DEVELOPMENT OF SOIL AMENDMENTS TO REDUCE EXPOSURES TO HAZARDOUS ENVIRONMENTAL CHEMICALS

A Dissertation

by

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ABSTRACT

During natural and manmade disasters such as floods and chemical spills, hazardous chemicals can be mobilized in the environment and affect water and food sources. Practical and economically-feasible technologies are needed to reduce the bioavailability of these chemicals and the risk of potential exposures. To solve this problem, we have developed and characterized novel, broad-acting sorbents that can effectively bind hazardous chemicals in the environment to reduce exposures. Calcium montmorillonite clays were modified with L-carnitine and choline to increase the lipophilicity of clay surfaces. Calcium montmorillonite clays were also processed and activated with sulfuric acid to increase the surface area and porosity of the clay. These sorbents were tested as soil amendments to determine the safety of the sorbents for plants and ability of the sorbents to reduce translocation of chemicals to plants.

In this study we characterized sorbent-chemical relationships using *in vitro*, *in vivo*, and *in silico* methods. Isothermal analyses indicated parent, modified, and processed calcium montmorillonite clays were highly effective sorbents of a variety of environmental chemicals with different chemical properties. Acid-processed clay (APM) had high capacity and affinity for the lipophilic chemical dieldrin and could significantly reduce dieldrin bioavailability from soil. The parent calcium montmorillonite clay significantly protected *Hydra vulgaris* from aminomethylphosphonic acid (AMPA) toxicity and reduced AMPA residues in corn sprouts. Nutrient-modified clays were effective sorbents of the amphiphilic compounds perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) based on isothermal results, soil and plant studies, and molecular dynamics simulations. Our results indicate that, upon further investigation, application of optimized sorbents to soil and added in higher levels after disasters can reduce chemical bioavailability and exposures to environmental chemicals.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professors Timothy Phillips (Department of Veterinary Integrative Biosciences), Stephen Safe (Department of Veterinary Physiology and Pharmacology), Thomas McDonald (Department of Environmental and Occupational Health), and Natalie Johnson (Department of Environmental and Occupational Health).

The molecular dynamics simulations depicted in Chapter 4 were conducted in part by Asuka Orr of the Department of Chemical Engineering.

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1. INTRODUCTION

Hazardous chemicals such as pesticides (Goncalves & Alpendurada, 2005) and PFAS (Brusseau et al., 2020) are prevalent in the environment, especially in soil, and can be detected in high levels after disasters such as hurricanes and droughts (Horney et al., 2018). Anthropogenic disasters such as chemical spills can also contribute to chemical contamination of water and soil (Thompson et al., 2016). The health hazards posed by environmental contaminants are compounded by these disasters, which mobilize contaminants in soil, sediment, and water, and threaten food supplies. Major exposure routes to these chemicals include contact with contaminated soil and consumption of contaminated food products (Khan et al., 2008; Pruvot et al., 2006). Translocation of chemicals to plants is concerning because a variety of plants have demonstrated uptake of chemicals from soil, resulting in contaminated produce (Murano et al., 2009). Exposure to chemicals that are commonly present in the environment can cause serious health issues such as respiratory illnesses, neurological disorders, and cancers (Järup, 2003; Kim et al., 2017).

Hydrophobic chemicals commonly occur as environmental contaminants and are largely organic in nature. An example of a prevalent hydrophobic contaminant in the environment is the pesticide dieldrin. Dieldrin is a legacy organochlorine insecticide that was developed in the 1940s as an alternative to dichlorodiphenyltrichloroethane (DDT) and was widely applied in agricultural settings. It is the major breakdown product of aldrin, another widely used insecticide (Ferguson & Korte, 1977). Aldrin readily breaks down to dieldrin in the environment and can be metabolized to form dieldrin in the body. Dieldrin is more resistant to degradation than aldrin and is classified as a persistent organic pollutant

(POP) as it is highly lipophilic and can sorb strongly to soil and sediment (Beyer & Gale, 2013; Martijn et al., 1993). Due to toxicity concerns, the Environmental Protection Agency (EPA) banned the use of aldrin and dieldrin. However, dieldrin is still detected in soil and sediment even years after the discontinuation of its use. Dieldrin is ranked #18 on the Agency for Toxic Substances and Disease Registry (ATSDR) Substance Priority List (SPL), indicating severe risks to public health based on prevalence in the environment, toxicity, and potential for exposure (ATSDR, 2019). Produce such as cucumbers, zucchini, and melons have been shown to take up dieldrin from soil (Donnaruma et al., 2009; Otani et al., 2007). Importantly, dieldrin can bioaccumulate up the food chain, presenting a risk of exposure to humans who consume contaminated food products. Dieldrin has been detected in the fat of cattle, ducks, and swine (Corrigan & Seneviratna, 1990; Falandysz & Kannan, 1992).

In contrast, hydrophilic contaminants are water soluble and can easily move through the environment. An important chemical that is hydrophilic and commonly present in the environment is aminomethylphosphonic acid (AMPA). AMPA is the major metabolite of the widely used herbicide glyphosate (PMG). PMG is the active ingredient of commercially-available pesticides like Roundup® and is the most widely used herbicide in the world, especially for genetically-modified (GM), Roundup-Ready® crops. Over 1.6 billion kg of PMG has been applied in the US since 1974, 2/3 of which has been applied in the last 15 years (Benbrook, 2016). PMG application in the US represents only 19% of global use, suggesting PMG contamination of water and soil is a global issue. Approximately 70% of PMG is broken down by microorganisms in soil to form AMPA (Bai & Ogbourne, 2016). Importantly, AMPA is the most frequently detected PMG metabolite in plants (Reddy et al., 2008). AMPA has been detected in GM crops as well as non-target plants such as cucumbers, cabbage, and tomatoes (Cessna et al., 2000; Duke et al., 2003). Recent toxicity data suggest chronic and sub-chronic exposures to AMPA, through consumption of contaminated food products, may present a risk to humans and animals (Bai & Ogbourne, 2016; Mesnage et al., 2015; Séralini et al., 2014).

