Oxidative Coupling of Acetaminophen Mediated

by Fe³⁺-Saturated Montmorillonite

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Abstract:

The wide usage of acetaminophen as human medicine has resulted in its ubiquitous occurrence in various environmental compartments. However, the information for the transformation of acetaminophen in soil is still limited. In this study, oxidative coupling of acetaminophen in bulk solution mediated by Fe^{3+} -saturated montmorillonite was observed under different environmental conditions. In the absence of natural phenolic acids, acetaminophen could be fully eliminated from the solution within 72 h at pH 3.5, acetaminophen dimer was identified as the major reaction product. Reduction of montmorillonite associated Fe^{3+} coupled with the oxidation of acetaminophen was considered as the main mechanism for acetaminophen transformation on Fe^{3+} -saturated montmorillonite. The clay associated Fe^{3+} showed higher reactivity than Fe^{3+} in solution due to the strong complexation between surface Fe^{3+} and acetaminophen. The cross-coupling reaction between acetaminophen and phenolic acids was also observed when phenolic acids were present in the system. While with the increase of phenolic acid concentration, the competition for the reactive sites between acetaminophen and phenolic acids significantly suppressed acetaminophen removal. These results demonstrated the importance of transition metal saturated clay minerals for the abiotic transformation of anthropogenic micropollutants.

Key words: Acetaminophen; Fe^{3+} -saturated montmorillonite; Oxidative coupling; Phenolic acid.

1. Introduction

Pharmaceuticals have been widely used not only for human but also for animals. However, due to the abuse of these chemicals, a large amount of pharmaceuticals have been released into the environment and caused severe adverse ecological effects (Carlsson et al., 2006). Acetaminophen is an antipyretic and analgesic ingredient used in many over-the-counter and prescription drugs with the global production of more than 100,000 tons per year (Anderson, 2008; Joncour et al., 2014; Zhao and Pickering, 2011). Moreover, acetaminophen cannot be completely metabolized by human body and has high water solubility (Amidon et al., 1995), which renders a considerable portion of acetaminophen entering into municipal wastewater treatment plant, and finally transporting to various environmental compartments (Kim et al., 2007). As a result, acetaminophen has been frequently detected in surface water, groundwater and soils with the concentrations ranging from ppt to ppm levels (Bedner and MacCrehan, 2006). Some prior studies demonstrated that the environmental residues of acetaminophen might interfere with normal embryonic development, survival and endocrine systems of aquatic organisms (Kim et al., 2007). Once released into the soil, acetaminophen would go through various abiotic/biotic processes which could significantly affect its bioavailability and toxicity (La Farre et al., 2008; Wu et al., 2012).

Clay minerals are important soil components with the unique sequestering and catalytic properties for many organic contaminants (Gao and Pedersen, 2005; Kowalska et al., 1994; Wilson, 1999). Montmorillonite, a 2:1 layered aluminosilicate mineral, is one of the most common clay minerals, which is widely distributed in soils, subsoils, and sediments with strong swelling capacity, large surface area and high cation exchange capacity (CEC) (Beermann and Brockamp, 2005). Prior researches have indicated that when the interlayer cations of

montmorillonite are exchanged with some transition metal ions (e.g., Cu^{2+} and Fe^{3+}), it can catalyze the degradation or polymerization of aromatic compounds (Gu et al., 2008; Jia et al., 2014; Joseph-Ezra et al., 2014). The mechanism for the reaction catalyzed by transition metal saturated montmorillonite involves a single electron transfer from aromatic compounds to the interlayer cations, resulting in the formation of organic radicals that are highly susceptible to undergo further polymerization or degradation reaction (Boyd and Mortland, 1986). However, only a few studies have investigated the interaction between pharmaceuticals and Fe^{3+} -montmorillonite. Liyanapatirana *et al.* (2009) observed that under relatively dehydrated condition (40% relative humidity), Fe^{3+} -montmorillonite can oxidize triclosan to produce several polymers with different molecule weights. So far, most of the prior studies on the transformation or polymerization reactions catalyzed by Fe^{3+} -montmorillonite were conducted under relatively low moisture conditions, it is still not clear whether the similar reaction can also occur in aqueous solution with pharmaceuticals. Furthermore, as the ubiquitous substance in the environment, phenolic acids are involved in various oxidative coupling reactions (Haider and Martin, 1967; Weber and Huang, 2003). The polymerization of phenolic compounds to form humic substances catalyzed by clay minerals has been extensively studied (Shindo and Huang, 1982, 1984). Therefore, the presence of phenolic acids might substantially affect the fate of acetaminophen in soil. However, to the best of our knowledge, there were still few studies conducted to investigate the influence of phenolic acids on the transformation of pharmaceuticals on clay mineral surface.

