Abiotic transformation of lamotrigine by redox-active mineral and phenolic compounds

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Abstract

The anticonvulsant drug lamotrigine is a recalcitrant environmental pollutant. It was detected in drinking water, surface water, reclaimed wastewater, arable soils and even in edible crops. In this work, we studied the mechanisms of lamotrigine transformation by a common redox soil mineral, birnessite, in a single-solute system and in bisolute systems with vanillic acid or o-methoxyphenol. In the single-solute system, 28% of lamotrigine was transformed and 14 transformation products (TPs) were identified. Based on a detailed analysis of the TPs, we suggested that lamotrigine is transformed mainly by oxidation, addition and dechlorination reactions. In the bisolute systems, the redox-active phenolic compounds both enhanced the elimination and transformation of lamotrigine. Vanillic acid was more efficient, generating 92% transformation of lamotrigine (58 TPs were identified), whereas o-methoxyphenol induced 48% transformation (35 TPs were identified). In the bisolute system with phenolic compounds, lamotrigine has possibly been transformed mainly via addition reactions with phenolic compounds and their oxidation products (protocatechuic acid, quinone and oligomers). Thus, masses of the formed TPs were elevated as compared to the parent compound. The current study demonstrates the important role of redox-active minerals and naturally occurring phenolic compounds in abiotic removal and transformation of a recalcitrant environmental pollutant.

Introduction

Lamotrigine is an anticonvulsant drug, which is used to treat epilepsy and bipolar disorders. In humans, 70% of the administered dose of lamotrigine is recovered from urine with 90% as the metabolite lamotrigine-*N*2-glucuronide, which is a result of conjugation.[1] The concentrations of this metabolite range from 0.103 nM to 32.539 nM in influents and from 0.019 nM from 2.193 nM in effluents of wastewater treatment plants.[2–4] Lamotrigine has been detected in groundwater, surface water and even in drinking water at concentrations of 0.005-5.746 µg/L.[3,5] In wastewater, lamotrigine is found in concentrations of 0.013-1.254 µg/L.[4] Moreover, its concentrations in wastewater effluents are higher than that in influents, due to deconjugation of lamotrigine-*N2*-glucuronide into lamotrigine and its transformation into OXO-lamotrigine.[3–6] Lamotrigine has been shown to be persistent in soils irrigated with treated wastewater, where it accumulates in the topsoil in concentrations of up to 4 mg/kg soil, decreasing with depth.[7,8]

The lamotrigine molecule is composed of a 1,2,4-triazine ring with two substituting amine groups attached to 2,3-dichlorobenzene (Table S1). Abiotic transformation of lamotrigine occurs mainly on the triazine ring, as was demonstrated by applying electrochemistry, photo-oxidation and ozonation, and confirmed by mass spectrometry (MS).[5,9–12] The triazine ring is susceptible to electrophilic attacks, due to its electron enrichment by substituting amine groups. Lamotrigine's benzene moiety is relatively deactivated by two electron withdrawing chlorine atoms. Nevertheless it forms conjugates via the benzene ring (as well as via the triazine moiety) due to biotransformation induced by the white-rot fungus *Pleurotus ostreatus*.[13]

Environmental fate of organic pollutants is governed by both biotic and abiotic processes.[14–16] When introduced to soils, organic pollutants can be transformed by redox-active manganese oxides, and particularly by birnessite (δ-MnO_2), a naturally

abundant manganese oxide.[12,17–21] These interactions, can be affected by phenolic compounds, occurring in soil solutions as reactive components of dissolved organic matter and are susceptible to oxidation by manganese oxides.[22–24] Adsorption and transformation of phenols by metal oxides can result in their oxidation or reduction, forming two types of products - phenoxy radicals and benzoquinones.[25,26] Phenoxy radicals can enhance oxidation of pollutants via oxidative cross-coupling mechanism, whereas quinones and hydroquinones acts as electron shuttles and degrade pollutants by direct redox reactions while undergoing redox cycling.[24,27,28]

The objective of the current study was to elucidate the abiotic transformation pathways of lamotrigine reacting with birnessite in single- and bisolute systems containing vanillic acid or o-methoxyphenol. Vanillic acid and o-methoxyphenol were selected as reactive precursors of dissolved organic matter that may affect transformation of lamotrigine. We hypothesized that the environmental persistence and fate of lamotrigine will be significantly affected by its surface-induced interactions with birnessite. The presence of phenolic compounds, which are oxidized on birnessite surface forming radicals, is expected to enhance and change the mechanism of lamotrigine transformation.

Materials and methods

Materials

Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine; ≥98%) was purchased from TCI (Zwijndrecht, Belgium), labeled lamotrigine-$^{13}C_3$ was purchased from TRC (Ontario, Canada), vanillic acid (4-hydroxy-3-methoxybenzoic acid; ≥97%) was obtained from Sigma Aldrich (Rehovot, Israel), and o-methoxyphenol (2-methoxyphenol; >99%) was purchased from Acros Organics (Geel, Belgium). The structures and selected properties of lamotrigine and phenolic compounds are shown in Table S1. All used solvents were HPLC and LC-MS grades.