Per- and polyfluoroalkyl substances (PFAS) are emerging chemicals of concern due to their ubiquitous presence in the environment and their potential toxicity. Their wide presence is due to their association with both water and oils (amphiphilic) and the strength of carbon-fluorine bond. PFAS have been produced and used since the 1950s for a variety of purposes such as protective coatings for paper and textiles, production of semi-conductors, and components of fire-fighting foams (Lindstrom et al., 2011). PFAS have been widely used due to their unique characteristics, including thermal, chemical, and biological stability, water and oil repellency, and surfactant properties (Teaf et al., 2019). The two most widely detected PFAS in the environment are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). These compounds have been detected in surface waters, sediments, and soils all over the world and have the potential to persist in soils and bioaccumulate (Zareitalabad et al., 2013). PFOA and PFOS can translocate to food products such as cucumbers, tomatoes, maize, and oats (Bao et al., 2020; Ghisi et al., 2019). PFAS have been detected in humans and animals, suggesting that the presence of these compounds in the environment presents a risk to public health.

The widespread presence of hazardous chemicals in environmental media necessitates effective strategies to reduce exposures to these chemicals. Sorbents are commonly studied and often used to reduce chemical exposures due to their potential to tightly bind chemicals, thus reducing chemical bioavailability from soil, sediment and water.

Chemicals can bind to interlayer, basal, and edge surfaces as well as in pores and to sorbent frameworks, allowing sorbents to effectively sequester and immobilize chemicals and reduce their bioavailability. However, there is minimal data concerning the efficacy of sorbents for binding chemicals in soil, the characterization of these binding reactions, and their effects on plant growth and uptake of chemicals from soil. This study represents a novel approach to characterizing chemical property-specific relationships that will help develop combinations of sorbents that can bind a variety of chemicals and real-life chemical mixtures in soil. A better understanding of the capacities and affinities of sorbents for various chemicals could enhance soil amendment techniques.

Natural, processed and modified clays and carbonaceous materials such as activated carbon have structural and chemical properties that make them effective sorbents of diverse chemicals. Many sorbent strategies use activated carbons to bind chemicals in soil (Denyes et al., 2016). Natural clays such as montmorillonites, which have been shown to tightly bind a variety of chemicals in water, could greatly increase the efficacy of sorbent amendments in soil (Wang et al., 2017; 2019a; 2019b). Additionally, novel sorbents developed in our laboratory including acid-processed clays (APMs) and nutrient-modified clays are broad-acting and can more effectively bind some chemicals than activated carbon (Wang et al., 2019b; 2021a). APMs are synthesized by processing and activating calcium montmorillonite (CM) clay with sulfuric acid, resulting in diverse binding sites and enhanced surface areas and porosities compared to the parent clay (Kumar et al., 1995). Also, L-carnitine and choline modifications can increase the hydrophobicity of interlayer surfaces, increasing the affinities for hydrophilic chemicals (Wang et al., 2017). Processed and modified clays or combinations of activated carbons and clays have not been fully explored in soil applications.

This study was designed to develop and characterize novel, broad-acting sorbents that can effectively bind hazardous chemicals, reducing exposures to chemicals in soil (Figure 1). We evaluated binding potentials of activated carbon, calcium montmorillonite clay, nutrient-modified calcium montmorillonite clays, and acid processed montmorillonite clays with chemicals on the ATSDR Substance Priority List and emerging chemicals of concern. Dieldrin, AMPA, PFOA, and PFOS were chosen as representative chemicals due to their diverse chemical properties (i.e. lipophilic, hydrophilic, and amphiphilic). To evaluate the efficacy of sorbents in binding these chemicals, we used 1) isothermal analyses to evaluate sorbent capacities and affinities for chemicals and potential binding mechanisms, 2) bioassays to determine sorbent safety for living organisms and efficacy in reducing chemical toxicity, 3) soil studies to determine sorbent ability to reduce chemical bioavailability from soil, and 4) plant studies to investigate the uptake of chemicals by plants and sorbent ability to reduce chemical translocation. Utilizing optimal sorbents as soil amendments could present a promising and feasible strategy to reduce the bioavailability of chemicals from soil and thus reduce potential exposures to these chemicals.

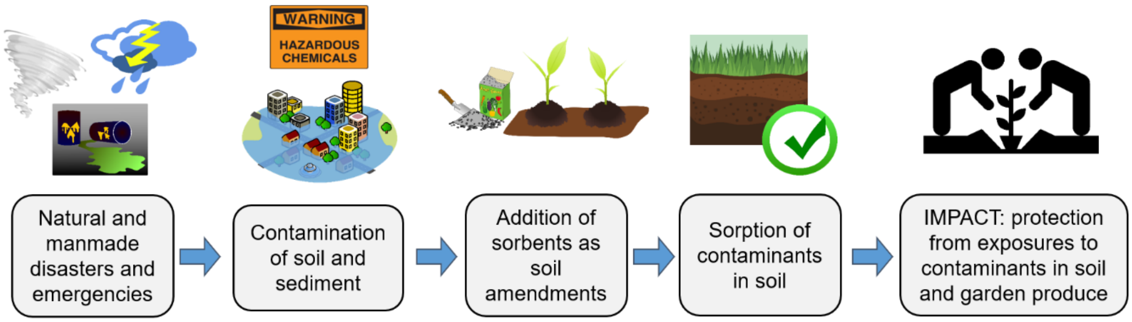

Figure 1. Application of sorbents to soil can protect against exposures to hazardous environmental chemicals, especially after disasters.

1.1 Dieldrin

Dieldrin is a synthetic organochlorine insecticide that was originally produced in 1948. It is a white powder with a mild chemical odor. Dieldrin is the major breakdown product and active compound of aldrin, another organochlorine insecticide. It is formed from the epoxidation of the double bond of aldrin (Figure 2). Dieldrin and aldrin are ranked #18 and #25 on the ATSDR Substance Priority List of chemicals that are ranked according to their prevalence, toxicity, and potential to result in human exposure (ATSDR, 2019).

1.1.1 Sources and prevalence

Dieldrin is a legacy pesticide that was developed as an alternative to DDT and was applied in agricultural settings worldwide throughout the 1950s to the 1970s. It was primarily used on cotton, corn, and citrus crops (Honeycutt & Shirley, 2014). Dieldrin and aldrin were also used to treat termite infestations and for timber preservation until 1987 when the manufacturer voluntarily cancelled its registration for use (ATSDR, 2002). In 1966, the use of these pesticides was at an all-time high with 19 million pounds of aldrin and 1 million pounds of dieldrin applied that year. This use decreased to 10.5 million and 670,000 pounds of aldrin and dieldrin in 1970, respectively. This decrease was attributed to increased insect resistance and the development of alternative pesticides. Many of the hazardous waste sites on the National Priority List (NPL) have been affected by spills, leaks, or improper disposal of aldrin and dieldrin. Aldrin and dieldrin have been detected in 207 and 243 of the 1,613 NPL sites, respectively (ATSDR, 2002). Because of the widespread use of both aldrin and dieldrin along with mishandling of the chemicals resulting in further contamination, exposure to dieldrin residues in the environment is still a concern.