In this study, Fe^{3+} -saturated montmorillonite was prepared and mixed with acetaminophen in aqueous solution, the transformation kinetic, degradation mechanism and reaction pathway of acetaminophen in the presence of two representative natural phenolic acids

were systematically investigated. Our results indicated that the high catalytic activity of Fe^{3+} -montmorillonite for oxidative coupling of acetaminophen was attributed to the associated Fe^{3+} . The cross-coupling reaction between acetaminophen and ubiquitous phenolic acids leads to the formation of oligomers, and the competition for the reactive sites between acetaminophen and phenolic acids was also observed. The results from current study demonstrated the importance of clay minerals on the abiotic transformation of anthropogenic micropollutants.

2. Materials and method

2.1 Chemicals

Acetaminophen, ferulic acid (FA) and *p*-coumaric acid (PCA) were purchased from Sigma-Aldrich with purity > 98%. Selected physiochemical properties of these compounds are provided in the Supporting Information (Table S1). The stock solution of acetaminophen (1.0 mM) and phenolic acids (2.0 mM) were prepared in Milli-Q water and stored at 4°C for further use. The montmorillonite used to prepare Fe^{3+} -saturated clay mineral was obtained from Feng Hong Inc. (Zhejiang Province, China), and the CEC of this montmorillonite was 770 mmol kg^{-1} measured by the ammonium acetate method at pH 7.0 (Sparks, 1996). The major chemical compositions of this montmorillonite were analyzed by X-ray fluorescence spectrometer (ARL-9800, Switzerland) and listed in supplementary Table S2.

2.2 Preparation of Fe^{3+} or Na^{+} -saturated montmorillonite

The Fe^{3+} or Na^{+} -saturated montmorillonite was prepared following the methods of Arroyo *et al.* (2005). Briefly, the montmorillonite was initially suspended in Milli-Q water, and the pH of the suspension was adjusted to 6.8 with 0.5 M sodium acetate buffer (pH 5.0) to

remove carbonate impurities. Clay-sized particles ($< 2 \mu\text{m}$) were collected by centrifugation for 15 min at 3295 g , and then resuspended in 0.1 M FeCl_3 or 0.5 M NaCl solution for 8 h to exchange interlayer cations. This step was repeated 6 times to fully saturate the exchangeable sites of montmorillonite by Fe^{3+} or Na^+ . The newly obtained clay mineral was repeatedly washed using Milli-Q water until Cl^- was fully removed as indicated by the negative test with AgNO_3 , then freeze-dried and grounded to pass a 100-mesh nylon sieve, finally stored in the desiccator for experimental use. The iron contents in the montmorillonite before and after Fe^{3+} saturation were measured using an atomic absorption spectrophotometer after digestion with the mixture of HF , HNO_3 and HClO_4 at 300 $^\circ\text{C}$ for 1.5 h. The measured iron contents in the original montmorillonite and Fe^{3+} -montmorillonite were 0.76% and 3.63%, which represented the structural iron and the total iron including structural iron and exchangeable iron, respectively. Hence, the exchangeable iron content can be calculated as $\sim 2.87\%$. To compare the reactivity of Fe^{3+} associated with different clay mineral, another common 2:1 type of non-swelling clay mineral (illite) was also prepared by the same procedure. Different from Fe^{3+} -montmorillonite, due to its higher surface charge density than montmorillonite, most of the structural charges originated from isomorphic substitution in illite are compensated by fixed K^+ , which cannot be replaced by the added Fe^{3+} , so exchanged Fe^{3+} ion on Fe^{3+} -illite are mainly located on external surfaces .

2.3 Degradation of acetaminophen catalyzed by Fe^{3+} -montmorillonite

To initiate the degradation reaction, 100 μL of 1.0 mM acetaminophen stock solution was mixed with 1.9 mL clay suspension containing 20 mg montmorillonite in a 8 mL-glass vial with the initial acetaminophen concentration of 50 μM . The pH of the clay suspension was pre-adjusted to 3.5, 5.0, 7.0, and 9.0, respectively using 0.1 M NaOH and HCl . The preliminary

