

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES
JOURNAL OF
ENVIRONMENTAL
SCIENCES
www.jesc.ac.cn

Decreased bioavailability of aminomethylphosphonic acid (AMPA) in genetically modified corn with activated carbon or calcium montmorillonite clay inclusion in soil

Sara E. Hearon¹, Meichen Wang¹, Thomas J. McDonald², Timothy D. Phillips^{1,*}

¹ Veterinary Integrative Biosciences Department College of Veterinary Medicine and Biomedical Sciences, Texas A&M University College Station, TX 77845, USA

² Department of Environmental and Occupational Health School of Public Health, Texas A&M University, College Station, 400 Bizzell St College Station, TX 77843, USA

ARTICLE INFO

Article history:

Received 11 May 2020

Revised 24 June 2020

Accepted 24 June 2020

Available online 27 July 2020

Keywords:

Glyphosate

AMPA

Activated carbon

Calcium montmorillonite

Sorbent

ABSTRACT

The widespread use of pesticides has resulted in detectable residues throughout the environment, sometimes at concentrations well above regulatory limits. Therefore, the development of safe, effective, field-practical, and economically feasible strategies to mitigate the effects of pesticides is warranted. Glyphosate is an organophosphorus herbicide that is degraded to aminomethylphosphonic acid (AMPA), a toxic and persistent metabolite that can accumulate in soil and sediment and translocate to plants. In this study, we investigated the binding efficacy of activated carbon (AC) and calcium montmorillonite (CM) clay to decrease AMPA bioavailability from soil and AMPA translocation to plants. Adsorption isotherms and thermodynamic studies on AC and CM were conducted and showed tight binding (enthalpy values > -20 kJ/mol) for AMPA with high capacities (0.25 mol/kg and 0.38 mol/kg, respectively), based on derivations from the Langmuir model. A hydra assay was utilized to indicate toxicity of AMPA and the inclusion of 1% AC and CM both resulted in 90% protection of the hydra ($**p \leq 0.01$). Further studies in glyphosate-contaminated soil showed that AC and CM significantly reduced AMPA bioavailability by 53% and 44%, respectively. Results in genetically modified (GM) corn showed a conversion of glyphosate to AMPA in roots and sprouts over a 10-day exposure duration. Inclusion of AC and CM reduced AMPA residues in roots and sprouts by 47%–61%. These studies collectively indicate that AC and CM are effective sorbents for AMPA and could be used to reduce AMPA bioavailability from soil and AMPA residues in GM corn plants.

© 2020 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

* Corresponding author.

E-mail: tphillips@cvm.tamu.edu (T.D. Phillips).

Introduction

Pesticides have been instrumental in the success of large-scale agricultural practices and in keeping commercial and residential areas free of annual and perennial weeds. An estimated 3 billion tons of pesticides are applied each year, worldwide (Pimentel, 2009). Pesticide use has been so widespread that pesticide residues have been detected throughout the environment, sometimes in concentrations well above regulatory limits. For example, a study in India measured organochlorine pesticides (OCPs) in drinking water and found 37% of samples exceeded the total pesticide level of 500 ng/L limit set by a European Economic Community Directive (EECD) for drinking water (Kaushik et al., 2012). Another study analyzed European agricultural soils for 76 pesticides, including legacy pesticides that have been banned, and found that 83% of samples contained 1 or more pesticide residues, and 58% contained mixtures of pesticides (Silva et al., 2019). This environmental contamination is of concern especially in cases of natural or anthropogenic disasters such as floods and chemical spills during which chemicals can be mobilized and redistributed to affect water, soil, and sediment. Exposure to these chemicals can occur through direct contact with contaminated soil and water or consumption of contaminated water and food products.

Glyphosate (phosphonomethylglycine; PMG) is one such chemical that has been applied in large quantities for agricultural and commercial purposes. It is an organophosphorus compound and the active ingredient of many broad-spectrum herbicides such as Roundup™. In agricultural applications, PMG is used as a non-specific, systemic herbicide before planting and during pre- and post-emergence phases of plant growth. In the US alone, 1.6 billion kg of PMG have been applied since its development in 1974, two-thirds of which has been in the last 10 years. One factor contributing to the dominant and increasing use of PMG is the availability of genetically modified (GM), PMG-resistant crops, which makes up 56% of global PMG application (Benbrook, 2016).

PMG is broken down by microorganisms in soil and metabolized in plant tissue to form its major metabolite aminomethylphosphonic acid (AMPA), a more toxic and more persistent molecule than PMG. PMG half-life and degradation to AMPA are affected by pH and temperature and depend largely on soil type and content, ranging from 3.5 days in loess topsoil to 110 days in clay topsoil under oxic conditions (Bergstrom et al., 2011; Yang et al., 2015). Studies suggest that 48%–70% of PMG is broken down to AMPA in soil, primarily through C–N cleavage (Bai and Ogbourne, 2016; Sun et al., 2019). A study in agricultural topsoils found 21% and 42% of samples contained PMG and AMPA, respectively, and measured maximum concentrations of 2 mg/kg for both compounds (Silva et al., 2018). PMG and AMPA toxicity are topics of debate among various regulatory and scientific entities. The International Agency for Research on Cancer (IARC) reclassified PMG as “probably carcinogenic to humans” in 2015, while the US Environmental Protection Agency (USEPA) concluded that PMG is not likely to be carcinogenic to humans (Guyton et al., 2015; USEPA, 2020). These agencies have

not made a determination on the carcinogenicity of AMPA, but IARC considered studies that provide evidence of AMPA's potential to cause genotoxic effects and oxidative stress (Benbrook, 2019). Early studies suggest the acute toxicities of both compounds are relatively low. However, more recent work shows the potential for chronic health effects for humans and animals (Bai and Ogbourne, 2016; Mesnage et al., 2015; Séralini et al., 2014).

Bioavailable fractions of PMG and AMPA in soil and water can translocate to plants such as vegetable crops, resulting in potential exposures for humans and animals. AMPA has been detected in GM crops as well as non-target plants. Reductions in photosynthetic rates have been observed in willow plants and tea plants exposed to AMPA (Gomes et al., 2016; Tong et al., 2017). A study in Argentina measured PMG and AMPA in stems, leaves, and grains of GM soybean crops and found PMG concentrations up to 5.2 mg/kg and 1.8 mg/kg in stems/leaves and grains, respectively, and AMPA concentrations up to 5.7 mg/kg and 1.7 mg/kg in stems/leaves and grains, respectively (Arregui et al., 2004). Moreover, AMPA has phytotoxic effects and can significantly reduce shoot dry weights in GM soybeans (Ding et al., 2011; Reddy et al., 2004).

In addition to AMPA's negative effects on plant growth, chronic exposure to AMPA residues through consumption of contaminated plant products could be harmful to human health. Microbial degradation and adsorption to soil particles are used as primary remediation strategies for PMG, but these processes result in the production of AMPA and contribute to the persistence of PMG in soil (Sidoli et al., 2016; Zhan et al., 2018). Effective, field-practical, and economically feasible sorbent strategies could be used to reduce AMPA bioavailability from soil and potential exposures through direct contact with contaminated soil or consumption of contaminated food products.

Materials such as activated carbon (AC) and clay-based sorbents have been used to bind various contaminants in the environment to reduce their bioavailability. Studies from our laboratory have shown that montmorillonite-based clays are highly effective sorbents for mycotoxins, pesticides, PAHs, and PCBs (Hearon et al., 2020; Phillips et al., 2019; Wang et al., 2019a, Wang et al., 2019b; Wang et al., 2020). We have recently shown that calcium montmorillonite (CM) clay can tightly bind PMG to active surfaces based on adsorption studies and computational modeling (Wang et al., 2019c). Studies have investigated the use of biochars derived from birch wood, eucalyptus, and pecan, cherry, and apple wood chips as potential soil amendments to reduce PMG leaching from soil (Hagner et al., 2015; Hall et al., 2018; Junqueira et al., 2019). PMG sorption onto surfaces of montmorillonite clays has also been studied (Khoury et al., 2010; Morillo et al., 1997). However, there is minimal data reporting the use of sorbents that can effectively bind AMPA to reduce its bioavailability in soil and reduce plant uptake from soil. Therefore, this study characterized the adsorption efficacy of AC and CM for AMPA using in vitro isothermal analyses, thermodynamic studies, and the safety and efficacy of these materials using a living organism (*Hydra vulgaris*). Plant studies were also used to evaluate the efficacy of sorbents to mitigate uptake of AMPA residues in genetically modified (GM) corn roots and sprouts.