Birnessite (δ-MnO_2) was synthesized following the procedure of McKenzie.[29] Briefly, concentrated HCl was added dropwise to a boiling solution of potassium permanganate and stirred for 10 min. The suspension was then centrifuged (15,200 *g*, 15 min) and decanted. The precipitated birnessite was washed with deionized water several times until the supernatant was colorless. The birnessite was oven-dried at 35 °C and then freeze-dried. Birnessite characterization is described in Karpov et al. and in the SI. [30]

Removal of lamotrigine and phenolic compounds by birnessite

Two types of batch experiments were performed to study removal of lamotrigine by birnessite: (i) a single-solute system, and (ii) a bisolute system in the presence of vanillic acid or o-methoxyphenol, each added separately in concentrations 20 times higher than lamotrigine. This ratio was used in a previous study of pollutant removal in the presence of birnessite and phenolic compounds and was found efficient in preliminary experiments for the studied system.[22]

Experiments were performed at initial lamotrigine concentrations of 4 nM (1024 ng/L) or 4 µM (1024 µg/L). The low concentration (4 nM) was used to examine lamotrigine behavior in environmentally relevant conditions, and the high concentration (4 µM) was applied when studying transformation by the birnessite surface and for characterization of transformation products (TPs). Moreover, high concentrations of organic compounds can also exist in the microenvironments of minerals surfaces due to adsorption of pollutants and natural organic molecules.[31]

Birnessite powder (10 mg) was weighed into a 15 mL polystyrene falcon tubes (Greiner Bio-One™ CELLSTAR™), then suspended in 5 mL deionized water, sonicated for 15 min and agitated for 2 h. The initial pH of the suspension was adjusted to 5.5 using 0.01 M HCl before the analytes were added, since preliminary experiments showed efficient removal of lamotrigine at these conditions. Besides, this pH was detected

under different environmental conditions: in discharged wastewater and in MnO_2-containing soil rhizosphere.[32,33] Deionized water and analytes were added to adjust the final volume to 10 mL. Birnessite concentration was 1 g/L in all experiments. Reaction tubes were agitated at 250 rpm at 25 °C in the dark. The reaction was terminated by centrifugation after 10 min, 0.5, 1, 4, 24, and 72 h. At the end of the reaction, the pH of suspension was 6.5. The tubes were centrifuged (at 3,220 *g*, 7 min), and the supernatants were decanted and filtered through 0.22 µm filters (MS PTFE syringe filter, Membrane Solutions).

Control samples of lamotrigine alone, phenolic compounds alone, lamotrigine in the presence of each phenolic compound and aqueous suspension of birnessite alone were kept in the same conditions and analyzed using the same corresponding procedures. All experiments were performed in triplicates. Data presented are means with standard errors.

Extraction of surface-bound lamotrigine and its transformation products

To detect the lamotrigine remaining on the birnessite surface and to reveal surface-bound TPs, extractions were performed. Extraction from the birnessite surface was performed by adding 5 mL of MeOH and 200 mM $CaCl_2$ (1:3) to the reaction tubes after supernatant decantation. Suspensions were sonicated for 15 min and agitated for 4 h. The suspensions were then filtered using 0.22 *µ*m filter (MS PTFE syringe filter, Membrane Solutions). Preliminary experiments demonstrated lamotrigine extraction efficiency of 89±10% (at lamotrigine initial concentration 4 µM). Further information about the analytical instruments and methods is described in SI. Total lamotrigine mass balance for each studied system was calculated as the sum of lamotrigine concentration in solution and in the extract (normalized to extraction volume).

Detection of lamotrigine and transformation products

Supernatants obtained for low initial lamotrigine concentrations of 4 nM were analyzed for lamotrigine by LC-MS. Sample preparation, cleanup procedures and analytical methods are described in SI.

Supernatants and extracts obtained in experiments with the high lamotrigine concentration of 4 µM, were filtered and analyzed without further treatment by HPLC. The dissolved Mn concentration was analyzed by ICP-OES. Non-targeted analysis was performed to detect lamotrigine TPs by ultrahigh-performance liquid chromatography high resolution mass spectrometry (UPLC-HRMS) (detailed analytical methods are described in SI).