Dieldrin has been detected in soil and sediment even years after discontinuation of use, especially in areas surrounding hazardous waste sites (Erturk et al., 2007; O'Shea et al., 2001). Dieldrin is classified as a persistent organic pollutant (POP) and is highly lipophilic (logP 5.40), so it can strongly sorb to organic matter and soil particles and persist in the environment. Dieldrin is more persistent and more resistant to degradation than aldrin. Early studies on dieldrin presence in soil and sediment measured dieldrin in ppb levels (ATSDR, 2002). Agricultural soil samples from Portugal, Canada, and India contained average dieldrin concentrations of 286, 450, and 780 ng g^{-1} (Goncalves & Alpendurada, 2005; Wan et al., 2005; Singh, 2001). In the US, soil remediation standards for dieldrin are very low, leading dieldrin to be a cleanup "driver" in many hazardous waste sites in states like New Jersey, Connecticut, Texas, and California (Stern, 2014).

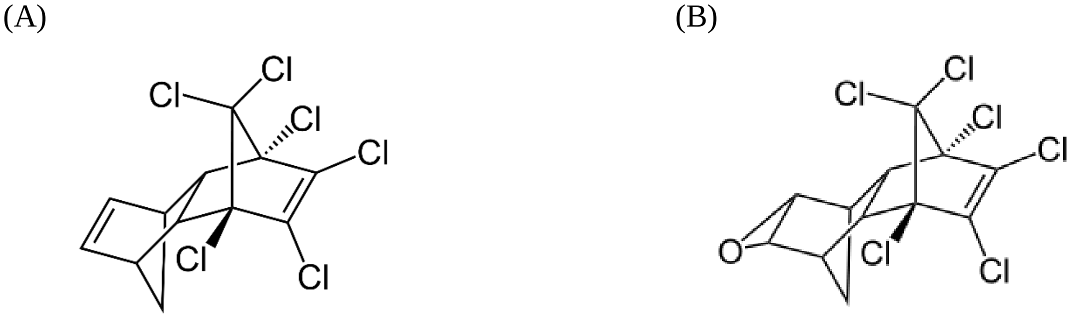

Figure 2. Chemical structure of aldrin (A) and dieldrin (B).

1.1.2 Toxicity

Concerns about dieldrin toxicity led to bans on dieldrin application and use. However, due to the widespread use and persistence of dieldrin, residues are still present in the environment. People can be exposed through either contact with contaminated soil or consumption of contaminated food and water. The acceptable daily intake of dieldrin is 0.0001 mg kg^{-1} day^{-1} and the reference dose is 0.005 mg kg^{-1} day^{-1} (Blaylock, 2005).

Dieldrin can be readily absorbed from the gastrointestinal and respiratory tracts as well as through the skin (Blaylock, 2005). Dieldrin has been shown to have neurotoxic, carcinogenic, and reproductive effects in cell culture and animal models. The most common short-term toxic effects of high exposures to dieldrin involve convulsions resulting from central nervous system excitation. Dieldrin causes this excitability through inhibition of gamma-aminobutyric acid (GABA)-regulated chloride transport, which blocks chloride influx into neurons (Honeycutt & Shirley, 2014). Many studies have investigated the role of chronic exposure to dieldrin in the development of Parkinson’s disease (PD) and suggested that dieldrin exposure may be a factor in PD etiopathogenesis (Kanthasamy et al., 2005; Richardson et al., 2006). Dieldrin may promote nigral dopaminergic degeneration through oxidative stress, mitochondrial dysfunction, protein aggregation, or apoptosis. Long-term exposure to lower levels of dieldrin can result in headaches, dizziness, vomiting, or muscle spasms (ATSDR, 2017).

Dieldrin can also have adverse effects on reproductive systems. Cell culture studies have indicated that exposure to low, biologically relevant concentrations of dieldrin can affect fetal human Leydig cells, reducing testosterone secretion and leading to dysregulation of reproductive development (Abramovich et al., 2007). Although hepatic toxicity has not been observed in humans, the liver was the most sensitive target organ in chronic-duration animal studies (Bachowski et al., 1997; Koshlukova & Reed, 2014).

There is not a consensus between regulatory agencies as to the carcinogenicity of dieldrin. The EPA classifies dieldrin as a “probable human carcinogen” based on cancer potency estimates derived from tumorigenesis in rats, while the International Agency for Research on Cancer (IARC) determined that dieldrin is not classifiable as to its carcinogenicity in humans due to no conclusive evidence that dieldrin can cause cancer in humans (Stern, 2014).

1.1.3 Translocation to plants

Dieldrin has been shown to translocate from soil to various vegetable plants, making consumption of contaminated produce an important exposure pathway. Although dieldrin sorbs strongly to soil particles, it can be taken up by plants, especially cucurbit species. An early study on plants grown in aldrin-contaminated silt loam soil found that dieldrin residues penetrated through cucumber root systems and were translocated to the fruits (Lichtenstein et al., 1965). Cucurbits are able to dissolve or desorb dieldrin from soil particles and take up dieldrin from the roots into plant compartments (Otani et al., 2007). Field studies of cucumbers grown in dieldrin-contaminated soil found dieldrin residues up to 0.07 mg kg^{-1} in plant compartments including the fruits (higher than the maximum safeguard level of 0.01 mg kg^{-1}) (Donnarumma et al., 2009). Melons and zucchini also demonstrated high uptake capabilities in these studies. Saito et al. (2012) compared dieldrin residues is 19 different vegetable crops grown in a contaminated field (0.068 to 0.125 mg kg^{-1} dieldrin). By comparing bioconcentration factors, they found that cucurbit vegetables including melons, watermelons, pumpkins, zucchini, and cucumbers had high concentrations of dieldrin while non-cucurbits including broccoli, cauliflower, cabbage, and celery did not have detectable levels of dieldrin. Importantly, it was noted that dieldrin concentrations were not accumulated in one particular part of cucumber plants, but rather similar concentrations were measured in leaves and fruit tissues. They concluded that dieldrin residues in cucumber fruits resulted from translocation of dieldrin from soil.