1. Materials and methods

1.1. Reagents

High pressure liquid chromatography (HPLC) grade acetonitrile and pH buffers (4.0, 7.0 and 10.0) were purchased from VWR (Atlanta, GA). Aminomethylphosphonic acid (AMPA) analytical standard was purchased from Acros Organics (Geel, Belgium). Glyphosate (PMG) analytical standards, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) and acetic acid were purchased from Sigma-Aldrich (Saint Louis, MO). HPLC grade acetone and water + 0.1% formic acid were purchased from Fisher Scientific (Hampton, NH). Activated carbon (AC) derived from coconut shell (mesh size: 100–325; iodine number: 1100 mg/g; bulk density: 30–33 lbs/ft.³) was obtained from the General Carbon Corporation (Paterson, NJ). Calcium montmorillonite (CM) was obtained from Engelhard Corp. (Cleveland, OH). CM has an average total surface area of 850 m²/g, an external surface area of approximately 70 m²/g, and a cation exchange capacity of 97 cmol/kg (Grant and Phillips, 1998). CM has a general formula of (Ca)_{0.3}(Al,Mg)₂Si₄O₁₀(OH)₂·nH₂O (Marroquin-Cardona et al., 2011). Deionized water (18.2 MΩ) was generated in the laboratory using an Elga™ automated filtration system (Woodridge, IL) and was used in all experiments.

1.2. In vitro studies: isothermal adsorption analyses

The AMPA stock solution was prepared by dissolving pure powder in deionized water to yield 1000 µg/mL. AC, CM, and collapsed-CM were used as sorbents for adsorption isotherms. Collapsed-CM was used to evaluate potential binding mechanisms and the importance of CM interlayer surfaces in binding AMPA and was prepared by heating the clay at 200 °C for 30 min, then 800 °C for 1 hr (Grant and Phillips, 1998). Sorbents were added at 0.0005% W/V to AMPA solutions with an increasing concentration gradient. To achieve the 0.0005% inclusion level, 10 µL of 0.5 mg/mL clay suspension in water was added to each sample. Sorbent suspensions were mixed vigorously during transfer to ensure equal distribution of clay to each sample. The concentration gradients of AMPA solutions were achieved by adding a calculated amount of AMPA stock solution to a complementary volume of mobile phase in 1.5 mL centrifuge tubes to a total volume of 1 mL. Additionally, 3 controls were tested (mobile phase, AMPA solution, and 0.0005% sorbent solution). All samples were agitated at 1000 rpm for 2 hr at ambient temperature (25 °C) and high temperature (37 °C) to investigate the thermodynamics of the reaction. Samples were then centrifuged at 2000 × g for 20 min to separate sorbent/AMPA complexes from the solution. The supernatants were collected for analysis.

PMG and AMPA were analyzed using a Waters Acquity® Ultra Performance Liquid Chromatography (UPLC)-Mass Spectrometry (MS)/MS (LC-MS/MS) equipped with a BEH C18 column (50 × 2.1 mm) following a method described by Pereira (2006). Separation was obtained using a mobile phase of water with 0.1% formic acid (eluate A) and acetonitrile with 0.1% formic acid (eluate B) (5%–100% of eluate B in 10 min) at 0.3 mL/min with a 40 µL injection volume and a negative

electrospray ionization mode at 4.5 kV spray voltage. Nitrogen gas was used as the collision gas and curtain gas, and argon gas was used as the nebulizer gas and heater gas. The source temperature was kept at 225 °C for PMG and 450 °C for AMPA. The MS was operated under multiple reaction monitoring (MRM) mode and the monitored precursor and product ions were 168 and 63/81 (PMG) and 110 and 63/79 (AMPA). The unit mass resolution was used for the ion mass analyzer. Empower analyst software was used to control the LC-MS/MS system and acquire the data. To ensure consistency of the detection methods and linearity of peak concentrations for PMG and AMPA, standard solutions of the analytes were prepared in distilled water at concentrations between 10 µg/mL and 0.05 µg/mL to measure standard curves for each set of samples. The standard curves for PMG and AMPA were linear ($r^2 > 0.99$). The limit of detection for both analytes was 0.05 µg/mL.

1.3. Data calculations and curve fitting

AMPA was detected by LC-MS/MS and quantified using standard calibration curves. AMPA concentrations in solution were calculated from peak area at the retention time. The amount adsorbed for each data point was calculated from the concentration difference between test and control groups. The resulting data was input into a Microsoft Excel program developed in our laboratory and plotted using Table-Curve 2D to derive values for each parameter. The best fit for the data was the Langmuir model, which was then used to plot equilibrium isotherms for each analysis. The isotherm equation was entered as a user-defined function,

$$\text{Langmuir model (LM)} \quad q = Q_{\max} \left(\frac{K_d C_w}{1 + K_d C_w} \right)$$

where q (mol/kg) is toxin adsorbed, Q_{\max} (mol/kg) is the maximum capacity, K_d is distribution constant, and C_w is toxin equilibrium concentration. Estimates for the Q_{\max} and K_d were derived from a double logarithmic plot of the data. The plot will normally display a break in the curve, where the value of the X-axis is an estimate of K_d , and the value of the Y-axis is an estimate of Q_{\max} . The K_d value is derived from the Langmuir equation by solving for K_d :

$$K_d = \frac{q}{(Q_{\max} - q)C_w}$$

The enthalpy (ΔH) is a parameter of the thermodynamics of the binding reaction, indicating total heat released or absorbed. It is calculated by the Van't Hoff equation, comparing individual K_d values at two temperatures (25 °C and 37 °C):

$$\Delta H = \frac{-R \ln \left(\frac{K_{d2}}{K_{d1}} \right)}{\left(\frac{1}{T_2} \right) - \left(\frac{1}{T_1} \right)}$$

where R (ideal gas constant) = 8.314 J/(mol K), and T (absolute temperature) = 273 + t (°C).

1.4. In vivo studies: hydra assay

Hydra vulgaris were obtained from Environment Canada (Montreal, QC) and maintained at 18 °C. Using a hydra classification method, morphology of the hydra was rated over time as an indicator of solution toxicity. The morphological scoring of hydra in this assay is objective and repeatable (Brown et al., 2014). A score of 0 to 10 was assigned individually to hydra in each experimental group. Scores of 7 to 9 indicated minor toxicity, 4 to 6 indicated moderate toxicity, and 0 to 3 indicated severe toxicity. The assay included monitoring at shorter time intervals during the first two days (0, 4, 20, and 28 hr) and 24-hr intervals during the last three days (44, 68, 92 hr). Control groups included hydra media (18.2 MΩ water, 4 mg/L EDTA, 115 mg/L N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid (TES), and 147 mg/L CaCl₂ adjusted to pH 6.9–7.0). Chemical exposure groups included 20 µg/mL AMPA solution in hydra media based on the minimum effective dose (MED) that caused 100% mortality of the hydra in 92 hr. Sorbent treatment groups consisted of 20 µg/mL AMPA plus 1.0% W/V sorbent inclusion. The sorbent inclusion level was based on a preliminary dosimetry study that evaluated sorbent inclusion levels between 0.25% and 2% and determined that 1% was the optimal dose. Each group contained 3 adult hydra in 4 mL of test solution and were kept at 18 °C. Solutions were not changed during the testing period. All test groups in disposable culture tubes were capped and agitated at 1000 r/min for 2 hr followed by centrifugation at 2000 × g for 20 min. Groups of hydra were then exposed to each solution and the hydra morphological responses for each group were scored and recorded at each time point. The average score for each group was used to determine the toxicity rating at each time point.

1.5. Soil studies

Garden soil (with a composition including compost, processed forest products, sphagnum peat moss, a wetting agent, and fertilizer containing 0.09% total nitrogen, 0.05% available phosphate, and 0.07% soluble potash) was obtained from The Scotts Miracle-Gro Company (Marysville, OH). Soil was air-dried and sieved through a 1 mm screen before use. Each 1 g soil sample in a disposable culture tube was spiked with 2 mL of 1.0 µg/mL PMG/acetone solution and thoroughly mixed to ensure even distribution of PMG. Samples were left uncapped in a fume hood for 7 days allowing the metabolism of PMG and evaporation of the acetone (Giesy et al., 2000). Sorbents were added at 1% W/W to soil samples and thoroughly mixed. The sorbent inclusion level was based on a preliminary study that evaluated doses of 0.5%, 1%, 2%, and 5% and determined that 1% was the optimal dose. Samples were hydrated by adding 4 mL of distilled water and then slowly agitating at 200 r/min for 24 hr. A soil extraction method (Chamkasem and Harmon, 2016) with modification was used to extract AMPA from soil samples. Briefly, 4 mL of 50 mmol/L acetic acid/10 mmol/L Na₂-EDTA (572 µL acetic acid and 0.74 g Na₂-EDTA in 200 mL water) was added to each sample before agitating at 1000 r/min for 1 hr. Samples were centrifuged at 2000 × g for 20 min and the supernatants were transferred to new culture tubes. The supernatants were then passed through Strata C18-

E (55 µm, 70A) columns preconditioned with 2 mL methanol and 2 mL water. Extracts were analyzed for AMPA using LC-MS/MS. Calibration curves were conducted for each group of extracts to ensure linearity of peak concentrations and consistency of the extraction method. Controls for each group of extracts included blank soil, soil spiked with PMG, and soil with sorbent. Peak areas of AMPA from samples that included sorbents were compared to the AMPA control samples to determine the percent reduction in AMPA bioavailability from soil.