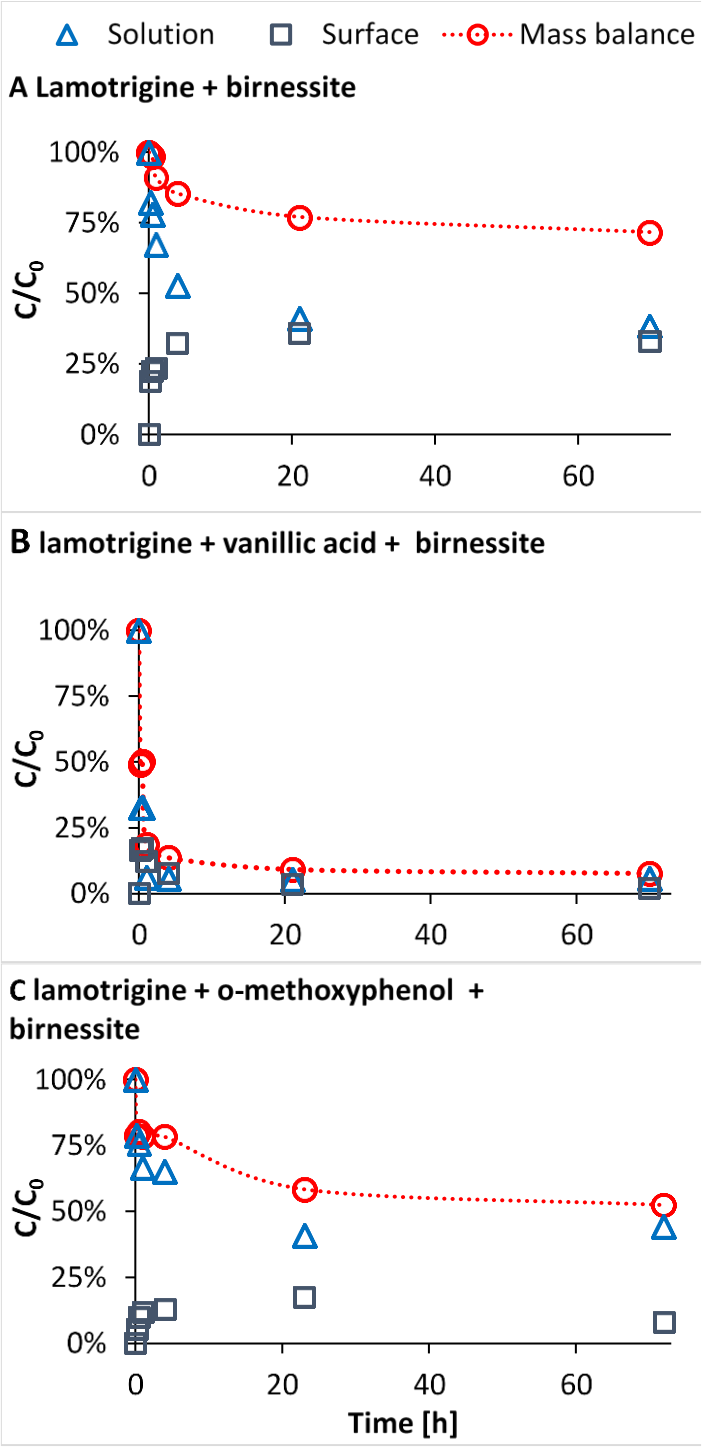
Results and discussion

Removal of lamotrigine from single- and bisolute systems

At the initial concentration of 4 nM, about 80% of lamotrigine was eliminated from the solution, regardless of the presence and type of phenolic compounds (Figure S1). It appears that at this low concentration, interactions between organic molecules (primary analyte and the phenolic acids) on the mineral surface are negligible. Most likely, enough surface area was left for the molecules to be adsorbed separately from one another, thus independent reactions of lamotrigine and phenolic compounds with birnessite occurred.

At the initial concentration of 4 µM, the presence of both phenolic compounds enhanced lamotrigine removal from solution, with o-methoxyphenol being less efficient than vanillic acid (Figure 1). For the single-solute system, mass balance of lamotrigine showed that 28% of the initially added lamotrigine was eliminated from the system after 72 h, while in the bisolute system with vanillic acid, 92% was eliminated (Figure 1, A and B). The initial concentrations of both lamotrigine and phenolic compounds in the

control solutions (without birnessite) did not change during this time. Vanillic acid and o-methoxyphenol were not detected in the solution or in the extracts from birnessite after 10 min interaction-time with the mineral. At pH 5.5 (the initial reaction pH) about half of the lamotrigine molecules (pK_a = 5.7) existed in solution as organic cations, whereas more than 90% of the vanillic acid (pK_a = 4.16) was negatively charged (Table S1).[34,35] In these conditions, electrostatic interactions between vanillic acid and lamotrigine, i.e. formation of ion pairs, both in solution and on the mineral surface, facilitated lamotrigine removal from the solution and its adsorption by birnessite.



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175 Figure 1. Relative concentration of lamotrigine (100% = 4 μM) in supernatant (triangles)
176 and in extracts from birnessite surface (squares) in single-solute system (A), and in
177 bisolute systems with vanillic acid (B), and o-methoxyphenol (C). Mass balance
178 (circles) is the sum lamotrigine in both phases. Average data is presented, standard
179 errors were within 4% of the average values.

180

For the bisolute system with o-methoxyphenol, the mass balance of lamotrigine showed elimination of 48% of its initial concentration (Figure 1C). The lamotrigine–o-methoxyphenol–birnessite system clearly demonstrated the difference between the removal efficiency of the compound from solution and its transformation by the mineral surface. The lamotrigine fraction remaining in the supernatant in the presence of o-methoxyphenol was 44% (Figure 1C), which is comparable to that of lamotrigine alone, i.e. in the single-solute system (38%) (Figure 1A). However, the lamotrigine fraction on the mineral surface in the presence of o-methoxyphenol was fourfold less than in the single-solute system, which indicates higher intensity of lamotrigine transformation on the mineral surface induced by o-methoxyphenol.

Transformation of lamotrigine in a single-solute system

Transformation of lamotrigine by birnessite was investigated at the high initial lamotrigine concentration of 4 μM, to facilitate detection and identification of TPs. Lamotrigine was transformed by birnessite and each of the 14 TPs were detected in both the solution and on the birnessite surface (Table 1, extended data are shown in Table S3 and Figure S2). Some of the TPs were detected also in the control samples as impurities (Table S3), but were considered as part of the transformation pathway and were included in the mechanism if their peak intensity was higher in the samples compared to the controls. According to the levels of confidence for identifying molecular structures described in Schymanski et al., [36] all identified TPs were tentative structural candidates, i.e. there was insufficient information to propose only one structure. It is important to note that the possibility of the existence of undetected short-lived intermediates or unstable TPs cannot be excluded. The proposed mechanism of lamotrigine transformation by birnessite involves three major pathways: oxidation, addition, and dechlorination (Figure 2).

207 Table 1. Transformation products (TP) of lamotrigine as measured in a single-solute
208 system (lamotrigine + birnessite).

TP name	Accurate mass m/z (Δ ppm)	Proposed formula $[M+H]^+/[M+Na]^+$	Modification from lamotrigine
		Oxidation	
TP257a	256.9997 (0.0)	$C_9H_7N_4OCl_2$	-NH+O
TP257b	256.9997 (0.0)	$C_9H_7N_4OCl_2$	-NH+O
TP272	272.0106 (1.8)	$C_9H_8N_5OCl_2$	+O
		Addition	
TP298_2	298.0626 (0.0)	$C_{12}H_{14}N_5Cl_2$	$+C_3H_6$
TP299	299.0470 (1.3)	$C_{12}H_{13}N_4OCl_2$	$+C_3H_5O$-N
TP332	332.0461 (2.7)	$C_{15}H_{12}N_5Cl_2$	$+C_6H_4$
TP399	399.9694 (1.0)	$C_{15}H_{10}N_5Cl_4$	$+C_6H_2Cl_2$
TP494	494.9829 (3.8)	$C_{18}H_{11}N_8OCl_4$	$+C_9H_3N_3OCl_2$
		Dechlorination	
TP222a	222.0540 (2.7)	$C_9H_9N_5Cl$	+H-Cl
TP222b	222.0542 (1.8)	$C_9H_9N_5Cl$	+H-Cl
TP266	266.0790 (1.9)	$C_{11}H_{13}N_5OCl$	$+CH_2O+CH_3$-Cl
		Other reactions	
TP256	256.0152 (2.0)	$C_9H_8N_5Cl_2$	-
TP289	289.9757 (3.4)	$C_9H_7N_5Cl_3$	+Cl-H
TP298_1	298.0256 (6.0)	$C_9H_{11}N_5OCl_2Na$	$+H_2O+H_2$

