humification pathways by poorly crystalline Al and Fe oxides and Mn oxide, which has been widely studied. Treatments containing an equimolar amount of glucose and glycine (Maillard reaction) or catechol, glucose and glycine (polyphenol-Maillard reaction) in the presence or absence of metal oxides, were conducted under environmentally relevant conditions, for 15 days under sterile conditions. The humification products were examined using C and Al K-edge, and Al, Fe and Mn L-edge NEXAFS spectroscopy. The redox-reactive Mn and Fe oxides enhanced humification in the Maillard and integrated catechol-Maillard systems to a greater extent than Al oxide. The Mn oxide sequestered substantially more C during humification than the other oxides, and the humic substances formed were more carboxylic in nature. Humification altered the surface chemistry of the oxides, especially the Mn oxide. The findings of this study are of fundamental importance in understanding the role of metal oxides in abiotic humification pathways and C stabilization in soils and sediments.

## Key Words

Oxidative polymerization, sesquioxides, birnessite, soft x-ray absorption spectroscopy, organo-mineral complexes

## Introduction

In the environment, humification is pivotal in transforming biomolecules originated from organized structures typical of organisms to randomly polymerized, heterogeneous humic substances characteristic of biogeochemical systems. Abundant research evidence at the molecular level shows that mineral colloids, acting as electron acceptors, can enhance the oxidative polymerization and/or polycondensation of biomolecules such as amino acids, sugars, and polyphenols, derived from the breakdown of biological residues and from biological metabolites (Huang and Hardie 2009). The polyphenol pathway and Maillard reaction are regarded as important pathways in natural humification (Huang and Hardie 2009). Furthermore the significance of linking these two pathways into an integrated polyphenol-Maillard pathway has been shown using birnessite ( $\delta$ -MnO<sub>2</sub>) as catalyst (Jokic *et al.* 2004; Hardie *et al.* 2007). Little is known on the abiotic catalysis of the Maillard reaction and especially the integrated polyphenol-Maillard reaction by poorly crystalline Al and Fe(III) oxides, which are common in nature. Sesquioxides, especially poorly crystalline Fe and Al oxides, also play a critical role in long-term carbon stabilization in soils (Kögel-Knabner *et al.* 2008). Therefore, the objective of this study was to investigate the catalysis of the Maillard reaction and integrated polyphenol-Maillard reaction by poorly crystalline Al and Fe(III) oxides, and to investigate the effect of humification reactions on C sequestration and surface chemistry of the oxides. A further aim of the study was to compare the reaction processes and products of the Al- and Fe-oxide-catalyzed Maillard and catechol-Maillard reaction systems with that of previously- investigated, Mn oxide-catalyzed systems.

## Methods and Materials

Poorly crystalline Al and Fe(III) oxides were synthesized according to the method described by Huang *et al.* (1977). Manganese(IV) oxide (birnessite) was synthesized according to the method described by McKenzie (1971). The BET specific surface areas of the Al, Fe and Mn oxide was 221.1 m<sup>2</sup>/g, 164.7 m<sup>2</sup>/g and 63 m<sup>2</sup>/g, respectively. Treatments containing an equimolar amount (0.05 mol) of glucose and glycine (Maillard reaction) or catechol, glucose and glycine (polyphenol-Maillard reaction) in the presence of 2.5 g metal oxides, and absence (control), were conducted under environmentally relevant conditions, i.e., pH 7.0 and 45° C, for a period of 15 days under sterile conditions. At the end of the reaction period the pH and Eh of the

systems was determined, and then the solution and solid phase were separated using ultracentrifugation. The extent of humification in the supernatant was measured using visible absorbance. The humic acid (HA) fraction from the supernatant and organo-mineral solid residue (SR) were isolated and characterized using C and Al K-edge and Al, Fe and Mn L-edge NEXAFS spectroscopy, on the SGM and PGM beamlines at the Canadian Light Source (CLS), Saskatoon, SK, Canada. The organic C content of the SR was determined using a dry combustion method.