1.6. Plant uptake studies

Compost (with a composition including aged forest products, sphagnum peat moss, perlite, sandy loam, and fertilizer containing 0.30% total nitrogen, 0.45% available phosphate, and 0.05% soluble potash, and 1.00% calcium derived from fish emulsion, crab meal, shrimp meal, earthworm castings, bat guano, kelp meal and oyster shell) was obtained from Fox-farm Soil & Fertilizer Company (Humboldt County, California). Hybrid Seed Corn (217-76STX) was obtained from Channel Bio, LLC (St. Louis, MO). Compost was air-dried and sieved through a 1 mm screen before use. These studies were conducted using compost and garden soil to evaluate any differences between the two media. For each study, six experimental groups including: soil or compost control; chemical control with 2 µg/mL PMG; two sorbent controls at 1% in base media; and two treatment groups of 1% sorbent and 2 µg/mL PMG were set up with six planters in each group. The dose of 1% sorbent in these studies was determined from the hydra and soil bioavailability studies. Each planter contained 10 g of soil/compost hydrated with 20 mL water. GM corn seeds were planted with at least 2 seeds per planter to ensure an adequate number of viable sprouts. Planters were monitored and watered throughout the study to ensure adequate hydration. Seeds were allowed to germinate and sprout for 7 days before planters were moved to hydroponic systems containing 1.5 L nutrient solution (potassium nitrate, calcium nitrate, monopotassium phosphate, potash, magnesium sulfate, and trace elements at 2.9–3.2 EC and 6.1 pH) on day 7 (Fig. 1). Hydroponic systems were used to simulate flood scenarios during which roots might be exposed to higher levels of water and potentially higher levels of contaminants such as pesticides. GM corn roots had significantly grown into the nutrient solutions after 3 days. Experimental groups were administered 500 mL of nutrient solution containing 5 µg/mL for chemical controls, 1% sorbent for sorbent controls and 5 µg/mL plus 1% sorbent for treatment groups. After exposure periods ranging from 1 day to 10 days, planters were removed from hydroponic systems. Roots and sprouts were separated, washed thoroughly and carefully with deionized water, and air-dried before weights were measured. Samples were then ground to a powder-like texture.

Extraction methods described by Chamkasem and Harmon (2016) were followed with modification. Briefly, 5 mL of 50 mmol/L acetic acid/10 mmol/L Na₂-EDTA was added to each sample in disposable culture tubes. Samples were homogenized for 5 min using a 150 Homogenizer and 7 mm Generator Probe (Fisher Scientific, Waltham, MA). The tubes were capped and agitated at 1000 rpm for 20 min and then centrifuged at

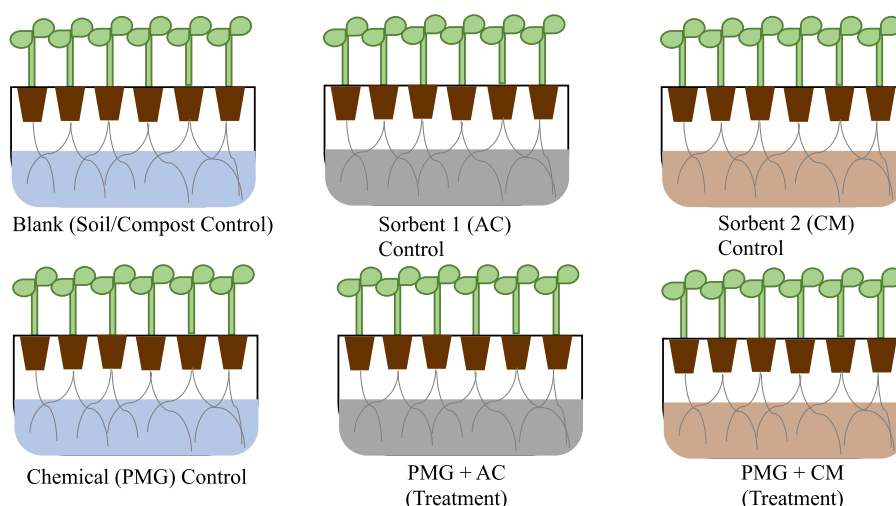


Fig. 1 – Plant uptake experimental design. These studies were used to determine sorbent ability to reduce plant uptake of chemicals from soil. Our hydroponic system simulates a flood scenario, during which plant roots would be exposed to high levels of chemical contaminants in floodwaters.

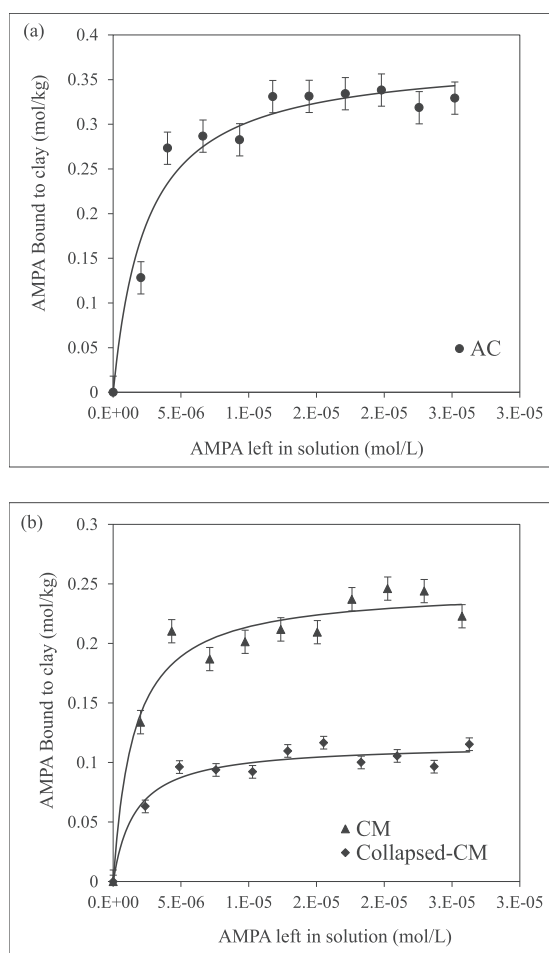


Fig. 2 – (a) Langmuir plots of AMPA on AC at 25 °C indicating binding of AMPA. (b) Langmuir plots of AMPA binding to CM and collapsed CM at 25 °C. Q_{\max} value was significantly decreased with collapsed CM clay, indicating interlayer surfaces are important in AMPA binding to CM clay.

2000 \times g for 20 min. The supernatants were passed through Strata C18-E (55 μ m, 70A) columns preconditioned with 2 mL methanol and 2 mL water. 2 mL of the extracts were added to autosampler vials and analyzed using LC-MS/MS.

1.7. Molecular model

The molecular model of CM clay and AMPA was constructed following a method described by Wang et al. (2017). Briefly, the molecular model for CM clay was drawn in ISIS Draw 2.0 and then imported into HyperChem 8.0. The model was constructed using the unit cell coordinates of muscovite, which were then converted to orthogonal coordinates in an Excel spreadsheet (constructed from a public domain C program). The unit cells were replicated in three-dimensional space by applying the symmetry operations for a C2/c space group. Finally, the d_{001} spacing of the model was set to the corresponding dimensions of the exchanged montmorillonite (21 Å). The AMPA structure was energy-minimized using the semiempirical quantum mechanical AM1 method. AMPA was inserted into the interlayer and on the external surface to illustrate the proposed sites of AMPA adsorption to CM.

1.8. Statistical analysis

A one-tailed t-test was used to determine statistical significance. Each experiment was conducted in triplicate to derive means and standard deviations for the following: (1) Q_{\max} and K_d values from isothermal analyses, (2) toxicity scores from the hydra assay, (3) concentrations of AMPA in soils and plants, and (4) root weight and sprout length and weight. These were compared using a Tukey test. T-values and degrees of freedom were used to determine the p-value. Results were considered significant at $p \leq 0.05$.

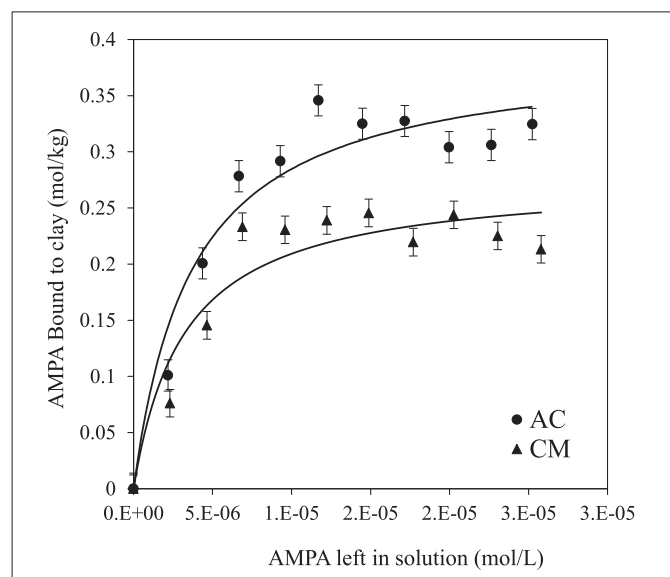


Fig. 3 – Langmuir plots of AMPA on CM and AC at 37 °C (CM: $K_d = 3.10E+05$; AC: $K_d = 2.70E+05$). K_d values for these plots were compared to those conducted at 25 °C to determine the enthalpy (ΔH) of the binding reactions. ΔH values for CM and AC were -48.2 and -26.6 kJ/mol, respectively.