1.1.4 Remediation strategies

Due to the persistence and prevalence of dieldrin in the environment, various remediation strategies have been investigated for their potential to either reduce or eliminate dieldrin residues available for exposure. Phytoremediation, biodegradation, and immobilization/sorption are among the most widely tested strategies for dieldrin remediation due to their relatively low cost and minimal environmental impacts.

Phytoremediation has been evaluated as a strategy to remove dieldrin residues from soil due to its minimal disruption of the environment. This technique involves the use of various plant species to extract, degrade, or immobilize contaminants. Dieldrin can strongly sorb to soil and has low water solubility, so translocation of dieldrin to plants seems unlikely. However, cucurbit species have been shown to readily uptake dieldrin into leaves and fruits. These plants can desorb dieldrin from soil and take it up into roots, and translocate to aerial parts of the plant within the xylem (Matsumoto et al., 2009). A study focused on identifying ideal plant species for phytoremediation of dieldrin concluded that since cucurbit plants such as zucchini demonstrated much higher uptake of dieldrin than non-cucurbits such as tomato and cabbage, cucurbit species would be good candidates for phytoremediation of soil (Otani et al., 2007). It has been reported that dieldrin has been detected in the pulps of squash, melons, and cucumbers (Jorgenson, 2001).

Biodegradation strategies using microorganisms to degrade dieldrin residues in soil under both anaerobic and aerobic conditions have also been evaluated. Batch cultures with methanogenic granular sludge degraded 88% of dieldrin (9 ug mL^{-1}) over 3 months in anaerobic conditions to produce two monodechlorinated products, aldrin, and two monodechlorinated derivatives of aldrin (Baczynski et al., 2004). Matsumoto et al. (2008) developed an efficient method to isolate aerobic dieldrin-degrading bacteria from soil using 1,2-epoxycyclohexane (ECH) to obtain novel *Burkholderia* and *Cupriavidus* species that degraded 49% and 38% of dieldrin, respectively. It is important to note that these biodegradation methods are optimized under very specific experimental conditions and may not be effective under all environmental conditions.

Carbon- and clay-based sorbents as well as various magnetic nanoparticles have been evaluated for their potential to bind and immobilize dieldrin in soil and water. Activated carbon (AC) derived from bamboo reduced 76-94% of dieldrin residues in drinking water with adsorption fitting the Freundlich trend, indicating possible partitioning of dieldrin molecules on and off sorbent surfaces (Thuy et al., 2012). A novel trolein-embedded AC sorbent had a high affinity for dieldrin in aqueous solution, with adsorption occurring through a spontaneous, endothermic reaction (Ru et al., 2007). Adsorption of dieldrin in water on a nanocomposite of graphene oxide and iron oxide magnetic nanoparticles fit the Langmuir trend, with a capacity of 1 mg g^{-1} (Shrivas et al., 2017). A highly porous, hydrophobic silica aerogel also had a strong affinity for dieldrin and removed 92% of dieldrin from water (Liu et al., 2009). Fewer studies have focused on using sorbents to bind dieldrin in soil. AC was tested for efficacy in reducing 0.07 mg kg^{-1} dieldrin bioavailability from soil, and was able to reduce dieldrin translocation to plants by 50% with 800 mg kg^{-1} AC inclusion (Hilber et al., 2009). In a field study, wood chip charcoal (0.5 kg m^2) was able to reduce dieldrin bioavailability from soil and translocation to cucumber plants by up to 85% (Saito et al., 2011).

1.2 Aminomethylphosphonic acid

Aminomethylphosphonic acid (AMPA) is the major metabolite of the widely used herbicide glyphosate (PMG). It is a weak organic acid with a phosphonic acid group (Figure 3). PMG is mineralized and metabolized by microorganisms in soil within days to form AMPA, which comprises up to 70% of PMG degradation products (Bai & Ogbourne, 2016). Less is known about the metabolism of PMG to AMPA in plants, but some studies have suggested oxidative cleavage of the C – N bond (Reddy et al., 2008). Because AMPA is the primary metabolite of PMG and the two compounds are often detected and studied together, it is important to consider PMG application, prevalence, environmental fate, and toxicity when discussing AMPA.

1.2.1 Sources and prevalence

PMG was synthesized in 1950 and commercialized in 1974 for use in agricultural, commercial, and residential use. PMG is the most heavily used pesticide in agricultural settings and the second most used pesticide for homes and gardens and in commercial settings, accounting for 40% of all herbicide use (Battaglin et al. 2014). Its popularity grew due to its low cost, efficiency, rapid absorption by plants, and perceived low toxicity as well as the development of glyphosate-resistant crops. PMG is most often used as a post-emergence pesticide and is applied after the crops and accompanying weeds have emerged from the soil, and may be applied at additional time points during the growing season (Benbrook, 2016). PMG has been used in more than 130 countries on more than 100 crops, including genetically-modified crops that are developed to resist PMG toxicity (Battaglin et al., 2014). About 80% of all genetically-modified crops are PMG-resistant (Dill et al., 2008). Additionally, the growing popularity of "no-till" farming for crops that are not genetically-modified has contributed to the increased application of PMG. Non-agricultural applications of PMG include residential and urban uses, which can result in contamination of drainage systems and areas downstream of wastewater treatment plants (Wittmer et al., 2011).

PMG and AMPA tend to sorb to soil particles and accumulate in topsoil. AMPA has been shown to be more persistent in the environment than PMG. The environmental fate of the two compounds largely depends on soil conditions (pH, phosphate fertilization, electrical conductivity) as well as weather and climate. A study using batch laboratory and lysimeter transport studies investigated leaching of PMG and AMPA from agricultural soils, and found that adsorption of PMG to soil particles contributes to its slow degradation rate in soils, especially those with clay content (Bergström et al., 2011). AMPA kinetics suggested that AMPA is more persistent than PMG, but may degrade at faster rates. Importantly, this study noted that PMG and AMPA residues remained in the topsoil despite large amounts of precipitation. Another study evaluated PMG and AMPA mobility in environmental media and found that, despite the sorption abilities of the two compounds on soil particles, 10-20% of residues were lost from topsoil with 96% of the loss occurring through surface runoff and only 4% through flow through in the soil (Daouk et al., 2013). PMG and AMPA residues in a low phosphorous soil after a single application of PMG were monitored, and almost 2 years after application, 19% of PMG and 48% of AMPA were detected in the topsoil, indicating the persistence of PMG and metabolism to AMPA in the soil (Laitinen et al., 2009). In addition to the persistence of AMPA in soil, it can be highly mobile in water and can be redistributed as a result of rainfall and surface runoff (Grandcoin et al., 2017).