experiments showed that the pH variation during the reaction was within ± 0.2 (Figure S1), and the greatest acetaminophen transformation was observed at pH 3.5, so all the remaining experiments were conducted at pH 3.5. To investigate the effect of natural phenolic acids on the transformation of acetaminophen, FA or PCA was added into the reaction system to obtain FA concentrations of 5, 50, 100, 500 and 1000 μM and PCA concentration of 100 μM , respectively. Acetaminophen transformations mediated by Fe^{3+} -montmorillonite (10.0 g L^{-1}), Fe^{3+} -illite (33.3 g L^{-1}), and FeCl_3 (0.42 g L^{-1}) were also conducted to compare the oxidation efficiency of different iron species, the Fe^{3+} amount in three systems was kept the same of 2.57 mM. All the reaction vials were covered with aluminum foil to avoid light interference. The experiment was conducted on a horizontal shaker (250 rpm) at 25 ± 0.5 °C. At given time intervals, three vials were sacrificed and centrifuged at 1250 g for 20 min. The supernatants were pipetted into autosampler vials for acetaminophen measurement. After decanting the supernatant, the clay paste in each vial was extracted with 1 mL methanol for 2 h (Polubesova et al., 2010), and centrifuged at 1250 g for 20 min. The acetaminophen concentrations in the supernatant and methanol extract were determined by a high performance liquid chromatography (HPLC) system, whereas the transformation products were analyzed using an Agilent G6410B triple quad mass spectrometry (MS/MS) with infusion injection. Similar experiment was also conducted under anaerobic condition with all reaction solution deoxygenated by continuously purging with N_2 ($\geq 99.999\%$) for 1 h. Sample preparation and degradation experiments were all conducted in an anaerobic chamber. At the given time intervals, samples were taken from the anaerobic chamber and processed following the same method as described above.

The amounts of ferrous iron produced during the reaction of Fe^{3+} -montmorillonite and acetaminophen were measured according to method developed by Sørensen *et al.* (1982) with

some modification. Briefly, 500 μL of reaction suspension was immediately mixed with 500 μL ferrozine solution (100 mM). After shaken for 1 h, the suspension was filtered through a 0.22 μm -filter membrane, and then the absorbance of the filtrate at 562 nm was measured to obtain the concentration of ferrozine-complexed Fe^{2+} using a SpectraMax M2 Microplate Reader. Basal spacing of Fe^{3+} -montmorillonite before and after it was dispersed in solution were determined by X-ray powder diffraction (XRD) analysis (X'TRA, ARL, Switzerland). Specifically, 0.1 g original Fe^{3+} -montmorillonite was suspended in 1 mL Milli-Q water, after shaken vigorously for 30 seconds, 100 μL clay slurry was immediately pipetted on a glass slide. For the measurement of swelled clay, 100 μL of clay suspension that went through 72 h reaction with acetaminophen was used. After clay suspension was adhered on glass slide, XRD analysis was carried out with the graphite monochromated $\text{CuK}\alpha$ radiation ($\lambda=1.5406 \text{ \AA}$). The X-ray generator was operated at 40 kV and 40 mA, and the diffraction patterns were collected between 4° and 30° at a scanning rate of 1° min^{-1} . The dielectric properties of Fe^{3+} -montmorillonite suspension and FeCl_3 solution were measured by the method of He and Zhao (2005) to compare their different catalytic abilities for acetaminophen transformation. The measurement was carried out using 4191A radio-frequency impedance analyzer (Hewlett-Packard) with the frequency range of 1-500 MHz. A dielectric measurement cell with concentric cylindrical platinum electrodes was used. The effective area and the distance between the electrodes were 78.5 mm^2 and 8 mm, respectively. The volume for Fe^{3+} -montmorillonite suspension and FeCl_3 solution was 1 mL to submerge the electrodes. The dielectric permittivity and conductivity were obtained by calculation of measured capacitance and conductance at different frequency and then the errors of the experimental data were corrected following the Schwan's method (Schwan, 1963). All the measurements were carried out at room temperature ($25 \pm 1^\circ\text{C}$).

2.4 Analytical Procedures

The concentrations of acetaminophen and phenolic acids were determined by a Waters HPLC (Waters Alliance 2695, Milford, MA) equipped with a photodiode array detector. The separation was performed on a C₁₈ column (Inertsil ODS-SP, 5 μ m, 4.6 $\times$ 150 mm, GL Sciences Inc., Japan) at 35 $^{\circ}$ C. The mobile phase was a mixture of water (containing 0.2% formic acid) and acetonitrile at 85:15 (v/v) with the flow rate of 1.0 mL min⁻¹. The detection wavelengths were set at 250 nm for acetaminophen, 310 nm for FA and 315 nm for PCA, respectively.