## Results

The presence of the redox-reactive Mn and Fe oxides significantly enhanced browning (humification) in all of the reaction systems, most notably in the catechol-Maillard system (Table 1). The presence of Al oxide in the Maillard system actually appeared to decrease visible absorbance compared to the control system (Table 1). This is most likely due to the sorption of humic polymers on the surface of the Al oxide, as indicated by its darkening in colour and the gain in organic C after humification reactions (Table 1). The Mn oxide-catalyzed catechol-Maillard system showed the greatest accumulation of humified organic C in the solid residue (2.41 g) compared to the Al oxide (0.34 g) and Fe oxide (0.45 g) systems (Table 1). The data indicate that much of the humic substances in the Fe oxide remained suspended in the supernatant, whereas, in the Mn oxide system, the supernatant and solid phase contained considerable amounts of humic substances (Table 1). In the Al oxide system most of the humic substances were found sorbed in the solid phase as the visible absorbance of the supernatant was even lower than that of the control system (Table 1). This could be attributed to the availability of dissolved metals for coprecipitation, as well as, the relative affinity of the dissolved metals for organic functional groups of the humic polymers. The Mn oxide-catalyzed systems had the lowest redox status (Table 1) which indicates that more oxidative polymerization took place than in the other systems. The Fe oxide systems had the second lowest redox status, while the control and Al oxide systems were the highest. Even though the visible absorbance was higher at 600 nm in the Fe oxide-catalyzed catechol-Maillard system (Table 1), the redox status and C content of the solid phase indicated that the Mn oxide system was the strongest promoter of humification in the catechol-Maillard system. Thus, the order of reactivity of the oxides in both the Maillard and catechol-Maillard systems was Mn oxide > Fe oxide >> Al oxide. The stronger catalytic ability of the Mn and Fe oxides compared to the Al oxide can be attributed to the higher redox potential of the Mn and Fe oxide reaction systems compared to the Al oxide system which is not subject to redox reactions. The standard electrode potential values of the overall redox reaction of catechol oxidation by Mn(IV) oxide and Fe(III) oxide are +0.509 V and +0.071 V, resp. (Liu and Huang 2001), which also explains the order of catalytic reactivity observed in the metal oxide systems. Furthermore, the lower electronegativity of Mn (1.55) versus Fe (1.83) or Al (1.61) accelerates the formation of semiquinone free radicals from catechol (Liu and Huang 2001).

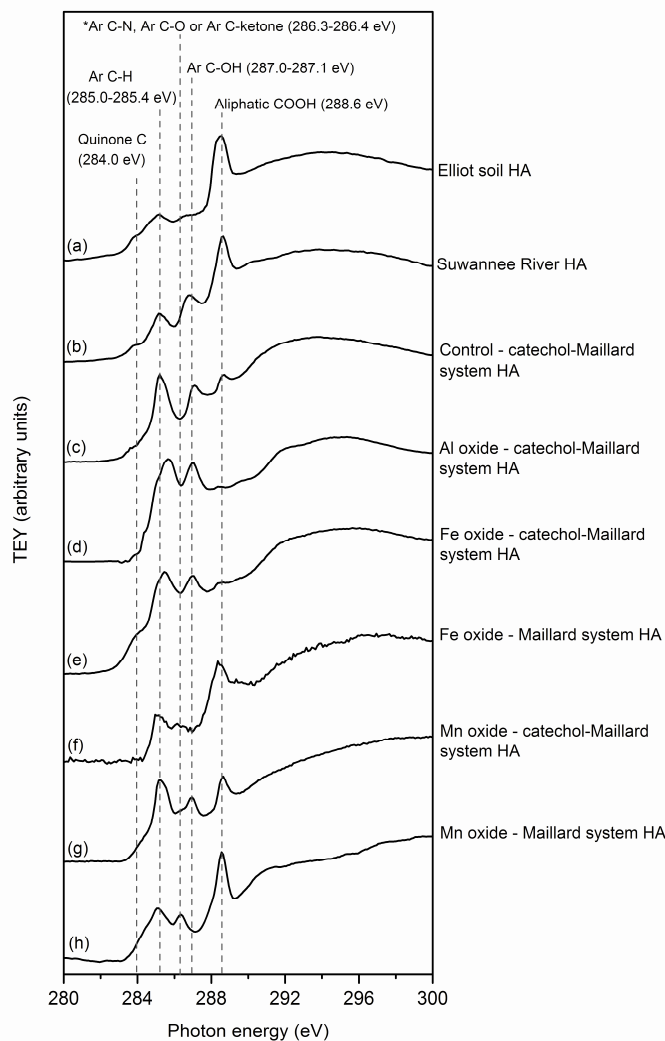
**Table 1. Visible absorbances of the supernatants (400 and 600 nm), redox status (pH + pE) and net organic C (g) gain in the solid residues in Maillard and integrated catechol-Maillard reaction systems with 2.5 g metal oxides.**