PMG and AMPA have been detected in soil and water all over the world. PMG and AMPA were detected in 21% and 42%, respectively, of 317 agricultural topsoils from the

European Union (EU) in concentrations up to 2 mg/kg (Silva et al., 2018). Samples with the highest frequencies of PMG and AMPA were those soils that had been used for permanent crops and root crops. In a study of 3,732 water and soil samples from all over the US, PMG and AMPA were detected in 39.4% and 55% of all samples, respectively (Battaglin et al., 2014). Importantly, PMG was detected without AMPA in 2.3% of samples, while AMPA was detected without PMG in 17.9% of samples.

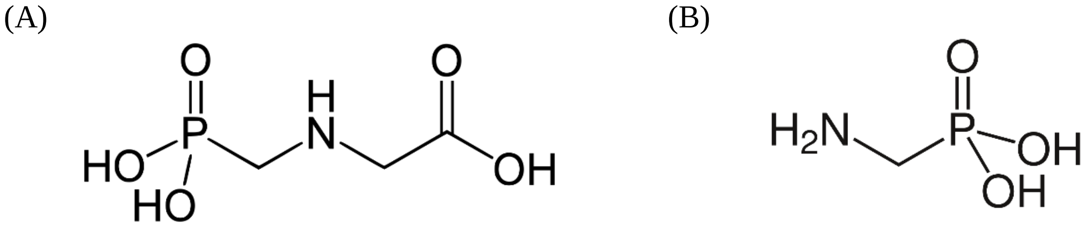

Figure 3. Chemical structures of PMG (A) and AMPA (B).

1.2.2 Toxicity

Exposure to PMG and AMPA can occur primarily through the consumption of contaminated water or food products. Recent studies on chronic and sub-chronic toxicity suggest exposure to AMPA resulting from accumulation up the food chain may also be of concern (Bai & Ogbourne, 2016).

The USEPA classified PMG as “not likely to be carcinogenic to humans”, while IARC classified PMG as “probably carcinogenic to humans (Group 2A) (USEPA, 2020). Major factors contributing to these differing conclusions are the assays included in the two evaluations. IARC placed much more weight on the results from AMPA toxicity assays than the USEPA, which may have contributed to their conclusion on the genotoxicity of PMG. These agencies have not decided 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). A study using an *in vitro* Comet assay in Hep-2 cells, a chromosome aberration test in human lymphocytes, and an *in vivo* micronucleus test in mice found statistically significant DNA damage, clastogenic effects, and increases in micronuclei (Manas et al., 2009). A recent study using human neuroblastoma SH-SY5Y cells found that exposure to 10 mM AMPA resulted in significant increases in production of reactive oxygen species (ROS) and nitric oxide and significant increases in caspase 3/7 activity, suggesting that AMPA can induce oxidative stress and cell death through apoptosis (Martinez et al., 2020). In addition to carcinogenicity, AMPA exposure may be toxic to the nervous system. *In vitro* studies using induced pluripotent stem cells (iPSCs) and human peripheral blood mononuclear cells (PBMCs) found that high doses of AMPA can cause blood-brain barrier alterations and decreased cell viability, respectively (Kwiatkowska et al., 2016; Martinez & Al-Ahmad, 2019). Results from another study using PBMCs suggested that PMG and AMPA may both affect epigenetic processes that regulate chromatin architecture such as the deacetylation of histone proteins (Wozniak et al., 2021).

1.2.3 Translocation to plants

In addition to toxicity concerns for humans, there is growing concern about the toxicity of AMPA to plants and crops. Gomes et al. (2016) evaluated the mechanisms by which AMPA exerts is phytotoxic effects and found that AMPA induces accumulation of reactive oxygen species in willow leaves by inhibiting antioxidant enzymes and disturbing chlorophyll biosynthesis. This disturbance of chlorophyll biosynthesis may occur as a result of AMPA competition with glycine in photorespiration processes or with δ-aminolevulinic acid (ALA) synthetase, which deprives plants of key substrates in the synthesis of chlorophyll (Gomes et al., 2014). Genetically-modified (GM) soybean injury resulting from PMG and AMPA application was investigated to compare the toxicities of the two compounds. AMPA at 0.12 and 0.50 kg ha^{-1} induced injury in the GM and non-GM soybeans, while PMG induced injury at 13.44 kg ha^{-1}, indicating that AMPA was more toxic to these plants than PMG (Reddy et al., 2004). This suggests that soybean injury resulting from PMG application may have been due to degradation of PMG to AMPA. Toxicities previously attributed to PMG exposure may actually be the result of AMPA.

Various studies have evaluated the potential for AMPA translocation to GM plants and non-GM plants. A study comparing PMG and AMPA residues in GM, unmodified, and organic soybeans from Iowa, USA found that the highest residues of PMG and AMPA were detected in GM-soybeans (3.3 and 5.7 mg kg^{-1}, respectively) due to pesticide application during the growth of these plants (Bohn et al., 2014). PMG and AMPA residues were not detected in unmodified and organic soybeans, however, trace levels of other pesticides were detected, possibly due to previous applications of persistent pesticides (even in organic fields). Importantly, this study noted that organic soybeans had much healthier nutritional profiles (more total protein and zinc; less fiber) than the GM soybeans. A monitoring study in Argentina measured PMG and AMPA residues in GM soybean grains up to 4.4 and 5.7 mg kg^{-1}, respectively (Arregui et al., 2004). The highest concentrations measured were attributed to repeated PMG application during the growth cycle, especially when treatment occurred close to the flowering stage. Other studies have shown that non-target plants such as non-GM soybean and corn, cowpea, and tomatoes can also take up AMPA from soil (de Llasera et al., 2005; Reddy et al., 2008).

1.2.4 Remediation strategies

Strategies to reduce or eliminate AMPA from environmental media have not been widely studied. Many techniques to remediate PMG in water and soil have been explored, but its primary metabolite AMPA is not often the focus. Immobilization with sorbents, microbial and oxidative degradation techniques, and phytoremediation have been explored for PMG remediation of wastewater (Feng et al., 2020). Biodegradation of PMG in soil has been widely explored, however many of these processes result in the production of AMPA (Lopez-Chavez et al., 2021; Zhan et al., 2018).