To identify the reaction products, the same samples (both supernatant and extract) were also analyzed by triple quad mass spectrometry (MS/MS). The mass spectrometer was operated in positive electrospray ionization mode (ESI+). Before the analysis, the samples were concentrated to 100 μ L by evaporation under a nitrogen gas flow. The capillary and fragmentor potentials were set at 3.5 kV and 130 V, respectively. Nitrogen (purity $\geq$ 99.999%) was used as desolvation gas at the flow rate of 10 mL min⁻¹. The desolvation temperature and nebulizer pressure were set at 350 $^{\circ}$ C and 40 psi, respectively. The mass spectrometer was first operated at the full scan mode with the mass-to-charge ratio (m/z) from 50 to 1000. Once a possible product was identified, secondary MS spectra were then obtained for selected ions using collision induced dissociation. Nitrogen ($\geq$ 99.999%) was used as the collision gas.

3. Results and discussion

3.1 Transformation of acetaminophen in the presence of Fe³⁺-montmorillonite

No significant loss of acetaminophen was observed in Na⁺-montmorillonite system or pure acetaminophen aqueous solution in the pH range from pH 3.5 to 9 during the experimental 72 h (Figure S2). However, in the presence of Fe³⁺-montmorillonite, acetaminophen rapidly

dissipated in the reaction solution especially under acidic conditions and the removal of acetaminophen showed a strong pH-dependent behavior (Figure 1). As the solution pH increased, the removal of acetaminophen decreased. For instance, after 72 h reaction, ~100% and 88% of acetaminophen was removed at pH 3.5 and 5.5, respectively. However, only 13% and 23% of acetaminophen was removed at pH 7.0 and 9.0. To differentiate the adsorption and transformation processes, the adsorption of acetaminophen by Fe^{3+} -montmorillonite was measured by addition of methanol to extract acetaminophen from clay particle. Results showed that there is nearly no adsorption occurred at pH 3.5 and pH 5.0, while ~2% and 12% adsorption was observed at pH 7.0 and pH 9.0, respectively (Figure S3). These results indicated that acetaminophen transformation substantially determines the acetaminophen removal at low pH, i.e., 3.5 and 5.0. Negligible adsorption of acetaminophen on Fe^{3+} -montmorillonite at low pH was also presented by other studies (Liu et al., 2010; Lorphensri et al., 2006). Due to the high pKa value ($\text{pK}_a=9.7$) (Hałka et al., 2010), acetaminophen molecule exists as a neutral species in the pH range studied in this research. Furthermore, even acetaminophen is in neutral form, the oxygens in amide and hydroxyl groups could bear partial negative charge, thus, decreasing the adsorption on clay mineral. The increased adsorption at higher pH may be explained by the interaction between acetaminophen and iron hydroxide precipitation produced by the hydrolysis of Fe^{3+} as the increase of pH, similar result was observed by Stefansson (2007).

The calculated reaction rate of acetaminophen at pH 3.5 and 5.0 were 0.047 and 0.028 h^{-1} , respectively. At neutral or alkaline conditions, the reaction significantly decreased. The decrease of acetaminophen transformation with the increase of pH could be explained by the change of iron species in Fe^{3+} -montmorillonite system. With the increase of pH, Fe^{3+} would gradually hydrolyze to form FeOH^{2+} , $\text{Fe}(\text{OH})_2^+$ and finally insoluble $\text{Fe}(\text{OH})_3$ (Millero et al., 1995).

Among them, Fe^{3+} and FeOH^{2+} have much higher oxidation potential than $\text{Fe}(\text{OH})_2^+$ and $\text{Fe}(\text{OH})_3$, thus the highest acetaminophen removal was found at pH 3.5. In addition, as the pH further increases to neutral and alkaline condition, most of Fe^{3+} is converted to $\text{Fe}(\text{OH})_3$ precipitate (Deiana et al., 1995), which can reduce the vacant coordination sites of Fe^{3+} for interaction with acetaminophen. Therefore, it correspond to the lower catalytic capacity of Fe^{3+} -montmorillonite at higher pH (Aqil and Schmitt, 2010). Similar to our results, Polubesova *et al.* (2010) also observed that the adsorption and transformation of phenolic acids by Fe^{3+} -montmorillonite were significantly suppressed with the increase of pH. Yong *et al.* (1997) demonstrated that as the solution pH increased from 4.5 to 7.5, the ability of Fe^{3+} -montmorillonite to oxidize 2,6-dimethylphenol and 2-methylphenol decreased. The data presented above lead to the conclusion that Fe^{3+} -montmorillonite can degrade acetaminophen in acidic conditions (Figure 1). Similar result was also observed by Qin *et al.* (2014), in their study, 17 β -estradiol could quickly react with Fe^{3+} -montmorillonite suspension. They proposed that electron transfer from 17 β -estradiol to interlayer Fe^{3+} results in the formation of highly water insoluble oligomers. It has been well documented that as a swelling clay mineral, the interlayer of montmorillonite would expand in aqueous solution so that water molecule can diffuse into the interlayer of montmorillonite (Chappell et al., 2005), which in turn allows acetaminophen molecules to move into the interlayer space and react with Fe^{3+} . In fact, our XRD result confirms that the basal spacing of prepared Fe^{3+} -montmorillonite increased from 12.5 Å to 15.7 Å after it was dispersed in solution compared to the dried sample (Figure S4).