2. Results

Adsorption isotherms were plotted using a Microsoft Excel program developed in our laboratory and generated with TableCurve 2D software to derive sorbent-chemical binding parameters. The Langmuir equation was used to determine Q_{max} (capacity) and K_d (affinity) values for AMPA binding to AC and CM. All plots fit the Langmuir model with r^2 above 0.95. The Q_{max} and K_d values along with the curved shape of the Langmuir plot indicated saturable binding of AMPA by AC and CM (Fig. 2a and b), and saturable binding of AMPA on active sorbent surfaces. The isothermal plot for collapsed-CM clay had a Q_{max} value of 0.116 mol/kg (Fig. 2b). Compared to the Q_{max} value of 0.246 mol/kg for CM, collapsed CM clay showed 53% less binding than the intact CM clay.

Thermodynamic studies were used to determine the heat of sorption (enthalpy) of the binding reactions. Isotherms for AC and CM were conducted at two temperatures (37 °C and 25 °C) to calculate the enthalpy (ΔH) of the binding reaction of AMPA with sorbents (Fig. 3). The K_d values for AC and CM isotherms conducted at 25 °C were compared to K_d values from isotherms conducted at 37 °C using the Van't Hoff equation. ΔH values for AC and CM were -26.6 kJ/mol and -48.2 kJ/mol, respectively.

The hydra assay in Fig. 4a shows the efficacy of AC, CM, and a 50:50 mixture of AC:CM in protecting the hydra from AMPA toxicity. Groups of hydra were exposed to 20 µg/mL AMPA based on the minimum effective dose (MED) that caused 100% mortality of the hydra in 92 hr. The control group in hydra media (without sorbent or AMPA inclusion) and sorbent controls had scores of 10 throughout the exposure period. Treatment

groups received 1% sorbent inclusion, which resulted in 90% protection of the hydra with all 3 tested sorbents ($p \leq 0.01$), indicating significant protection of hydra from AMPA toxicity with addition of AC, CM, and 50:50 AC:CM. The hydra assay in Fig. 4b shows toxicity of 20 µg/mL PMG over the 92 hr exposure period.

Soil studies were conducted to determine the potential of AC, CM, and 50:50 AC:CM to reduce the bioavailability of AMPA from soil. Soil samples (1.0 g) were spiked with 2 mL of 1.0 µg/mL AMPA solution in acetone. Recovery of AMPA from soil samples was 75%, which is consistent with % recoveries reported in the literature (Druart et al., 2011; Laitinen et al., 2009). AMPA recovery from soil samples in Fig. 5 shows that 1% W/W AC, CM, and 50:50 AC:CM significantly reduced AMPA bioavailability by 52.7%, 43.5%, and 56.6% respectively ($p \leq 0.01$).

A time course study was conducted to investigate PMG and AMPA uptake by GM corn sprouts and metabolism of PMG to AMPA over a 10-day exposure period (Fig. 6). All experimental groups were exposed to 2 µg/mL PMG in the soil and were flooded with 5 µg/mL PMG solution. The 6 groups were removed from hydroponic systems on days 1, 2, 4, 6, 8, and 10 after exposure. Throughout the 10-day exposure period, average growth rates of root dry weight, sprout dry weight and sprout length were 1.5 mg/day (Fig. 6a), 45.6 mg/day (Fig. 6b), and 29 mm/day (Fig. 6c). These measurements for treatment groups were not significantly different than the control groups. Over the 10-day exposure period, PMG concentrations decreased from 9.3 to 5.6 µg/g in roots and from 20.5 to 5.4 µg/g in sprouts (Fig. 6d and e). AMPA concentrations increased from 1.9 to 3.4 µg/g in roots and from 9.1 to 11.3 µg/g in sprouts (Fig. 6f and g).

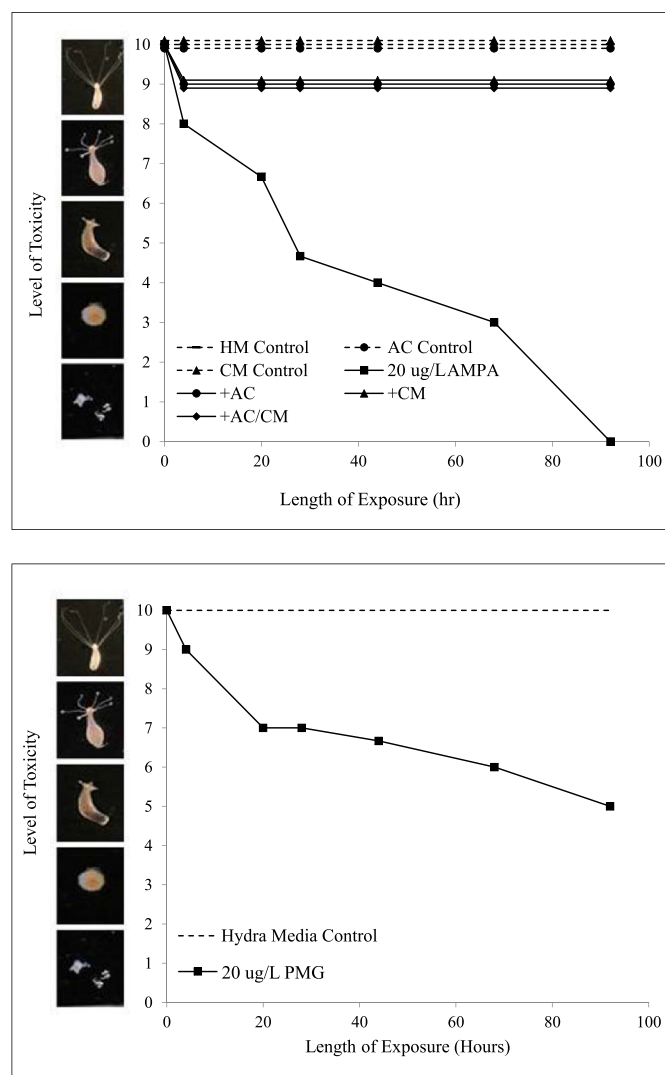


Fig. 4 – (a) Hydra assay showing AMPA toxicity at 20 µg/mL and the protection of hydra with inclusion of 1.0% W/V AC, CM, and 50:50 AC:CM. Control groups with no AMPA (hydra media and sorbent controls) are included for comparison and show scores of 10 throughout the test period. Sorbent inclusion at 1.0% was able to significantly protect hydra from AMPA toxicity (90% protection). ($p \leq 0.01$). (b) Toxicity of 20 µg/mL PMG resulting in moderate toxicity to the hydra.**

Plant studies were conducted using both compost and garden soil to evaluate potential differences in GM corn uptake of PMG and AMPA from media with varying compositions (Fig. 7). No significant differences were seen between root/sprout dry weights or sprout length measurements for control groups (blank soil, AC control, CM control) and treatment groups (PMG control, PMG + AC, and PMG + CM). Plant growth was favored and less adversely affected by PMG in compost in terms of root and sprout dry weight ($*p \leq 0.05$) (Fig. 7a and c). In corn roots, uptake of AMPA in the PMG control group was 80% higher in the compost group than in the soil group ($**p \leq 0.01$) (Fig. 7e). AMPA was non-detectable in the soil treatment groups. Based on the limit of detection of 0.05 µg/g, uptake of AMPA in the PMG + 1% AC and PMG + 1% CM treatment groups was at least 80% and 78% higher in the compost study than the soil study ($**p \leq 0.01$). In corn sprouts, uptake of AMPA in PMG control,

PMG + 1% AC treatment, and PMG + 1% CM treatment groups was 48%, 54%, and 61% higher, respectively, in the compost study than in the soil study ($**p \leq 0.01$) (Fig. 7d). In terms of reduction of AMPA uptake with sorbent inclusion in the compost study, 1% AC resulted in 55% and 56% reduction, and 1% CM resulted in 58% and 47% reduction in AMPA residues measured in corn roots and sprouts, respectively. In the soil study, 1% AC and 1% CM resulted in 61% and 60% reduction in AMPA residues measured in corn sprouts, respectively. In corn roots, 0.11 µg/g of AMPA was measured in the PMG control group, and was non-detectable in the AC and CM treatment groups. Based on the limit of detection of 0.05 µg/g, 1% AC and CM inclusion significantly reduced AMPA residues in sprouts (at least 55%). The results of these studies suggest AC and CM are able to significantly reduce AMPA uptake in GM corn roots and sprouts.