Electrochemical treatment of wastewater using birnessite thin films coupled with oxidative treatments can effectively mineralize AMPA (70 – 115 mg AMPA mineralized per g birnessite) (Ndjeri et al., 2013; Pensel et al., 2016). Clay sorbents have been used to bind and immobilize AMPA. Molecular mechanics of AMPA binding to montmorillonite clay indicated that AMPA was attracted to interlayer surfaces through covalent bonding with the AMPA phosphonate moiety (Rennig et al., 2008). Adsorption of AMPA onto goethite also occurred through the phosphonate moiety binding to the goethite surface (Barja & Dos Santos Afonso, 2005). Immobilization with sorbents has also been utilized to reduce AMPA bioavailability from soil. In a greenhouse pot experiment, biochar amendments in soil reduced leaching of AMPA by up to 46% (Hagner et al., 2015). Adsorption of AMPA with cow dung and rice husk biochars in an acidic soil fit the Freundlich adsorption model, removing 33% and 31% more AMPA than the control soil, respectively (Garba et al., 2018). In addition to immobilization with sorbents, bioremediation of AMPA has also been explored. *Trichoderma harzianum* has been found to have AMPA-degrading activity and achieved 69% of AMPA degradation after 10 days of incubation (Bouchiat et al., 2016).

1.3 Per- and polyfluoroalkyl substances

PFAS are recently of critical concern due to their widespread distribution and persistence in the environment and their potentially negative effects on human health. PFAS are synthetic chemicals that are amphiphilic, containing both a hydrophilic functional group and a hydrophobic carbon chain tail (Figure 4). PFOA has a carboxylic acid functional group, while PFOS has a sulfonic acid functional group. PFOA and PFOS are the most commonly detected in the environment and thus were chosen as the representative compounds in this study.

1.3.1 Sources and prevalence

PFAS, especially PFOA and PFOS, are prevalent in soil and water across the globe ranging up to ppm levels in contaminated sites. PFAS are used in various industries as components of refrigerating agents, hydraulic fluids, floor polishes, metal plating, and food packaging. Gluge et al. (2020) estimated over 200 use categories for PFAS across chemical, electronic, energy, mining, manufacturing, and construction industries. PFOA has been widely used as an industrial surfactant and in the production of Teflon products. PFOS is a common component of aqueous film-forming foams (AFFF) and textiles, carpets, and papers. PFAS have such a wide range of applications due to their unique chemical properties and abilities to repel oil, grease, and water and reduce friction and resist temperature extremes. PFAS are released to the environment through fluoropolymer manufacturing and improper disposal, biosolid application, wastewater treatment effluents, landfill leachate, and degradation of PFAS precursors (Sunderland et al., 2019).

PFOA and PFOS have been voluntarily phased out by many large manufacturers. However, these compounds are still widely detected throughout the environment, including surface water, groundwater, and drinking water. PFOA has been detected in wastewater effluents up to 0.12 µg L^{-1}, in AFFF release sites averaging 405 µg L^{-1}, and up to 6,570 µg L^{-1} in fire training and response locations (Eschauzier et al., 2013). A study in Australia found average PFAS concentrations up to 100 ng L^{-1} in groundwater, with PFOA and PFOS detected in 82% and 96% of samples, respectively (Vo et al., 2020). Hu et al. (2016) estimated that water supplies for 6 million US residents exceed USEPA lifetime health advisory limits (70 ng L^{-1}) for PFOA and PFOS.

More recently, the focus has shifted to PFAS contamination of soil due to data suggesting that soil is an important reservoir for PFAS and the potential for leaching to groundwater and accumulation in plants. Analyses on the fate and transport of PFAS suggest that soils serve as significant long-term sources of PFAS based on distribution coefficients (Anderson et al., 2019; Zareitalabad et al., 2013). Additionally, PFOA and PFOS do not easily degrade in soil (Saikat et al., 2013). A study collected data from over 30,000 soil samples taken from background sites as well as primary- and secondary-source sites all over the world and detected PFAS in almost every sample (Brusseau et al., 2020). PFOA and PFOS background concentrations ranged from 0.003 to 162 µg kg^{-1} and 0.01 to 124 µg kg^{-1}, respectively, while contaminated site concentrations ranged from 2 to 50,000 µg kg^{-1} and 0.4 to 460,000 µg kg^{-1}, respectively. Rankin et al. (2016) measured PFOA and PFOS in North American background soils at concentrations up to 2.7 and 3.1 ng g^{-1}, respectively. Estimates for the global load of PFAS in soil ranges from 1500 to 9000 metric tons for 8 of the commonly detected PFAS, with PFOA and PFOS accounting for approximately 1000 metric tons (Washington et al., 2019).

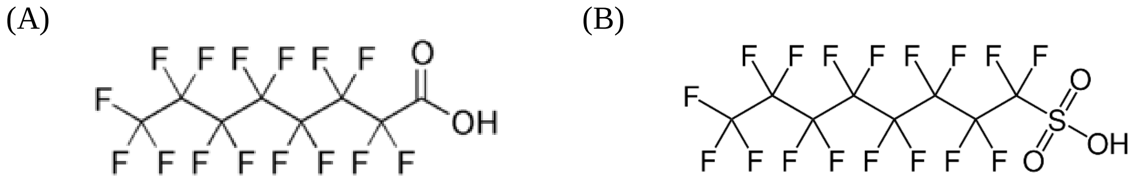

Figure 4. Molecular structures of PFOA and PFOS.

1.3.2 Toxicity

Exposure to PFAS can occur through a variety of pathways due to the widespread distribution of PFAS in the environment and their presence in many household products. Consumption of drinking water contaminated with PFAS is thought to be a main route of exposure, presenting a health risk even at low concentrations (Teaf et al., 2019). Exposure to PFAS through food and food packaging is also a significant exposure pathway, along with indoor air and house dust. Additionally, people can be exposed to PFAS through the consumption of crops grown in contaminated soil (Costello & Lee, 2020). PFAS have been detected in the serum of over 98% of the US population, indicating widespread exposure (Lewis et al., 2015). Data on PFAS toxicity in humans is limited, but toxicological data from animal studies coupled with epidemiological evidence suggest PFAS exposure could be associated with various adverse health outcomes.