As acetaminophen molecule consists of a benzene ring with one hydroxyl group and one amide group in a *para* (1, 4) pattern. The lone pair electrons on the hydroxyl and carboxyl oxygen, acetamido nitrogen and the benzene ring render acetaminophen as a conjugated

molecule (Lu et al., 2009), which is generally susceptible to be oxidized by losing one electron to form a radical cation. Therefore, it is expected that the electron transfer reaction between acetaminophen and Fe^{3+} can oxidize acetaminophen to form a radical and subsequently forming polymers through a self-coupling process. By analyzing the transformation products in the supernatant and on Fe^{3+} -montmorillonite surface, only one new reaction product in the supernatant was identified. The information of the molecular weight and the mass fragmentation pattern indicates that this transformation product corresponds to acetaminophen dimer with m/z of 301, the detailed discussion about this product will be presented below. Since there is no authentic standard commercially available, the amount of acetaminophen dimer formed is represented by the integrated peak area, which increases over the reaction time (Figure 2).

To compare the reactivity of Fe^{3+} associated with different clay minerals or in aqueous bulk solution, Fe^{3+} -montmorillonite (ferric ions exist in both external surface and interlayer space), Fe^{3+} saturated illite (ferric ions are only distributed on the external surface) (Jia et al., 2011), and FeCl_3 solution were mixed with acetaminophen solution, respectively. The amounts of exchangeable iron on both montmorillonite and illite or the total iron content in FeCl_3 solution were kept the same according to the CEC of the two clay minerals (i.e., 770 and 231 mmol kg^{-1} for montmorillonite and illite, respectively). Our control experiments showed that after 72 h, there is almost no decrease of acetaminophen in the presence of Na^+ -montmorillonite and Na^+ -illite at pH 3.5, demonstrating that clay associated Fe^{3+} is responsible for the degradation reaction (Figure 2). As shown in Figure 2, there is no significant difference ($p > 0.05$) for the degradation of acetaminophen on Fe^{3+} -illite and Fe^{3+} -montmorillonite, indicating that there are similar reactivity for Fe^{3+} associated with montmorillonite (including both interlayer and external surface adsorbed Fe^{3+}) and external surface adsorbed Fe^{3+} on illite. Moreover, when FeCl_3

solution was mixed with acetaminophen, similar transformation process was also observed. However, even at the same iron content, the degradation rate of acetaminophen in FeCl_3 solution is much lower than that in the presence of Fe^{3+} saturated clay mineral (Table S3). After 72 h, only 74.4% of acetaminophen can be degraded in FeCl_3 solution. Similar result has been reported by Klausen *et al.* (1995), they found that Fe^{2+} in aqueous solutions was unreactive, while in the presence of clay minerals (e.g., magnetite, goethite, and lepidocrocite), Fe^{2+} can readily reduce nitrobenzenes to the corresponding aniline products. The elevated reactivity of clay bound Fe^{3+} in this study might be explained by the different properties of water molecules in homogeneous bulk solution versus on heterogeneous clay surface. Previous studies had demonstrated that interlayer water in crystalline mineral or adsorbed water on mineral surface is quite different from water molecule in bulk solution (Aqil and Schmitt, 2010; Hueckel, 1992; Martin, 1960). Thoenen (2001) showed that the dielectric constant ϵ of water adsorbed on clay mineral is lower than that of bulk water, which may induce stronger electrostatic interactions between the exchangeable cations and the negative charges on clay mineral surface. Hynes and Coinceanainn (2004) also suggested that in bulk solution, the electron transfer between organic compound and Fe^{3+} in solution is through an outer-sphere mechanism, while on the clay surface, with lower dielectric permittivity for the adsorbed water, inner-sphere complexes would become dominant for the interaction (Polubesova *et al.*, 2010). In this study, we measured the frequency-dependent dielectric permittivity of FeCl_3 solution and Fe^{3+} -montmorillonite system at the same Fe^{3+} content, it clearly shows that the dielectric constant of the bulk solution (FeCl_3 solution) is indeed higher than that of water adsorbed on clay mineral surfaces (Figure S5). The higher ϵ suggests that the bulk water has greater ability to shield charges, which might diminish the

interaction between acetaminophen molecule and Fe^{3+} in bulk solution, suggesting that the iron on clay surface is more reactive.