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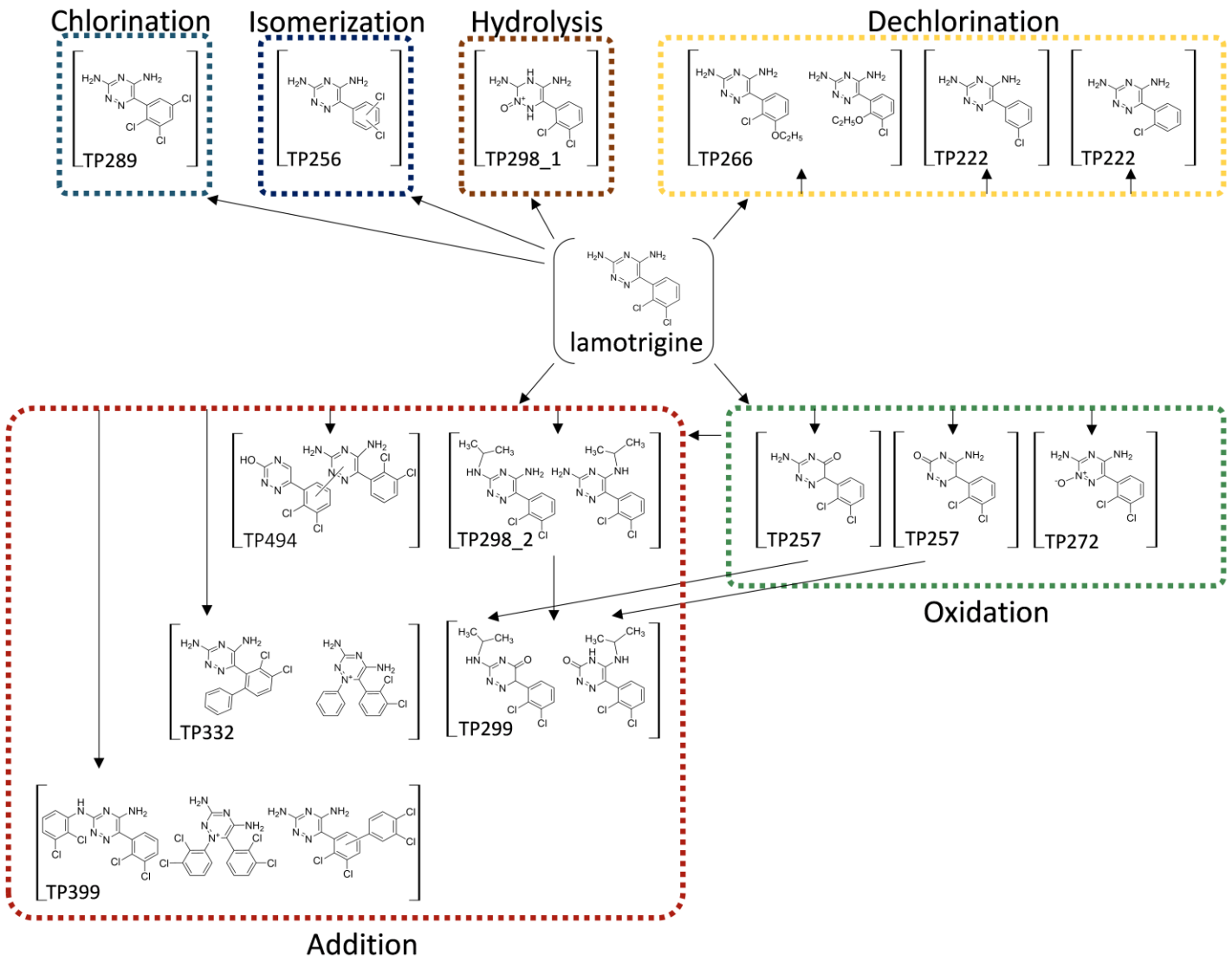

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211 Figure 2. Proposed mechanism of lamotrigine transformation by birnessite.

212 Oxidation: Oxidation of lamotrigine is initiated by either the addition of oxygen to the
213 triazine ring or substitution of one of the amine groups by a hydroxyl group. The oxygen
214 atom could originate from reactive oxygen species resulting from the interaction of
215 birnessite with dissolved oxygen.[37] The addition of oxygen to a triazine ring resulted in
216 the formation of TP272 (Figure 2). The fragmentation pattern includes a radical
217 fragment with m/z 242.0132 formed by a loss of NO (Figure S2, E), indicating the
218 addition of oxygen to the triazine nitrogen to form lamotrigine-*N2*-oxide. Lamotrigine-
219 *N2*-oxide is known as a human metabolite that is formed in treated wastewater and in
220 advanced chemical oxidation processes such as ozonation and reaction with hydroxyl
221 radical.[2,4,9] Oxidation of lamotrigine by white-rot fungus also results in the formation of
222 lamotrigine-*N2*-oxide, which is the only TP formed in both this biotic and single-solute
223 abiotic systems.[13]
224 Another pathway of lamotrigine oxidation is by substitution of one of lamotrigine's
225 amine group by a hydroxyl group. This can occur on each of the two amine positions,
226 forming two isomers: TP257a and TP257b (Figure 2 and Table 1). A fragment of
227 lamotrigine-N2-glucoronide which was detected by Zonja et al. and was identified as
228 OXO-lamotrigine, had the same m/z and fragmentation pattern (loss of CO) as TP257
229 isomers found in our system (Figure S2 F+H).[4,38] This finding emphasizes the
230 degradation strength of the abiotic system with birnessite, which oxidized lamotrigine
231 directly to OXO-lamotrigine while in the biologically induced degradation in wastewater
232 treatment plants, OXO-lamotrigine originates from lamotrigine-*N2*-glucoronide, and is
233 not a direct product of lamotrigine transformation. The two isomers exhibited similar
234 changes with time. Their highest intensity was obtained for the mineral surface extracts
235 after 24 h, and then their surface concentration gradually decreased. Concentrations
236 of these isomers in the solution were stable for 72 h (Table S3). This behavior suggests
237 an initial accumulation of OXO-lamotrigine isomers on the surface by adsorption to the