Animal studies suggest that PFOA and PFOS may be carcinogenic and may cause toxicity in the immune, liver, and endocrine systems, although there is high variability in the results of these studies (Arrieta-Cortes et al., 2017; Lilienthal et al., 2017). For example, PFOA has been associated with tumor development in the liver, testes, and pancreas of rats. In terms of exposure to PFOS, animal studies have suggested that chronic exposure can result in thyroid hormone disruptions and elevated cholesterol (Saikat et al., 2013). For toxicity in humans, several occupational studies have indicated potential links between PFAS exposure and various adverse health outcomes. Occupational exposures to PFAS can lead to individuals with serum PFAS concentrations exponentially higher than the general population. A study in Ohio with individuals potentially exposed to PFOA suggested that PFOA exposure could be related to high cholesterol, thyroid disease, ulcerative colitis, and pregnancy-induced hypertension (Teaf et al., 2019). PFOS exposure could be associated with disruptions in the blood-brain barrier and neurodevelopmental effects (Rappazzo et al., 2017; Wang et al., 2011). Studies have suggested PFOA exposure could be related to kidney and testicular cancer, however, further reviews found current evidence of these associations to be inconsistent (Barry et al., 2013; Kennedy et al., 2004). Epidemiological studies have found associations between PFOS exposure and bladder, rectal, thyroid, and pancreatic cancers (Alexander & Olsen, 2007; Eriksen et al., 2009; Olsen et al., 2004). PFOA and PFOS have both exhibited key characteristics of carcinogens, including epigenetic alterations, oxidative stress, chronic inflammation, immunosuppression, alteration of cell proliferation and cell death (Temkin et al., 2020). Additionally, toxicological and epidemiological studies have suggested that PFOA and PFOS can cause immunotoxicity through antigen-specific antibody responses (DeWitt et al., 2019). IARC has classified PFOA as “possibly carcinogenic to humans” citing limited evidence in humans, while the USEPA considers there to be suggestive evidence of PFOA carcinogenicity (Teaf et al., 2019). The USEPA also considers there to be suggestive evidence of PFOS carcinogenicity, while IARC has not yet made a determination. Arrieta-Cortes et al. (2017) evaluated PFOS risk of carcinogenicity following the IARC monograph methodology, and concluded that based on the current evidence, PFOS is “not classifiable as to its carcinogenicity in humans”. More large-scale studies are necessary to evaluate the associations between PFAS exposure and adverse health outcomes.

1.3.3 Translocation to plants

Because of their unique chemical properties, PFAS such as PFOA and PFOS can be ubiquitous in the environment. This presents the potential for contamination of food products through exposure to contaminated water or soil. Uptake of PFAS by various plants has been widely studied, especially by plants exposed to high levels of PFAS. Plants irrigated with PFAS-contaminated water or those grown in PFAS-contaminated soil are especially vulnerable to exposure. Additionally, biosolids that are commonly applied to improve soil quality can contain high levels of PFAS, as PFAS are often resistant to most wastewater treatments (Alder & van der Voet, 2015; Blaine et al., 2014). Vegetables grown in greenhouses near a fluorochemical industrial park in China and watered with groundwater contaminated with PFAS showed uptake of PFAS into the tissues of tomatoes and cucumbers (Bao et al., 2020). Importantly, this study demonstrated bioaccumulation of PFAS in the edible parts of the vegetables. Garcia-Valcarcel et al. (2014) demonstrated uptake of PFAS by vegetables grown in hydroponic solutions spiked with up to 1 µg mL^{-1} PFAS. Transfer factors (ratio of PFAS in plant tissues to PFAS in nutrient solution) of PFOA and PFOS were 3.4 and 3.1, respectively. Another study investigated the risk of exposure to PFAS in home-grown vegetables (Lal et al., 2020). They found that even at the low dose of PFOS (1 µg mL^{-1}), the daily intake rates calculated for children aged 2-6 were higher than the standards set by Food Standards Australia New Zealand (FSANZ). In addition to vegetables, uptake of PFAS by cereals such as wheat and oats as well as root crops such as potatoes and carrots has been observed (Lechner & Knapp, 2011; Stahl et al., 2009).

1.3.4 Remediation strategies

As concerns about PFAS prevalence and toxicity grow, a variety of remediation strategies have been extensively investigated to reduce the risk of exposure to PFAS in soil and water. *Ex situ* and *in situ* technologies including adsorption, filtration (reverse osmosis or nanofiltration), thermal, chemical oxidation/reduction, soil washing, and bioremediation have shown promise in immobilizing or degrading PFAS in the environment (Shahsavari et al., 2021). This discussion is focused on the use of sorbents to immobilize PFAS in water and soil.

Both charcoal- and clay-based sorbents have been used to reduce PFAS contamination of water. Granular activated carbon was more effective in removing PFOA and PFOS from surface and wastewater (45 – 67%) compared to biochars derived from hardwood and pinewood (Inyang & Dickenson, 2017). Adsorption of PFOS on biochar derived from corn straw fit the Langmuir model indicating high capacity for PFOS (Guo et al., 2017). Adsorption occurred mainly through hydrophobic and electrostatic interactions. An organo-montmorillonite modified with hexadecyltrimethylammonium bromide (HDTMAB) was an effective sorbent of PFOS through hydrophobic interactions with interlayer surfaces, followed by rearrangement through electrostatic interactions between the HDTMAB modifications and PFOS (Zhou et al., 2010). These studies indicated that pH and solution ionic composition greatly affected sorbent efficacy.

In terms of sorbents used as soil amendments to reduce PFAS bioavailability from soil, biochar and activated carbons are the most widely studied and applied. A biochar derived from pine (5% inclusion) reduced PFOA and PFOS (1 mg mL^{-1}) bioavailability from loamy sand soil by 44% and 88%, respectively, and from sandy clay soil by 18% and 70%, respectively (Askeland et al., 2020). This study concluded that while biochar could bind PFAS effectively, the binding was weak and reversable. Biochar was also tested for the ability to sorb PFAS in soils with low and high organic matter content (Bjerkli, 2019). Results showed the biochar significantly reduced PFAS bioavailability at 0.5% inclusion in low OM content and 5% in soil with high OM content. A commercially-available carbon-based sorbent (RemBind®) at doses from 5% up to 30% has been shown to reduce PFOS bioavailability from highly-contaminated soil up to 99% (Braunig et al., 2021; Kabiri et al., 2021). Clay-based sorbents have also been tested for binding potential of PFAS. MatCARE™, a commercially-available clay-based sorbent, had a high capacity for PFOS and significantly reduced PFOS bioavailability with 10% w/w inclusion in soil (Das et al., 2013). The inclusion of montmorillonite (3%) reduced PFOS leaching by up to 40% from soil highly contaminated with AFFF (Hale et al., 2017).