For comparison, although there are also some structural irons in the lattice of Na^+ -saturated montmorillonite, no significant transformation of acetaminophen on Na^+ -montmorillonite was observed. Therefore, our results manifest that the structural iron might have no or less capacity to oxidize acetaminophen. The montmorillonite used in this study is a *cis*-vacant type smectite (Lantenois et al., 2008), the relatively low structural iron content (only ~0.76 wt%) and the inertness of structural iron collectively induce the weak catalytic activity for Na^+ -montmorillonite (Drits and Manceau, 2000; Neumann et al., 2011).

To study the effect of oxygen on the degradation process, the acetaminophen transformation mediated by Fe^{3+} -montmorillonite was also investigated under anaerobic condition. Our results showed that in the first 4 h, the removal of acetaminophen is nearly identical for both aerobic and anaerobic conditions, while as the reaction proceeds, the degradation of acetaminophen without oxygen almost stops, on the contrary, the aerobic reaction continues until complete dissipation (Figure 3). The measured Fe^{2+} production as the reaction proceeded also followed the similar trend. As the shown in Figure 3, Fe^{2+} concentrations in both aerobic and anaerobic systems continuously increased within the first 4 h, while as the reaction continued, Fe^{2+} generation almost suspended in anaerobic system compared to the continuous increase of Fe^{2+} in aerobic condition. According to these observations, we hypothesize that the role of oxygen in the reaction is to reoxidize Fe^{2+} , which is reduced by acetaminophen, to regenerate the reactive clay associated Fe^{3+} . Therefore, the presence of oxygen is necessary to sustain the degradation of acetaminophen, and the reaction would stop once all reactive Fe^{3+} is depleted under anaerobic condition.

Based on our results, the amount of Fe^{3+} is much higher than acetaminophen, indicating that most of the iron is not reactive. It may be explained that montmorillonite associated Fe^{3+} could form clusters, e.g. $[\text{Fe}(\text{OH})_{1-4}]_n^{-1-2+}$ or amorphous iron oxyhydroxides (Jia et al., 2011; Manjanna et al., 2009; Tzou, 1983), which may account for the reduction of the reactivity. On the other hand, when Fe^{3+} -montmorillonite was prepared, the high charge of Fe^{3+} can cause the flocculation of clay minerals (Goldberg and Glaubig, 1987; Manjanna et al., 2009), thereby decreasing the surface area. However, it is still difficult to quantitatively determine the extent to which the Fe^{3+} is deactivated and how the inactive sites become active again. Further studies may be needed to measure the reactive sites on Fe^{3+} -montmorillonite using appropriate probe molecules.

3.2 Effect of natural phenolic acids on the transformation of acetaminophen by Fe^{3+} -montmorillonite

The effect of two representative phenolic acids, i.e., FA and PCA on acetaminophen transformation was investigated (Figure 4). The presence of FA (100 μM) inhibited acetaminophen transformation, after 72 h, the transformation percentage of acetaminophen decreased to 94.7% compared to complete degradation of acetaminophen in phenolic acids-free system. However, for PCA, at the same concentration (100 μM), it did not show any significant effect on acetaminophen removal. Meanwhile, the continuous decrease of PCA and FA concentrations was also observed during the reaction, which indicates that FA and PCA themselves could also react with Fe^{3+} -montmorillonite. Polymerization of FA and PCA to form oligomers on the surface of Fe^{3+} -montmorillonite has been previously reported (Polubesova et al., 2010). The different reaction rates of the two phenolic acids on Fe^{3+} -montmorillonite might explain the discrepant effect on acetaminophen transformation (Figure 4). By comparing the