mineral followed by their further decomposition (Figure 2). We were unable to associate each isomer with a certain structure (both are marked as TP257 in Figure 2).

Addition: This pathway is characterized by the substitution of lamotrigine, initiated by the formation of TP298_2, TP399 or TP494 (Figure 2). TP298_2 is formed by adding C_3H_6 to lamotrigine, which can occur on both of the amine's nitrogens, allowing for two possible structures (Figure 2). Lamotrigine was detected as a fragment in the MS/MS spectra of TP298_2 (Figure S2, I), which indicates that the carbon chain was added to one of the amine groups. TP298_2 was observed mostly on the birnessite surface and was stable in solution during the experiment (Table S3). TP298_2 can be transformed into TP299 by substituting each amine group on the triazine ring by a hydroxyl, allowing for two structures (Figure 2). The fragmentation pattern of TP299 (Figure S2, K) contains TP257 (OXO-lamotrigine), which suggests another possible pathway for the formation of TP299 (Figure 2), by the addition of C_3H_6 to one of the TP257 isomers.

TP399 is a product of the addition of a dichlorobenzene ring to lamotrigine, which can originate from the cleavage of another lamotrigine molecule. The additional ring can be added to each part of the lamotrigine molecule. Since no fragments were detected in the MS/MS spectra for this TP, we suggest 3 possible structures (Figure 2). This TP was detected mainly on the mineral surface (the solution contained negligible amounts), and it disappeared with time (Table S3). TP332 is formed by addition of benzene ring to lamotrigine that can be attached to the triazine moiety or to the dichlorophenyl structure, as the fragmentation pattern shows (Figure S2 J).

TP494 is produced by binding of lamotrigine and deaminated OXO-lamotrigine. The two molecule parts are most likely attached via the triazine moiety, as the fragmentation pattern shows (Figures 2 and S2, L). It was observed mainly on the mineral surface, where proximity of two molecules is a prerequisite for the addition

reaction (Table S3). This reaction demands two lamotrigine molecules to adsorb and transform in close vicinity on the mineral surface, which is expected only at high concentrations and is less likely to occur at environmental concentrations of lamotrigine.

Dechlorination: Three dechlorination products were detected: TP266, and two isomers of TP222. TP266 is formed by substituting one chlorine atom by an ethoxide and exhibits an isotopic signature of one chlorine (Figure S2, D). Although both of lamotrigine's chlorines can be subjected to substitution, only one isomer of TP266 was detected, indicating that one of the chlorines is more susceptible to detachment (the two options for TP266 structure are shown in Figure 2). Substitution of the *ortho* position chlorine is more probable because it is activated as compared to the *meta* position.[9] Another possibility is that TP266 formation occurs in two steps: the first step is substituting chlorine by hydroxide, forming an intermediate with m/z 238, followed by the addition of ethyl that can originate from birnessite-bound hydrocarbons. This pathway cannot be verified because m/z 238 was not detected as a TP in this system. The suggestion is based on the observed fragment m/z 238.0460 in the fragmentation pattern of TP266 (Figure S2 D).

Both TP222a and TP222b exhibit an isotopic signature of one chlorine (Figure S2 A+B). Those isomers are impurities, because they appear both in the samples as well as in the lamotrigine controls, but their levels were higher in the samples and thus they participated in reactions with birnessite (Table S3). Elimination of chlorine atom and its substitution with hydrogen is known to occur via a radical mechanism, and thus we expect that TP222 formation involved a short-lived radical intermediate that was not detected.[39] Since both chlorines of lamotrigine can be removed, two isomers of TP222 are formed (Figure 2). These isomers can be products dechlorination of lamotrigine or its structural isomer TP256. Their fragmentation patterns (Figure S2 A+B) and

formation kinetics (Table S3) are similar. Therefore, we were unable to determine the retention time of each isomer. TP222 isomers were previously observed as products of lamotrigine degradation by photolysis.[9]

Other reactions: Three additional TPs, which did not belong to the abovementioned pathways were TP256, a structural isomer of lamotrigine, and TP289 and TP298_1, which are the products of lamotrigine chlorination and hydrolysis, respectively. TP256 is a structural isomer of lamotrigine that was present in the lamotrigine control sample as an impurity (Table S3). TP256 is mentioned in the literature as a product of radical reaction during lamotrigine photolysis.[9,10] Keen et al. hypothesized that this isomer is formed by a C-Cl bond cleavage, followed by the detached chlorine atom staying in the solvent cage, which is then attached to a different position on the benzene ring.[9]

TP289 is a product of lamotrigine chlorination and therefore shows an isotopic signature of three chlorine atoms (Figure S2 G). In the single-solute system, the additional chlorine atom can originate from dechlorination reactions of other lamotrigine molecules, like those resulting in the formation of TP266 or TP222. The fragmentation pattern of TP289 shows one fragment of m/z 244.9421 that corresponds to a trichlorobenzene ring substituted with a chain originating from the triazine moiety (Figure S2 G), which suggests that the chlorination occurs on dichlorophenyl. TP298_1 is formed by hydrolysis of lamotrigine's triazine ring, resulting in its de-aromatization.