| Treatment                        | Absorbance |        | pH + pE | Gain in org. C (g) |
|----------------------------------|------------|--------|---------|--------------------|
|                                  | 400 nm     | 600 nm |         |                    |
| Al oxide Maillard                | 0.15       | 0.00   | 11.65   | 0.12               |
| Al oxide Integrated <sup>a</sup> | 6.01       | 2.10   | 8.07    | 0.34               |
| Fe oxide Maillard                | 8.28       | 0.85   | 7.19    | 0.13               |
| Fe oxide Integrated              | 86.96      | 128.43 | 7.65    | 0.45               |
| Mn oxide Maillard                | 32.61      | 3.00   | 7.07    | 0.17               |
| Mn oxide Integrated              | 100.59     | 44.21  | 6.62    | 2.41               |
| Control Maillard                 | 0.74       | 0.12   | 11.44   | -                  |
| Control Integrated               | 2.84       | 0.42   | 9.05    | -                  |

<sup>a</sup>Integrated catechol-Maillard system

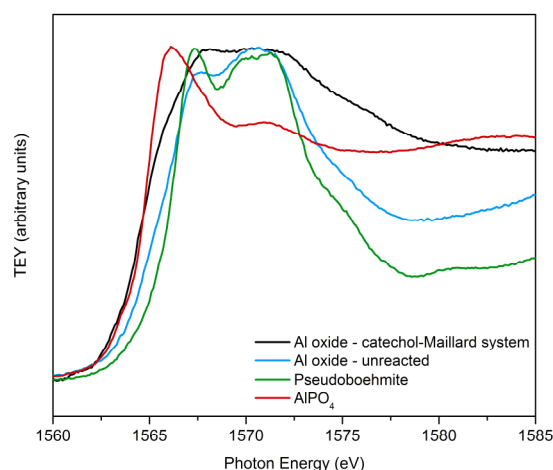
The metal oxides substantially influenced the chemical nature of the resultant humic products in the supernatant and solid residues in the Maillard reaction and integrated catechol-Maillard reaction humification pathways. The humic products (supernatant and solid phase) from the catechol-Maillard systems catalyzed by Mn oxide (Figure 1f and h) had a much higher content of aliphatic carboxylic groups (288.6 eV) than those from the control (Figure 1c) and Al and Fe oxide-catalyzed systems (Figure d and e), which were more aromatic in nature. This is largely attributed to the higher redox potential of the Mn oxide system, which implies more aggressive oxidation. The HA from the Fe oxide- and Mn oxide-catalyzed Maillard reaction systems appeared quite similar, as both were strongly aliphatic carboxylic in nature (Figure 1f and h). The solid residues from all the metal oxide-catalyzed Maillard systems were strongly aliphatic