reaction rate constants of acetaminophen, FA and PCA on Fe^{3+} -montmorillonite, we found that FA has the highest tendency to react with Fe^{3+} -clay, followed by acetaminophen and then PCA. The corresponding reaction rate constants for FA, acetaminophen, and PCA are 0.058, 0.047, and 0.001 h^{-1} , respectively (Table S3). Similar result was also obtained by Polubesova *et al.* (2010), who found that FA exhibits much higher reactivity with Fe^{3+} -montmorillonite than other phenolic acids with similar structures. The different effect from FA and PCA could be explained by the competition between the two phenolic acids and acetaminophen for the reaction sites on Fe^{3+} -montmorillonite. Since FA has a higher reaction rate constant than acetaminophen to interact with Fe^{3+} -montmorillonite, the reaction between FA and Fe^{3+} -montmorillonite could partially interfere with the reaction of acetaminophen molecules on clay mineral, thus reducing the transformation of acetaminophen. While for PCA, the suppression effect is limited as acetaminophen has higher reaction rate with clay than PCA. The reason for this discrepant effect from the two phenolic acids can be explained by the difference of their molecular structures. Compared to PCA, FA has one additional methoxy substitution, this group is considered as a ring activator since it can repel electrons and increase the electron density in the ring, thereby increasing the susceptibility of the ring to be oxidized (Morrison and Boyd, 1992), which is consistent with the results from Lehmann *et al.* (1987).

To further verify the competition between FA and acetaminophen for the reaction sites, different initial concentrations of FA were used to explore the effect on acetaminophen transformation. As shown in Figure 5, the acetaminophen transformation and acetaminophen dimer formation continually decrease as the increase of FA concentrations. The reaction rate constants of acetaminophen are 0.047, 0.042, 0.031, 0.023, 0.009 and 0.006 h^{-1} when the concentration of FA increases from 0 to 5, 50, 100, 500 and 1000 μM , respectively. While for

FA, with the concentration increases, the reaction rates firstly decrease from 0.056 h⁻¹ at 5 μM to 0.032 h⁻¹ at 50 μM, and then barely change even at the highest concentration of FA (0.039, 0.039 and 0.033 h⁻¹ at 100, 500 and 1000 μM, respectively) (Table S4). The different trends for the reaction rates of acetaminophen and FA further confirm that with the increase of FA concentrations, more reaction sites on Fe³⁺-montmorillonite would be occupied by FA, which would reduce the probability of acetaminophen to interact with Fe³⁺-montmorillonite. Our experimental observation is also consistent with the polymerization of acetaminophen by laccase, the presence of natural organic matter strongly suppressed the self-coupling of acetaminophen due to the competitive reaction between natural organic matter (NOM) and laccase (Lu and Huang, 2009).

3.3 Identification of reaction products and proposed transformation pathway of acetaminophen on Fe³⁺-montmorillonite

The transformation products of acetaminophen by Fe³⁺-montmorillonite in absence or presence of phenolic acids were shown in Figure 6 and 7. Only one product with m/z of 301 was detected for the reaction of acetaminophen with Fe³⁺-montmorillonite in the absence of phenolic acids. This reaction product corresponds to the self-coupling of two acetaminophen molecules, which is also consistent with previous studies on the acetaminophen transformation in other oxidative processes (Lu and Huang, 2009; Lu et al., 2009). Fe³⁺-montmorillonite mediates one-electron oxidation of the substrate to generate a radical, which then couples with another acetaminophen radical to form the dimer. However, in the presence of FA, a new peak (product 2) was identified in HPLC chromatogram (Figure 6A), which is not shown in the FA-free samples. Furthermore, the peak area of product 2 also increases as the reaction proceeds (Figure 6B). A reasonable hypothesis is that this peak likely corresponds to the cross-coupling product from the

reaction between FA and acetaminophen. Previous studies showed that cross-coupling reactions could occur between two different active substrates through radical-radical binding, nucleophilic addition, or between an active substrate and an inert chemical via hydrogen abstraction or free-radical addition (Chen et al., 2015; Weber and Huang, 2003). Since both FA and acetaminophen could react with Fe^{3+} -montmorillonite, it is expected that cross-coupling reaction would occur in the system containing the mixture of FA, acetaminophen and Fe^{3+} -montmorillonite. With the molar ratio of FA to acetaminophen increases from 0.1 to 20, the formation of product 2 initially increases to the maximum, then decreases with further increase of FA concentrations. At low concentration of FA (corresponding to the lower ratio of FA to acetaminophen), the amount of the formed FA active radical in the reaction system is relatively low, thus the reaction between FA and acetaminophen radicals to form product 2 appears to be limited. As the concentration of FA increases, more FA radical can be formed, which may favor the cross-coupling reaction between FA and acetaminophen and the generation of product 2. It is also expected that higher degree of cross-coupling products can be formed in the system when high concentrations of FA and acetaminophen radicals are present. Similar results were observed by Colosi *et al.* (2007), in their study, 2,2'-dichlorobiphenyl (PCB-4) was removed from solution via peroxidase-mediated NOM cross-coupling reaction. The PCB-4 removal rate reached a maximum, and then decreased with the increase of NOM concentrations. The increase of cross-coupling degree between NOM self-coupling products and PCB-4 was considered to account for the observed PCB-4 removal pattern (Colosi et al., 2007).