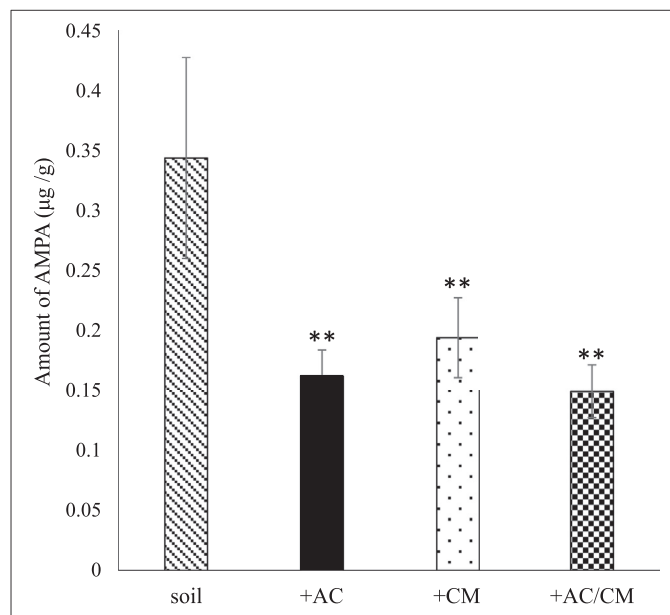


Fig. 5 – Soil studies showing significant reduction of AMPA bioavailability with inclusion of 1% AC, CM, and 50:50 AC:CM at 52.7%, 43.5%, and 56.6%, respectively. ($p \leq 0.01$)**

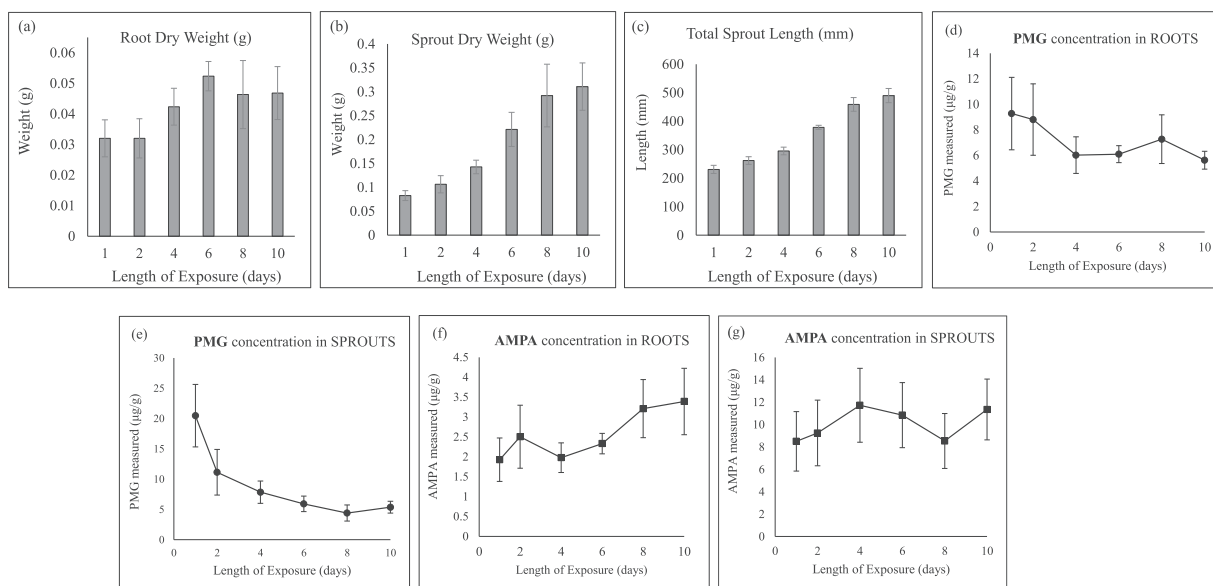


Fig. 6 – Time course plant study showing uptake of PMG and conversion to the metabolite AMPA. Root dry weight (a), sprout dry weight (b) and total sprout length (c) were measured over the duration of the 10-day exposure period. PMG and AMPA concentrations were measured in both the roots (d, f) and sprouts (e, g).

3. Discussion

AMPA (following the breakdown of PMG) can accumulate in soil and sediment. PMG and AMPA have $\log P$ values of -3.40 and -4.70, respectively, confirming that they are soluble in water and can be easily mobilized and redistributed, especially in cases of floods or storms. PMG and AMPA can be mobilized from soil and sediment by rainfall, redistributed

via runoff, and affect surface water and groundwater sources (Battaglin et al., 2014; Daouk et al., 2013; Scribner et al., 2007). Additionally, phosphate fertilization of soils has been shown to contribute to PMG remobilization due to competition between PMG and phosphate for adsorption sites in soil (Bott et al., 2011). Many studies have evaluated PMG bioavailability and strategies to reduce exposures, but few have focused on its major metabolite AMPA. There is a lack of information on safe and economical methods to reduce AMPA

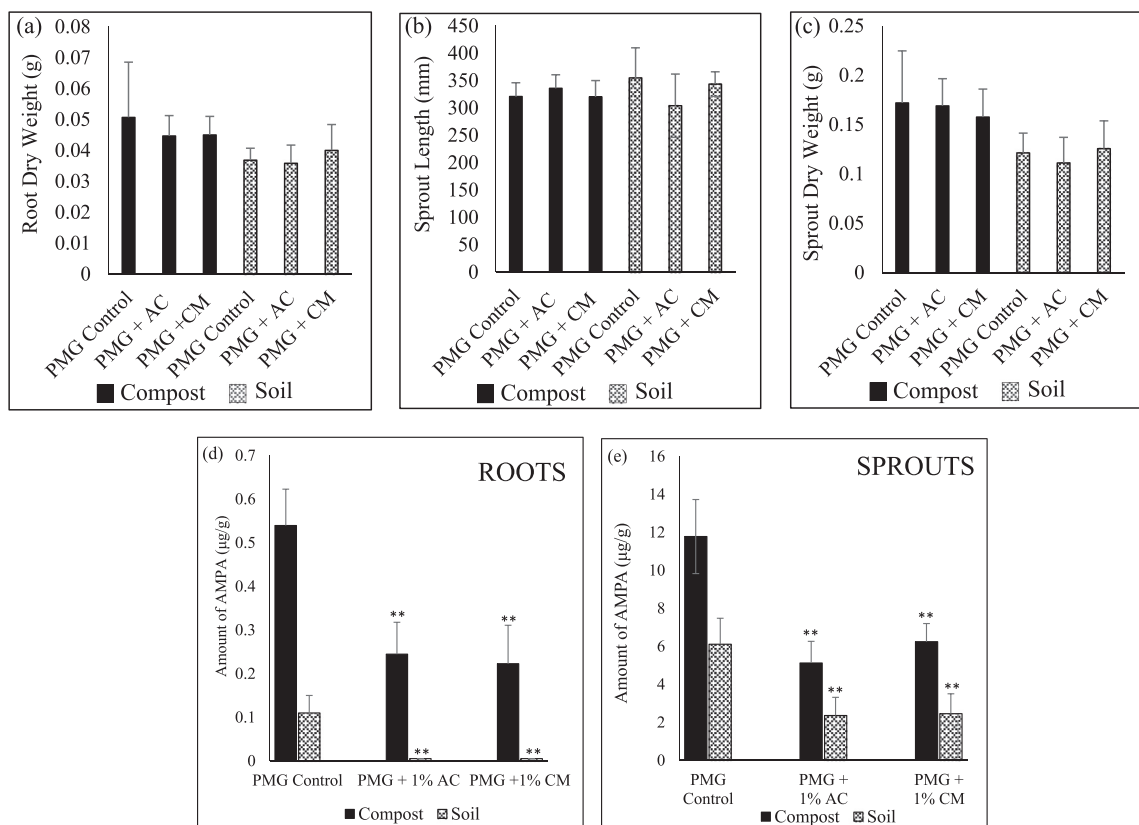


Fig. 7 – Plant uptake study showing AMPA residues in corn roots and sprouts for sprouts grown in both compost and soil. No significant differences were seen between groups for root dry weight (a), sprout dry weight (b), and total sprout length (c). 1% AC and CM were both able to significantly reduce AMPA residues in corn roots (d) and sprouts (e) in both the compost and soil studies ($p \leq 0.01$)**

residues in the environment. The use of activated carbon and clay sorbents to mitigate unintended exposures to AMPA in soil and plant products could be a promising strategy. We characterized AMPA sorption onto AC and CM to develop safe and effective soil amendments that can be used to reduce the bioavailability of AMPA in soil and plants.