Transformation of lamotrigine molecules containing triazine rings with amine substitutes by birnessite might be facilitated by the formation of manganese oxide-lamotrigine precursor complexes as suggested by Shin and Cheney for protonated atrazine, interacting with birnessite at low pHs.[40,41] Formation of these precursor complexes might induce both oxidative and non-oxidative transformations of the molecules.

315 In the experimental conditions of the current work, the pH changed from 5.5 to 6.5
316 (Figure S3). At pH 5.5, protonated lamotrigine (Table S1) can form precursor
317 complexes with surface hydroxyls of birnessite, whereas at pH 6.5, Mn(IV) can form
318 precursor complexes with non-protonated lamotrigine molecules by accepting nitrogen
319 electrons to its *d*-orbitals. Both precursor complexes might destabilize non-protonated
320 or protonated lamotrigine, resulting in both oxidative and non-oxidative lamotrigine
321 transformations. The pH of suspension increased during the reaction since
322 consumption of protons during redox reactions involving manganese dioxide occurs
323 when Mn(IV) is reduced.[42,43] Changes in the peak area of TPs was observed in the
324 supernatants and on the minerals surfaces with the increase in pH (Figure S4).
325 Reactions involved dechlorination, chlorination and isomerization (i.e. TP222, TP266,
326 TP289 and TP256, respectively) were correlated with pH and demonstrated a similar
327 trend: the TPs eliminated from solution and accumulated on the surface with increasing
328 pH (Figure S4, A-D,G). The TPs elimination from solution was more drastic than their
329 accumulation on the surface suggesting the tendency of TPs depletion from the
330 system. Although radical dehalogenation mechanism is not a pH-dependent process,
331 birnessite reactivity is pH-dependent, and can indirectly affect lamotrigine
332 transformation and TPs behavior.[39] Hence, the depletion of TPs with decreasing pH
333 can be explained by the impeding of birnessite reactivity with increasing pH. For other
334 reactions in the transformation mechanism, a clear trend of changes in TPs behavior
335 with pH was not observed (Figure S4, E-F,H-N). This implies that transformation of
336 lamotrigine can be affected by pH directly (due to changes in fraction of its charged
337 species (Table S1) or indirectly (via other pH-dependent-reactions occurring in the
338 system) or via reactions, which are not influenced by pH.
339 In the single-solute system, dissolved Mn was detected at a concentration of 5.80 μM
340 (versus 4.14 μM in the birnessite suspension) (Figure S5). The presence of dissolved

Mn in the birnessite control can be explained by the formation of Mn(IV) chlorides due to the addition of HCl to the mineral for adjusting the suspension's pH. The additional dissolved Mn in the single-solute system might be considered as Mn(II), which formed due to lamotrigine oxidation or other reactions involving dissolution of Mn in this system.

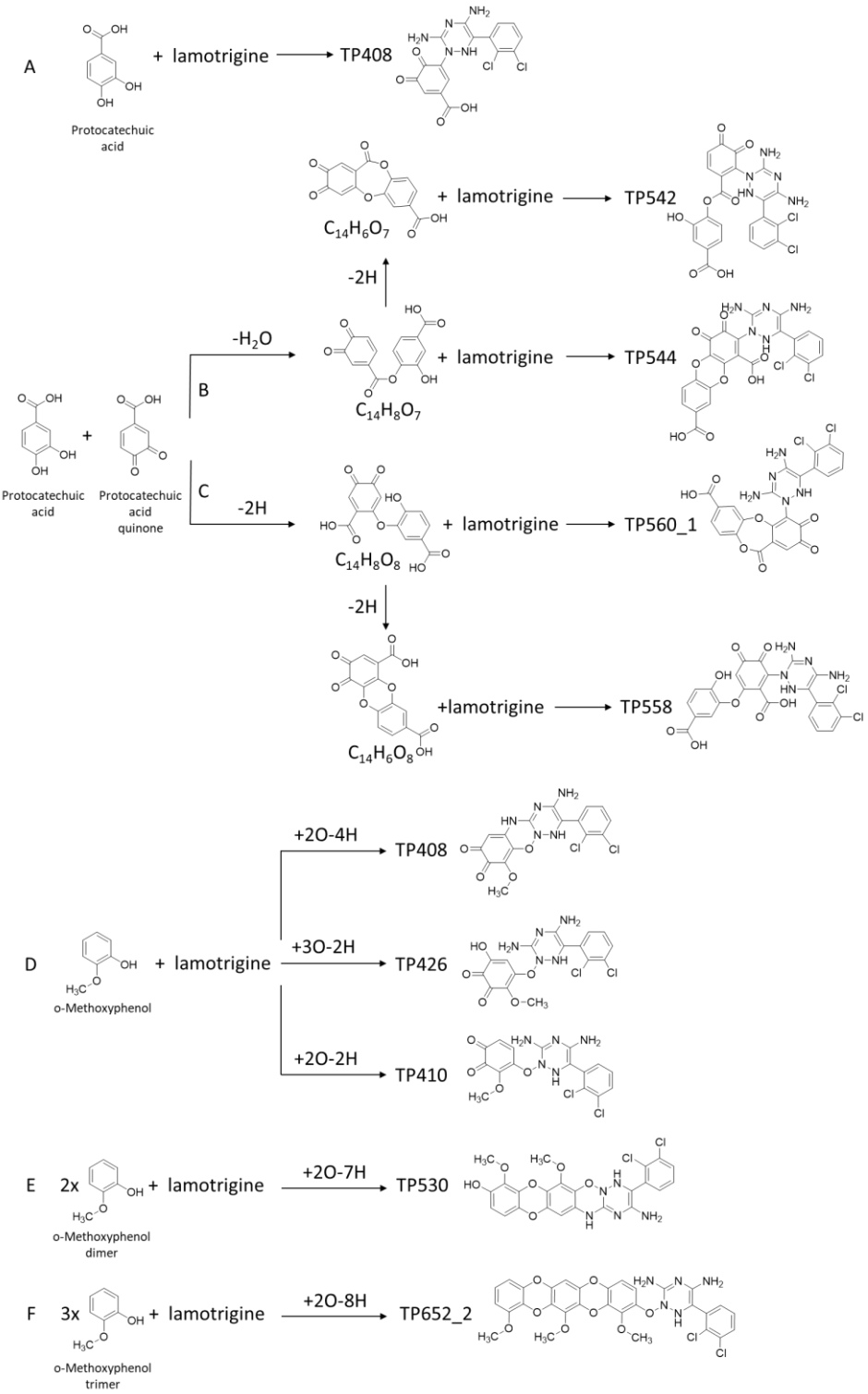
Transformation of lamotrigine in bisolute systems