carboxylic in nature (data not shown) in comparison the HA fractions isolated from the supernatants (Figure 1). This is attributable to the chemical partitioning of the humic substances between the solution and solid phases by the preferential coprecipitation of multivalent metals with humic substances possessing aliphatic carboxylic groups (Hardie *et al.* 2007) or to the preferential adsorption of these functional groups on the positively charged surfaces of Al or Fe oxides (Kögel-Knabner *et al.* 2008). The HA in the control system appeared to be relatively strongly aliphatic carboxylic in nature (Figure 1c). However, in contrast to the other systems, there were no metal oxides or dissolved multivalent metals in the control system, and therefore, the chemical partitioning of the humic polymers between the solution and solid phases based on their functional groups would not occur.



**Figure 1. Comparison of the effect of the presence of Al-, Fe- and Mn-oxide on the visible absorbance (400-600 nm) of the supernatant from (a) Maillard reaction and (b) the integrated catechol-Maillard system. The absorbances are scaled by the dilution factor.**

The Al K-edge (TEY) (Figure 2) and L-edge (FLY) (not shown) NEXAFS spectra of the Al oxide reacted in the catechol-Maillard system indicated that the residue had a greater content of tetrahedral-Al than the unreacted Al oxide. This indicates the effect of organic humic polymers bound to the Al (either through surface complexation or by co-precipitation with Al from solution) on Al speciation of the solid residues. There was very little change in the nature of the Al oxide reacted in the Maillard system (not shown). The Fe L-edge NEXAFS (TEY and FLY) spectra of the Fe oxides reacted in the Maillard and catechol-Maillard systems (not shown) indicate that the residues from these systems contained more Fe(II) than the unreacted Fe oxide. The Mn oxide-catalyzed Maillard system contained predominantly  $\text{MnCO}_3$  (rhodochrosite), which was confirmed by XRD (data not shown). This was attributed to the higher pH of the Mn oxide Maillard system (pH 8.0) as a result of the reductive dissolution of birnessite which is favourable for the formation of  $\text{MnCO}_3$ . The Mn oxide-catalyzed catechol-Maillard system contained only Mn(II), mainly as organically-complexed Mn(II) (not shown).



**Figure 2. Aluminum K-edge NEXAFS TEY spectra of (blue) the unreacted Al oxide catalyst, (black) the Al oxide after reaction in the integrated catechol-Maillard reaction system, and the reference compounds, (red)  $\text{AlPO}_4$  (4-coordinated), and (green) pseudoboehmite (6-coordinated).**

## Conclusions

The results show that the Al, Fe(III) and Mn(IV) oxides catalyzed humification to different extents in the Maillard and catechol-Maillard reaction systems. The Mn oxide was the strongest promoter of browning in the supernatants from the Maillard and catechol-Maillard systems. The greatest enrichment of organic C in the solid phase was found in the Mn oxide-catalyzed catechol-Maillard system (2.41 g), which was about 5 times greater than that of the Fe oxide system (0.45 g) and about 7 times greater than the Al oxide system (0.34 g). The humic products produced in the Mn oxide-catechol-Maillard system were the most aliphatic carboxylic in nature compared to the other metal oxide-catalyzed catechol-Maillard reaction systems, which is largely attributed to the higher redox potential of the Mn oxide system. The humification processes also affected the surfaces of the oxides to different extents. The Al oxide reacted in the catechol-Maillard system had a greater content of tetrahedral-Al after reaction with the biomolecules, indicating the effect of organic humic polymers bound to the Al on the Al coordination. The Fe oxide reacted in the Maillard and catechol-Maillard systems contained more Fe(II) than the unreacted Fe oxide. In contrast, the Mn(IV) oxide was found to be completely reduced to Mn(II) after reaction with the biomolecules. The order of catalytic reactivity of the oxides was as follows: Mn oxide > Fe oxide >> Al oxide. The findings of this study are of fundamental importance in understanding the role of metal oxides in abiotic humification pathways and C stabilization in soils and sediments.

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