Isothermal analyses were conducted to derive insight into AMPA binding to sorbent surfaces. Both isotherms fit Langmuir trends with high Q_{\max} and K_d values, indicating efficient binding of AMPA onto saturable binding sites. Q_{\max} values (0.376 and 0.247 mol/kg for AC and CM, respectively) indicated high capacities of both sorbents for AMPA, and K_d values ($4.09\text{E}+05$ and $6.05\text{E}+05$ for AC and CM, respectively) indicated high affinities of the sorbents for AMPA (Table 1).

CM clay has negatively charged interlayer surfaces, which are often important binding sites for various chemicals (Grant and Phillips, 1998; Wang et al., 2019c). To investigate the importance of these sites in AMPA binding to CM, the clay was heated to collapse interlayer surfaces. This heating process has been shown to significantly dehydroxylate the CM clay structure and collapse interlayer surfaces to decrease potential binding sites. There was a 53% decrease in Q_{\max} of the collapsed CM compared to the intact CM (under the experimental conditions in this study), suggesting that the interlayer accounts for a large portion of AMPA binding to CM. Other

Table 1 – Summary of Q_{\max} and K_d values for all AC and CM adsorption isotherms. *PMG Q_{\max} and K_d values are from isotherms conducted with 10 µg/mL PMG and 0.002% W/V sorbent inclusion (Wang et al., 2019a).

		Q_{\max}	K_d
AMPA	CM @ 25°C	0.247	6.58E+05
	CM @ 37°C	0.276	3.10E+05
	Collapsed-CM @ 25°C	0.116	6.18E+05
	AC @ 25°C	0.376	4.09E+05
	AC @ 37°C	0.390	2.70E+05
PMG*	CM	0.340	2.00E+05
	AC	0.550	5.64E+05

binding interactions may involve reactions at edge sites and on the basal surfaces (Fig. 8).

Thermodynamic studies were used to evaluate the binding reactions of AMPA onto sorbent active surfaces. The negative ΔH values for CM and AC (-48.2 and -26.6 kJ/mol, respectively) indicated that AMPA binding to sorbent surfaces involved exothermic reactions. The high negative value for CM indicated that AMPA binding to clay surfaces was tight and in-

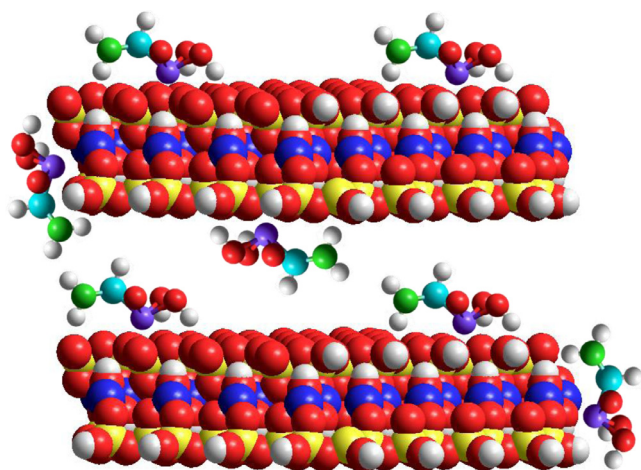


Fig. 8 – Molecular model of geometrically optimized CM clay layers and AMPA molecules showing predicted binding of AMPA onto CM surfaces. Binding sites may include interlayer surfaces, basal surfaces, and edge sites.

involved chemisorption mechanisms. This result agrees with a previous study where AMPA predominantly bound to CM via covalent interlayer surface complexes through the phosphate moiety (Renning et al., 2008). The lower negative value for AC indicated that AMPA binding to carbon surfaces might include weaker bonds such as hydrogen bonds, dipole-dipole interactions, and Van der Waals attractions (i.e., physisorption).

In the hydra assay, all 3 sorbents including AC, CM, and 50:50 AC:CM were not toxic to the hydra with inclusion levels as high as 1.0% W/V. All sorbents significantly protected the hydra from AMPA toxicity. Inclusion of 1.0% sorbent reduced severe toxicity (score of 0) of 20 µg/mL AMPA to minor toxicity (score of 9). These results agree with the high efficacy shown in isothermal and thermodynamic analyses, further indicating that AC and CM are effective sorbents of AMPA. Additionally, the hydra assay in Fig. 4b shows that 20 µg/mL PMG resulted in moderate toxicity (score of 5) to the hydra, suggesting that PMG may be less toxic to the hydra than AMPA.

All 3 sorbents tested at 1% inclusion were able to significantly reduce AMPA bioavailability from soil with 52.7%, 43.5%, and 56.6% reduction for AC, CM, and 50:50 AC:CM, respectively. The 50:50 AC:CM combination resulted in the highest reduction in AMPA bioavailability. This could be attributed to the contribution of diverse binding sites for AC and CM, resulting in an additive effect. The efficacy of sorbent treatment in soils is consistent with previous reports where clay-based soils showed reduced bioavailability of PMG and AMPA, whereas, sandy soils had more bioavailable PMG and AMPA (Bergstrom et al., 2011; Giesy et al., 2000). We expect that at exposure levels of PMG that are commonly detected in the environment, there will be a significant reduction in AMPA bioavailability. Since previous thermodynamic studies suggested that AMPA-sorbent complexes would be stable in a soil environment and carbon and clays are natural components of many soil types, inclusion of these materials as soil amendments should have limited adverse environmental effects (Ito et al., 2017).

To further investigate the sorbent treatment for plants, a time course study was conducted to evaluate PMG and AMPA levels in GM corn roots and sprouts over a 10-day exposure period. Measurements for root and sprout dry weight and sprout length were not significantly different between the control group (data not shown) and PMG exposure groups, indicating PMG did not have significant adverse effects on growth based on these measurements. On day 1, 1.93 and 9.11 µg/g of AMPA were measured in GM corn roots and sprouts, respectively. This indicates that PMG and AMPA could be taken up from soil and metabolism of PMG to AMPA could occur in plant tissues during the growth phase as well as the exposure phase. PMG levels steadily decreased in both roots and sprouts over the 10-day exposure period, and AMPA levels steadily increased in roots and remained relatively constant in sprouts. With a longer exposure period, further decreases in PMG residues and possible increases in AMPA residues are expected. By the end of the exposure period, 36.5% and 55.4% of the PMG measured in roots and sprouts on day 1 had metabolized to AMPA. These results are consistent with previous studies that showed up to 70% metabolism of PMG to AMPA (Sun et al., 2019).

Plant studies using GM corn were conducted to investigate effects of compost versus soil during plant growth and toxin uptake. No significant differences were seen in sprout length between compost and soil studies. Root dry weight was significantly higher in the compost study than the soil study for PMG control and PMG + AC treatment groups. Sprout dry weight was significantly greater in the compost study than the soil study for all three test groups. These differences could be attributed to higher nutrient content that is associated with compost. Importantly, AC and CM treatment did not have any negative effects on plant growth based on root and sprout dry weights and sprout length. AMPA residues were significantly higher in all compost groups than soil groups. This could be due to greater PMG sorption to soil particles than to compost particles, resulting in greater PMG available for metabolism and translocation to corn in the compost groups. Additionally, physical properties including hydraulic conductivity, moisture content, and microbial respiration could contribute to variable uptake of AMPA from compost and soil (Erban et al., 2018). Importantly, both sorbents administered in the hydroponic system at 1% significantly reduced AMPA residues in GM corn roots and sprouts grown in both garden soil and compost by 47% - 61%. Studies have investigated the use of biochars in soil at levels up to 5% W/W (Graber et al., 2010; Spokas et al., 2009). It is possible that sorbents at higher inclusion will result in more significant reduction based on previous dosimetry studies (Maki et al., 2016; Mitchell et al., 2014; Phillips et al., 2008). These results indicate that AC and CM could be used as soil amendments to reduce AMPA residues in GM corn roots and sprouts. Further studies with longer exposure periods (2-3 months) are needed to investigate PMG/AMPA uptake into GM corn kernels. It is important to note that plant studies were carried out under laboratory conditions that do not necessarily represent typical environmental conditions. These studies were meant to simulate uptake of PMG and AMPA from soil and water and to demonstrate sorbent ability to reduce translocation of these chemicals to GM corn roots and sprouts. Additionally, the hydroponic system allowed us to simulate a “flood” scenario during which plant roots may be exposed

to higher volumes of water. In this experimental setup, roots were directly exposed to nutrient solutions containing PMG. Previous plant studies conducted in our laboratory evaluated the effect of adding 5% compost (100 g) to the nutrient solution and found that it did not have any significant effects on levels of AMPA residues measured in roots/sprouts or the efficacy of the sorbents in reducing chemical translocation to plants. Further studies are needed to evaluate the efficacy of sorbents in field applications.