Transformation of lamotrigine in the presence of vanillic acid or o-methoxyphenol was studied at the high initial lamotrigine concentration of 4 µM, which enables to detect lamotrigine TPs above the detection limit. The presence of both phenolic compounds resulted not only in an increased elimination of lamotrigine, but also in its more extensive transformation. Though a direct reaction between lamotrigine and birnessite can occur both in the single- and bisolute systems, the presence of phenolic compounds can change the lamotrigine transformation pathways.

The impact of vanillic acid on both the removal and transformation of lamotrigine was more significant than that of o-methoxyphenol. Transformation of lamotrigine in the presence of vanillic acid resulted in 58 TPs versus 35 TPs formed in the presence of o-methoxyphenol (Table S4). It is interesting to note that in the bisolute systems, all TPs exhibited higher masses than lamotrigine except two isomers of TP222. Oxidation of vanillic acid and o-methoxyphenol by redox-active mineral surfaces has been reported to be accompanied by the formation of free radicals and polymerization, resulting in products with higher masses than the reactants.[44,45] It is interesting to note that out of all products detected, only two TPs (TP272 – lamotrigine-*N2*-oxide, and TP314) were identical to those in abiotic systems and a system of biological transformation of lamotrigine.[13]

The different impact of vanillic acid and of o-methoxyphenol on lamotrigine removal and transformation can be explained by the differences in their molecular structures (Table S1). Vanillic acid contains two electron-donating groups, hydroxy and methoxy, and a carboxylic electron-withdrawing group. Conversely, o-methoxyphenol contains only electron-donating substituents on its benzene ring. Thus, phenoxy radicals of vanillic acid are expected to be stabilized as compared to those originating from o-methoxyphenol, because the carboxyl group inductively withdraws electrons from the benzene ring impeding electron localization on the phenolic oxygen.[46] Though both o-methoxyphenol and vanillic acid can react in cross-coupling reactions with lamotrigine, the high reactivity of vanillic acid towards lamotrigine might be explained by the interactions of lamotrigine with the relatively stable radicals of vanillic acid. We suggest that the radicals of o-methoxyphenol tend to be self-coupling, which decrease their reactivity towards lamotrigine as compared to the cross-coupling of lamotrigine with vanillic acid. Kang et al. showed similar loss of the recalcitrant herbicide cyprodinil by birnessite in the presence of these phenolic compounds.[22] On the contrary, results of our study demonstrated the significant impact of the molecular structure and properties of phenolic compounds on lamotrigine transformation by birnessite.

In all systems containing phenolic compounds (with and without lamotrigine), the concentration of dissolved Mn was about 2.85 μM (Figure S5), which is lower than dissolved Mn in birnessite controls (birnessite in aqueous suspension) or in the single-solute lamotrigine-birnessite system. Oxidation of phenolic compounds was expected to produce larger amounts of dissolved Mn, but the opposite result indicates re-adsorption of dissolved Mn on the birnessite surface as a separate ion or complexed with organic compounds. Thus, the measured concentration of dissolved Mn in solution is lower than expected in the samples as compared to the controls.[41,47]



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392 Figure 3. Proposed pathways of lamotrigine transformation by birnessite in the bisolute
393 system with vanillic acid (A-D) and with o-methoxyphenol (D-F). Only one possible
394 structure of transformation products (TPs) is shown.

395

Lamotrigine transformation in the presence of birnessite and vanillic acid

All TPs, which were detected in the single-solute system except TP494, were also detected in the presence of vanillic acid. Hence, the presence of vanillic acid changes to some extent lamotrigine transformation mechanism by birnessite. As was described above, TP494 is a product of self-coupling of lamotrigine with its TP. In the bisolute systems, self-coupling reactions of lamotrigine are less likely to occur due to the presence of phenolic compounds at higher concentrations than lamotrigine. Cross-coupling reactions of lamotrigine with TPs formed by oxidation of phenolic compounds are more probable. Based on our data, we suggest that products of vanillic acid oxidation reacted with lamotrigine, which intensified lamotrigine transformation and resulted in formation of the detected TPs in this bisolute system (Figure 3).

We observed the presence of protocatechuic acid, and quinones of protocatechuic acid in vanillic acid-birnessite control system; these compounds are formed due to the oxidation of vanillic acid.[48,49] One possible reaction of lamotrigine in the bisolute system with vanillic acid is an addition of protocatechuic acid to lamotrigine, leading to the formation of one of the TP408 isomers (Figure 3A and Table S4).