In order to further verify our hypothesis, the transformation products of acetaminophen after 72 h were concentrated using solid phase extraction and analyzed by MS in positive model mode (pseudo-molecular ion MH^+), a series of compounds were identified in the reaction system

containing acetaminophen, Fe^{3+} -montmorillonite and FA, and the mass spectra of these products are presented in Figure 7. The peak with m/z value of 152 was considered as the acetaminophen residue (molecular mass 151) in the system, while another peak with m/z 195 was assigned to FA (molecular mass 194). In addition to these two reactants, other peaks were also detected and these products can be explained by the self-coupling reaction between acetaminophen molecules or cross-coupling reaction between acetaminophen and FA. For example, the reaction products with the molecule weights of 301, 344 and 688 correspond to self-coupling of two acetaminophen ($151+151-1$), cross-coupling between acetaminophen and FA ($151+194-1$), and cross-coupling of two acetaminophen and two FA ($2\times 151+2\times 194-2$), respectively. The presence of high molecule weight product (i.e., 688) indicates that higher level of cross-coupling between FA and acetaminophen might also occur. To elucidate the secondary fragmentation patterns of the transformation products, all of these compounds were analyzed by the secondary MS, and the mass spectra are given in Figure 7B-D. The secondary mass spectra of the transformation products are consistent with the possible fragmentation pattern of each product, further supporting our assignments.

Overall, the reaction between acetaminophen and Fe^{3+} -montmorillonite can be described that one electron is transferred from acetaminophen to ferric ion associated with the external surface or the interlayer of clay mineral. As the result, Fe^{3+} is reduced to Fe^{2+} , and an acetaminophen radical is produced. Furthermore, Fe^{2+} can be reoxidized to Fe^{3+} by oxygen to allow the reaction to proceed. The formed acetaminophen radical can couple with each other to generate acetaminophen dimer. When phenolic acids are present in the system, e.g. FA, these phenolic compounds can follow the similar reaction to produce phenolic radicals, the reaction

between different radicals would lead to the formation of cross-coupling products as observed in our study.

4. Environmental implications

In this study, our results demonstrate that a widely used pharmaceutical, acetaminophen can undergo polymerization reaction mediated by a natural clay mineral. The Fe^{3+} associated with montmorillonite showed high reactivity to oxidize acetaminophen through a single electron transfer reaction. Moreover, the presence of ubiquitous phenol acids could also strongly affect this process by competing reaction sites or forming cross-coupling products with these anthropogenic micropollutants. Due to the much lower aqueous solubility of the polymerization products compared to the parent compounds, this process may be considered as a natural detoxification reaction and a potential degradation route for acetaminophen and other organic pollutants in the soil environment.

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Figure Legends

Figure 1. pH-independent acetaminophen removal kinetics mediated via Fe^{3+} -saturated montmorillonite.

Figure 2. Acetaminophen transformation kinetics in different reaction systems containing Fe^{3+} -montmorillonite, Fe^{3+} -illite, and FeCl_3 individually.

Figure 3. Anaerobic and aerobic transformation of acetaminophen with Fe^{3+} -montmorillonite in solution at pH 3.5 and the produced Fe^{2+} during the reaction process.

Figure 4. Effects of ferulic acid (FA) and *p*-coumaric acid (PCA) on acetaminophen transformation catalyzed by Fe^{3+} -montmorillonite. Acetaminophen, FA and PCA in legend mean the concentration of each compound after they were reacted with Fe^{3+} -montmorillonite individually, and remaining legends mean the remaining concentration of target compound in the system containing acetaminophen, phenolic acid and Fe^{3+} -montmorillonite.

Figure 5. Acetaminophen transformation (A) and dimer formation (B) kinetics in aqueous systems containing Fe^{3+} -montmorillonite in the presence of the different amount of FA.

Figure 6. Comparison of HPLC profiles from different transformation system with Fe^{3+} - or Na^{+} -montmorillonite (A) and time course of formation of the new product (Product 2) (B) during acetaminophen transformation with Fe^{3+} -montmorillonite in the presence of different concentration of ferulic acid (FA).

Figure 7. MS spectra of products resulting from 72 h transformation of acetaminophen with Fe^{3+} -montmorillonite in the presence of FA. (A): total ions current chromatogram using positive ion mode (B)-(D): MS/MS spectra for the selected ions.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.