4. Conclusion

Results in this study indicate that activated carbon derived from coconut shell and calcium montmorillonite clay are effective sorbents of AMPA. The efficacy of this treatment is supported by isothermal, thermodynamic, and hydra results, showing that both sorbents can tightly bind AMPA with high capacity, affinity and enthalpy and can protect a living organism from AMPA toxicity. Soil and plant studies further showed that the sorbents at 1% inclusion can significantly reduce AMPA bioavailability from soil and uptake by plants and might be important in protecting plants from AMPA toxicity. The main novelty of this work is the use of AC and/or montmorillonite clay to sorb AMPA (an important metabolite of glyphosate) and reduce its translocation into genetically modified plants. The costs of AC and CM clays produced for environmental applications (AC) or animal feed (CM) are similar to the costs of commonly-used biochar products. Thus, AC and CM would be economically feasible for use as soil amendments. Future studies including ACs from different sources, CM, and a mixture of carbonaceous and clay-based sorbents will investigate the effects of optimal sorbents on nutrient utilization in plants and the mitigation of other environmentally relevant chemicals in lawns and gardens following natural disasters, chemical spills, and emergencies.

Acknowledgements

This work was supported by funding through NIEHS SRP (Superfund Hazardous Substance Research and Training Program), P42 ES0277704, and USDA Hatch 6215.

Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.06.029.

REFERENCES

- Arregui, M.C., Lenardón, A., Sanchez, D., Maitre, M.I., Scotta, R., Enrique, S., 2004. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. *Pest Manag. Sci.* 60 (2), 163–166. doi:10.1002/ps.775.
- Bai, S.H., Ogbourne, S.M., 2016. Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. *Environ. Sci. Pollut. Res.* 23 (19), 18988–19001. doi:10.1007/s11356-016-7425-3.
- Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *J. Am. Water Resour. Assoc.* 50 (2), 275–290. doi:10.1111/jawr.12159.
- Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* 28 (1), 3. doi:10.1186/s12302-016-0070-0.
- Benbrook, C.M., 2019. How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides. *Environ. Sci. Eur.* 31 (1), 2. doi:10.1186/s12302-018-0184-7.
- Bergström, L., Börjesson, E., Stenström, J., 2011. Laboratory and lysimeter studies of glyphosate and aminomethylphosphonic acid in a sand and a clay soil. *J. Environ. Qual.* 40 (1), 98–108. doi:10.2134/jeq2010.0179.
- Bott, S., Tesfamariam, T., Kania, A., Eman, B., Aslan, N., Römhild, V., Neumann, G., 2011. Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation. *Plant Soil* 342 (1), 249–263. doi:10.1007/s11104-010-0689-3.
- Brown, K.A., Mays, T., Romoser, A., Marroquin-Cardona, A., Mitchell, N.J., Elmore, S.E., Phillips, T.D., 2014. Modified hydra bioassay to evaluate the toxicity of multiple mycotoxins and predict the detoxification efficacy of a clay-based sorbent. *J. Appl. Toxicol.* 34 (1), 40–48. doi:10.1002/jat.2824.
- Chamkasem, N., Harmon, T., 2016. Direct determination of glyphosate, glufosinate, and AMPA in soybean and corn by liquid chromatography/tandem mass spectrometry. *Anal. Bioanal. Chem.* 408 (18), 4995–5004. doi:10.1007/s00216-016-9597-6.
- Daouk, S., De Alencastro, L.F., Pfeifer, H.-R., 2013. The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: Proof of widespread export to surface waters. Part II: the role of infiltration and surface runoff. *J. Environ. Sci. Health Part B* 48 (9), 725–736. doi:10.1080/03601234.2013.780548.
- Ding, W., Reddy, K.N., Zablotowicz, R.M., Bellaloui, N., Arnold Bruns, H., 2011. Physiological responses of glyphosate-resistant and glyphosate-sensitive soybean to aminomethylphosphonic acid, a metabolite of glyphosate. *Chemosphere* 83 (4), 593–598. doi:10.1016/j.chemosphere.2010.12.008.
- Druart, C., Delhomme, O., de Vaufléury, A., Ntcho, E., Millet, M., 2011. Optimization of extraction procedure and chromatographic separation of glyphosate, glufosinate and aminomethylphosphonic acid in soil. *Anal. Bioanal. Chem.* 399 (4), 1725–1732. doi:10.1007/s00216-010-4468-z.
- Erban, T., Stehlik, M., Sopko, B., Markovic, M., Seifrtova, M., Halesova, T., Kovaricek, P., 2018. The different behaviors of glyphosate and AMPA in compost-amended soil. *Chemosphere* 207, 78–83. doi:10.1016/j.chemosphere.2018.05.004.
- Giesy, J.P., Dobson, S., Solomon, K.R., 2000. Ecotoxicological risk assessment for Roundup® herbicide. *Rev. Environ. Contam. Toxicol.* 35–120. doi:10.1007/978-1-4612-1156-3_2.
- Gomes, M.P., Le Manac'h, S.G., Maccario, S., Labrecque, M., Lucotte, M., Juneau, P., 2016. Differential effects of glyphosate and aminomethylphosphonic acid (AMPA) on photosynthesis and chlorophyll metabolism in willow plants. *Pestic. Biochem. Physiol.* 130, 65–70. doi:10.1016/j.pestbp.2015.11.010.
- Graber, E.R., Meller Harel, Y., Koltun, M., Cytryn, E., Silber, A., Rav David, D., Tsechansky, L., Borenshtein, M., Elad, Y., 2010. Biochar impact on development and productivity of pepper and tomato grown in fertilized soilless media. *Plant Soil* 337 (1), 481–496. doi:10.1007/s11104-010-0544-6.
- Grant, P.G., Phillips, T.D., 1998. Isothermal adsorption of aflatoxin B1 on HSCAS clay. *J. Agric. Food Chem.* 46 (2), 599–605. doi:10.1021/jf970604v.

- Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K., 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol.* 16 (5), 490–491. doi:[10.1016/s1470-2045\(15\)70134-8](https://doi.org/10.1016/s1470-2045(15)70134-8).
- Hagner, M., Hallman, S., Jauhiainen, L., Kemppainen, R., Rämö, S., Tiilikka, K., Setälä, H., 2015. Birch (*Betula* spp.) wood biochar is a potential soil amendment to reduce glyphosate leaching in agricultural soils. *J. Environ. Manage.* 164, 46–52. doi:[10.1016/j.jenvman.2015.08.039](https://doi.org/10.1016/j.jenvman.2015.08.039).
- Hall, K.E., Spokas, K.A., Gamiz, B., Cox, L., Papiernik, S.K., Koskinen, W.C., 2018. Glyphosate sorption/desorption on biochars – interactions of physical and chemical processes. *Pest Manag. Sci.* 74 (5), 1206–1212. doi:[10.1002/ps.4530](https://doi.org/10.1002/ps.4530).
- Hearon, S.E., Wang, M., Phillips, T.D., 2020. Strong adsorption of dieldrin by parent and processed montmorillonite clays. *Environ. Toxicol. Chem.* 39 (3), 517–525. doi:[10.1002/etc.4642](https://doi.org/10.1002/etc.4642).
- Ito, A., Wagai, R., 2017. Global distribution of clay-size minerals on land surface for biogeochemical and climatological studies. *Sci. Data* 4 (1), 170103.
- Junqueira, L.V., Mendes, K.F., Sousa, R.N.d., Almeida, C.d.S., Alonso, F.G., Tornisiello, V.L., 2019. Sorption-desorption isotherms and biodegradation of glyphosate in two tropical soils aged with eucalyptus biochar. *Arch. Agron. Soil Sci.* 1–17. doi:[10.1080/03650340.2019.1686139](https://doi.org/10.1080/03650340.2019.1686139).
- Kaushik, C.P., Sharma, H.R., Kaushik, A., 2012. Organochlorine pesticide residues in drinking water in the rural areas of Haryana, India. *Environ. Monit. Assess.* 184 (1), 103–112. doi:[10.1007/s10661-011-1950-9](https://doi.org/10.1007/s10661-011-1950-9).
- Khoury, G.A., Gehris, T.C., Tribe, L., Torres Sánchez, R.M., dos Santos Afonso, M., 2010. Glyphosate adsorption on montmorillonite: An experimental and theoretical study of surface complexes. *Appl. Clay Sci.* 50 (2), 167–175. doi:[10.1016/j.clay.2010.07.018](https://doi.org/10.1016/j.clay.2010.07.018).
- Laitinen, P., Rämö, S., Nikunen, U., Jauhiainen, L., Siimes, K., Turtola, E., 2009. Glyphosate and phosphorus leaching and residues in boreal sandy soil. *Plant Soil* 323 (1), 267–283. doi:[10.1007/s11104-009-9935-y](https://doi.org/10.1007/s11104-009-9935-y).
- Maki, C.R., Monteiro, A.P.A., Elmore, S.E., Tao, S., Bernard, J.K., Harvey, R.B., Romoser, A.A., Phillips, T.D., 2016. Calcium montmorillonite clay in dairy feed reduces aflatoxin concentrations in milk without interfering with milk quality, composition or yield. *Anim. Feed Sci. Technol.* 214, 130–135. doi:[10.1016/j.anifeedsci.2016.02.014](https://doi.org/10.1016/j.anifeedsci.2016.02.014).
- Marroquín-Cardona, A., Deng, Y., García-Mazcorro, J., Johnson, N.M., Mitchell, N., Tang, L., Robinson, A., Taylor, J.F., Wang, J.-S., Phillips, T.D., 2011. Characterization and safety of uniform particle size NovaSil clay as a potential aflatoxin enterosorbent. *Appl. Clay Sci.* 54 (3–4), 248–257. doi:[10.1016/j.clay.2011.09.009](https://doi.org/10.1016/j.clay.2011.09.009).
- Mesnager, R., Arno, M., Costanzo, M., Malatesta, M., Séralini, G.-E., Antoniou, M.N., 2015. Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. *Environ. Health* 14 (1), 70. doi:[10.1186/s12940-015-0056-1](https://doi.org/10.1186/s12940-015-0056-1).
- Mitchell, N.J., Kumi, J., Aleser, M., Elmore, S.E., Rychlik, K.A., Zychowski, K.E., Romoser, A.A., Phillips, T.D., Ankrah, N.-A., 2014. Short-term safety and efficacy of calcium montmorillonite clay (UPSN) in children. *Am. J. Trop. Med. Hyg.* 91 (4), 777–785. doi:[10.4269/ajtmh.14-0093](https://doi.org/10.4269/ajtmh.14-0093).
- Morillo, E., Undabeytia, T., Maqueda, C., 1997. Adsorption of glyphosate on the clay mineral montmorillonite: effect of Cu(II) in solution and adsorbed on the mineral. *Environ. Sci. Technol.* 31 (12), 3588–3592. doi:[10.1021/es970341l](https://doi.org/10.1021/es970341l).
- Pereira, L., 2006. LC/MS of Glyphosate and AMPA. *Thermo Fisher Scientific, Inc., Runcorn, Cheshire, UK*.
- Phillips, T.D., Afriyie-Gyawu, E., Williams, J., Huebner, H., Ankrah, N.A., Ofori-Adjei, D., Jolly, P., Johnson, N., Taylor, J., Marroquín-Cardona, A., Xu, L., Tang, L., Wang, J.S., 2008. Reducing human exposure to aflatoxin through the use of clay: a review. *Food Addit. Contam. Part A* 25 (2), 134–145. doi:[10.1080/02652030701567467](https://doi.org/10.1080/02652030701567467).
- Phillips, T.D., Wang, M., Elmore, S.E., Hearon, S., Wang, J.-S., 2019. NovaSil clay for the protection of humans and animals from aflatoxins and other contaminants. *Clays Clay Miner.* 67 (1), 99–110. doi:[10.1007/s42860-019-0008-x](https://doi.org/10.1007/s42860-019-0008-x).
- Pimentel, D., 2009. Environmental and economic costs of the application of pesticides primarily in the United States. *Integr. Pest Manage.* 1, 89–111. doi:[10.1007/978-1-4020-8992-3_4](https://doi.org/10.1007/978-1-4020-8992-3_4).
- Reddy, K.N., Rimando, A.M., Duke, S.O., 2004. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *J. Agric. Food Chem.* 52 (16), 5139–5143. doi:[10.1021/jf049605v](https://doi.org/10.1021/jf049605v).
- Rennig, A., Slutter, A., Tribe, L., 2008. Interactions of aminomethylphosphonic acid and sarcosine with montmorillonite interlayer surfaces. *Int. J. Quantum Chem.* 108 (3), 538–543. doi:[10.1002/qua.21525](https://doi.org/10.1002/qua.21525).
- Scribner, E.A., Battaglin, W.A., Gilliom, R.J., Meyer, M.T., 2007. Concentrations of Glyphosate, Its Degradation Product, Aminomethylphosphonic Acid, and Glufosinate in Ground- and Surface-Water, Rainfall, and Soil Samples Collected in the United States, 2001–06: U.S. p. 111 Geological Survey Scientific Investigations Report 2007-5122.
- Séralini, G.-E., Clair, E., Mesnager, R., Gress, S., Defarge, N., Malatesta, M., Hennequin, D., de Vendômois, J.S., 2014. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environ. Sci. Eur.* 26 (1), 14. doi:[10.1186/s12302-014-0014-5](https://doi.org/10.1186/s12302-014-0014-5).
- Sidoli, P., Baran, N., Angulo-Jaramillo, R., 2016. Glyphosate and AMPA adsorption in soils: laboratory experiments and pedotransfer rules. *Environ. Sci. Pollut. Res.* 23 (6), 5733–5742. doi:[10.1007/s11356-015-5796-5](https://doi.org/10.1007/s11356-015-5796-5).
- Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils – A hidden reality unfolded. *Sci. Total Environ.* 653, 1532–1545. doi:[10.1016/j.scitotenv.2018.10.441](https://doi.org/10.1016/j.scitotenv.2018.10.441).
- Silva, V., Montanarella, L., Jones, A., Fernández-Ugalde, O., Mol, H.G.J., Ritsema, C.J., Geissen, V., 2018. Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. *Sci. Total Environ.* 621, 1352–1359. doi:[10.1016/j.scitotenv.2017.10.093](https://doi.org/10.1016/j.scitotenv.2017.10.093).
- Spokas, K.A., Koskinen, W.C., Baker, J.M., Reicosky, D.C., 2009. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. *Chemosphere* 77 (4), 574–581. doi:[10.1016/j.chemosphere.2009.06.053](https://doi.org/10.1016/j.chemosphere.2009.06.053).
- Sun, M., Li, H., Jaisi, D.P., 2019. Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil-water system. *Water Res.* 163, 114840. doi:[10.1016/j.watres.2019.07.007](https://doi.org/10.1016/j.watres.2019.07.007).
- Tong, M., Gao, W., Jiao, W., Zhou, J., Li, Y., He, L., Hou, R., 2017. Uptake, translocation, metabolism, and distribution of glyphosate in nontarget tea plant (*Camellia sinensis* L.). *J. Agric. Food Chem.* 65 (35), 7638–7646. doi:[10.1021/acs.jafc.7b02474](https://doi.org/10.1021/acs.jafc.7b02474).
- U.S. Environmental Protection Agency (USEPA), 2020. Glyphosate: Interim Registration Review Decision, Case Number 0178. Docket Number EPA-HQ-OPP-2009-0361.
- Wang, M., Maki, C.R., Deng, Y., Tian, Y., Phillips, T.D., 2017. Development of high capacity enterosorbents for aflatoxin B1 and other hazardous chemicals. *Chem. Res. Toxicol.* 30 (9), 1694–1701. doi:[10.1021/acs.chemrestox.7b00154](https://doi.org/10.1021/acs.chemrestox.7b00154).
- Wang, M., Hearon, S.E., Phillips, T.D., 2019a. Development of enterosorbents that can be added to food and water to reduce

- toxin exposures during disasters. *J. Environ. Sci. Health Part B* 54 (6), 514–524. doi:[10.1080/03601234.2019.1604039](https://doi.org/10.1080/03601234.2019.1604039).
- Wang, M., Safe, S., Hearon, S.E., Phillips, T.D., 2019b. Strong adsorption of Polychlorinated Biphenyls by processed montmorillonite clays: potential applications as toxin enterosorbents during disasters and floods. *Environ. Pollut.* 255, 113210. doi:[10.1016/j.envpol.2019.113210](https://doi.org/10.1016/j.envpol.2019.113210).
- Wang, M., Orr, A.A., He, S., Dalaijamts, C., Chiu, W.A., Tamamis, P., Phillips, T.D., 2019c. Montmorillonites can tightly bind glyphosate and paraquat reducing toxin exposures and toxicity. *ACS Omega* 4 (18), 17702–17713. doi:[10.1021/acsomega.9b02051](https://doi.org/10.1021/acsomega.9b02051).
- Wang, M., Hearon, S.E., Phillips, T.D., 2020. A high capacity bentonite clay for the sorption of aflatoxins. *Food Addit. Contam. Part A* 37 (2), 332–341. doi:[10.1080/19440049.2019.1662493](https://doi.org/10.1080/19440049.2019.1662493).
- Yang, X., Wang, F., Bento, C.P.M., Xue, S., Gai, L., van Dam, R., Mol, H., Ritsema, C.J., Geissen, V., 2015. Short-term transport of glyphosate with erosion in Chinese loess soil — a flume experiment. *Sci. Total Environ.* 512–513, 406–414. doi:[10.1016/j.scitotenv.2015.01.071](https://doi.org/10.1016/j.scitotenv.2015.01.071).
- Zhan, H., Feng, Y., Fan, X., Chen, S., 2018. Recent advances in glyphosate biodegradation. *Appl. Microbiol. Biotechnol.* 102 (12), 5033–5043. doi:[10.1007/s00253-018-9035-0](https://doi.org/10.1007/s00253-018-9035-0).