Another set of reactions is initiated by the dimerization of protocatechuic acid with its quinone followed by its addition to lamotrigine, resulting in the formation of TP544 and TP542 (Figure 3B). The first step of reaction is when protocatechuic acid binds to protocatechuic acid quinone through the oxygen of the quinone carboxylic group, accompanied by the loss of a water molecule, resulting in forming the product $C_{14}H_8O_7$ (Figure 3B). This product reacts with lamotrigine to form TP544. Further transformation of $C_{14}H_8O_7$ by the loss of two hydrogens and a ring closure lead to the formation of $C_{14}H_6O_7$ (Figure 3B), which forms TP542 by adding it to lamotrigine. The quinone can bind to protocatechuic acid through the phenol group of protocatechuic acid (Figure 3C) with a loss of two hydrogens. This results in the formation of $C_{14}H_8O_8$, which then

forms TP560_1 when added to lamotrigine (Figure 3C). This reaction can proceed to transform $C_{14}H_8O_8$ to $C_{14}H_6O_8$ by losing two hydrogens and a ring closure (Figure 3C). TP588 can be produced by adding $C_{14}H_6O_8$ to lamotrigine. Additional TPs (TP408, TP410 and TP426) formed in this system (Figure 3D) were also detected in the presence of o-methoxyphenol and are described in the section below. The similarity of TPs in the bisolute systems can be explained by the decarboxylation of vanillic acid by birnessite resulting in o-methoxyphenol formation followed by its reaction with lamotrigine to form the same TPs (Figure 3D).[45]

Lamotrigine transformation in the presence of birnessite and o-methoxyphenol

From the 35 TPs found in the presence of o-methoxyphenol, only 14 were observed in the single-solute system. TP299, TP399 and TP494, produced in addition reactions in the single-solute system were below the detection limit in this bisolute system. These findings confirm that the presence of phenols changes the lamotrigine transformation mechanism by birnessite.

Dimers and trimers of o-methoxyphenol were detected in the o-methoxyphenol-birnessite control systems, which confirms the participation of radicals in phenol oxidation.[22] Cross-coupling lamotrigine TPs with monomers/dimers/trimers of o-methoxyphenol, formed in the presence of birnessite, are shown in Figure 3D-F. TP530 and TP652_2 (Figure 3, E and F) were formed only in the presence of o-methoxyphenol, while TP408, TP410 and TP426 (Figure 3D) were also present in the bisolute system with vanillic acid. These TPs are products of addition reactions of lamotrigine with o-methoxyphenol monomers (TP408, TP410 and TP426), dimers (TP530) or trimers (TP652_2) followed by oxidation. Radicals formed by phenol oxidation can cross-couple with organic pollutants, as was also shown for lamotrigine in the bisolute systems.[24] Although we propose that TPs in the bisolute systems are formed by coupling between phenolic compound products and lamotrigine molecules

448 by addition only (i.e. without changing the lamotrigine molecular structure), we cannot
449 exclude that phenoxy radicals can cause significant structural changes of the
450 lamotrigine molecule.
451
452 **Environmental implications**
453 This work demonstrates fast and efficient abiotic transformation of lamotrigine by the
454 common soil redox-active mineral birnessite and elucidates the impact of natural
455 phenols on the transformation processes. The formed TPs can be adsorbed to solid
456 surfaces in the soil, transported through the soil profile to groundwater and/or face
457 biotic transformation. Although transformation of lamotrigine was observed in the
458 presence of birnessite alone, transformation was more extensive in the presence of
459 phenolic compounds. The impact of phenolic compounds depends on their different
460 structures, properties, and their various interactions with birnessite and lamotrigine.
461 Phenolic compounds can affect the mechanism of lamotrigine transformation by
462 preventing certain addition reactions occurring in the birnessite-lamotrigine system.
463 The interactions of lamotrigine with birnessite in the presence of phenolic compounds
464 was driven by oxidation of phenols by birnessite and resulted in formation of products
465 with higher molecular masses than lamotrigine. This might enhance TPs'
466 immobilization by binding to soil organic matter, increasing their half-life time. The
467 presence of the TPs after 72 h of incubation when the pH of the system was ~6.5
468 suggests that the formed TPs might be stable not only in acidic but also in neutral soils.
469 Most of the TPs were not detected previously and thus their toxicity is unknown.
470 Though glucuronide conjugate of lamotrigine is less toxic than the parent compound
471 the conjugation is reversible due to glucuronide hydrolysis.[4]
472 Abiotic transformation appears to be an important process affecting the environmental
473 fate of organic pollutants alongside biotic degradation. The persistence of

environmental pollutants is commonly determined by the compound's stability in biotic systems, leaving out abiotic factors, which also can influence pollutants' environmental fate, as suggested in this work. The similarity in TPs of biotic and abiotic transformations of lamotrigine might indicate close pathways of the pollutant degradations, whereas variations in the transformation products suggest differences in biotic and abiotic reaction mechanisms.[13] Elucidating the efficiency and mechanisms of these processes and detailed characterization of the TPs will help to select the optimal methods of soil and water decontamination.

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Supporting Information. Information is provided about molecular properties of lamotrigine, vanillic acid and o-methoxyphenol (Table S1); characterization of birnessite; sample preparation and chromatographic analytical methods; limits of detection (LOD) and quantification (LOQ) of the analytes (Table S2); removal of lamotrigine from solution by birnessite in single-solute and bisolute systems (Figure S1); fragmentation patterns of lamotrigine transformation products in the presence of birnessite (Figure S2); changes in pH of suspensions with time (Figure S3); plots of changes in TPs peak areas vs. pH of suspensions in single solute system (Figure S4); dissolved Mn released from birnessite surface in single- and bisolute systems of lamotrigine with or without phenolic compounds (Figure S5); and a list of lamotrigine transformation products detected in single- and bisolute systems (Table S4). The Supporting Information is available free of charge on the ACS Publications website.

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