1.4 Sorbent amendments to reduce exposures

The efficacy of sorbents in decreasing or eliminating levels of hazardous chemicals in the environment is well-documented in the literature. Carbon- and clay-based sorbents are widely use in water treatment and soil remediation of both organic and inorganic compounds. Contaminants can bind to active surfaces of sorbents through hydrogen bonding, dipole-dipole interactions, ion exchange, or covalent bonding (Kah et al., 2017). Carbon-based sorbents have been widely utilized due to their surface areas and porosity. However, alternative sorbents such as clays may present more environmentally-friendly strategies due to their lower costs and abundance in nature (Nafees & Waseem, 2013).

1.4.1 Carbonaceous sorbents

Activated carbon (AC) is utilized in industrial applications for metal finishing and purification, medical treatment of poisonings and overdoses, analytical chemistry, organic farming, gas purification and fuel storage, and environmental applications such as water filtration, spill cleanup, and soil remediation. Structural properties of AC and other carbon-based sorbents make them effective in these various applications. Activated carbons are produced from carbonaceous sources such as bamboo, coconut shell, or wood either through pyrolysis, oxidation, or chemical activation (Hagemann et al., 2018). Chemical activation is often the preferred method as it requires lower temperatures, shorter time, and produces a consistent product. The resulting materials have high surface areas and porosities that are ideal for the sorption of contaminants.

Carbon-based sorbents have been extensively studied as sorbents of environmental contaminants including pharmaceuticals, pesticides and heavy metals. These sorbents typically bind compounds through van der Waals or London dispersion forces, although some studies have indicated hydrogen bonding and electron donor-acceptor interactions may also be important depending on the chemical of concern (Kah et al., 2017; Zhang et al., 2016). Because of their abilities to bind environmental chemicals, carbon-based sorbents have been applied as soil amendments in the forms of granular or powder AC and biochars to reduce chemical bioavailability. Granular AC has a higher capacity than powder AC and can be reactivated for continued use, while powder AC has a much smaller particle size, is less expensive, and more commonly used in water filtration. Results from a field study using 2% inclusion of granular and powder AC in soil showed 69% and 76% reduction of PAH concentrations in soil pore water after 28 months, respectively (Hale et al., 2012). Granular and powder AC both significantly reduced chlorobenzene bioavailability from soil at 1% inclusion (Song et al., 2015). Biochars are carbon-rich materials that are derived from biomass and intended for application as soil amendments as they can improve soil fertility and reduce greenhouse gas emissions (Kookana, 2010). Biochar derived from eucalyptus wood chips significantly reduced chlorpyrifos and carbofuran bioavailability from soil and protected spring onions from exposure (Yu et al., 2009b). 5% inclusion of a waste timber biochar reduced PFAS leaching by 98-100% in soil with low organic content and 23-100% in soil with high organic content (Sormo et al., 2021). Biochars derived from wood chip, rice husk, and bamboo were tested for efficacy in reducing dieldrin translocation to cucumber fruits, and results showed that wood chip biochar was the most effective (Saito et al., 2011).

However, there are concerns about the safety of applying carbon-based sorbents to soil. Pyrolysis is an incomplete combustion method, and can result in the production of toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), dioxins, polychlorinated biphenyls (PBCs), and heavy metals (Yavari et al., 2015). Carbon-based sorbents have been shown to reduce microbial denitrification and nutrient availability (Bonaglia et al., 2019). They can also reduce macrofauna living in sediment and decreased survival and growth rates of benthic invertebrates (Janssen & Beckingham, 2013; Samuelsson et al., 2015). Nutrient immobilization by carbon-based sorbents can also be detrimental to plant health. Considering these factors, it is important to develop sorbents that can be more effective than carbon-based sorbents and can be used as alternatives to or in combination with ACs and biochar in environmental applications.

1.4.2 Calcium montmorillonite clay

Calcium montmorillonite (CM) clay is a dioctahedral smectite clay that is made up of silicate layers with both tetrahedral and octahedral sheets (Phillips et al., 2019). CM clay has a 2:1 structure, with a central octahedral sheet of alumina sandwiched by two tetrahedral sheets of silica. Interlayer cations like calcium and sodium can counteract the negatively charged interlayer surfaces (Phillips et al., 2002). Chemicals can bind to these active interlayer surfaces, making CM clay an efficient sorbent of many environmental chemicals. Clays such as montmorillonites have been used as sorbents to reduce exposures to compounds such as pesticides and heavy metals (Abollino et al., 2008; Peng et al., 2009; Polati et al., 2006). Montmorillonite clay is a natural component of many soil types, so no adverse environmental impacts are expected with adding this clay as a soil amendment (Ito & Wagai, 2017).

The CM clay used in this study has an average total surface area of 850 $m^2\ g^{-1}$, an external surface area of approximately 70 $m^2\ g^{-1}$, and a cation exchange capacity of 97 $cmol\ kg^{-1}$ (Grant & Phillips, 1998). The general formula for CM is $(Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$, and the compound contains some quartz, mica, calcite, orthoclase feldspars, and sanidine as impurities (Marroquín-Cardona et al., 2011). It has been extensively studied for safety and efficacy in humans and animals to reduce exposures to environmental contaminants. Studies in rats, broiler and leghorn chicks, swine, goats, and dairy cows demonstrated the safety of CM clay at doses from 0.25 to 2% w/w and efficacy in significantly reducing biomarkers of aflatoxin exposure (Phillips et al., 2019). A long-term study in rats (28 weeks) confirmed the safety of CM clay up to 2% w/w (Afriyie-Gyawu et al., 2005). Importantly, montmorillonite clays were effective in reducing biomarkers of aflatoxin exposure in human clinical trials and safe for consumption by adults up to 3 months and children up to 2 weeks (Afriyie-Gyawu et al., 2008; Mitchel et al., 2014). Collectively, these studies suggest that CM clay is safe for human and animal consumption and can bind aflatoxin in the gastrointestinal tract, reducing aflatoxin bioavailability and protecting against exposures.

In addition to being an effective sorbent of aflatoxin, CM clay has also been shown to tightly bind the widely used herbicides glyphosate and paraquat with adsorption fitting the Langmuir trend, indicating saturated binding sites (Wang et al., 2019c). CM clay was also able to bind coplanar polychlorinated biphenyl (PCB) congeners including PCB 77 and PCB 126 (Wang et al., 2019d).

1.4.3 Modified clays

Many environmental chemicals have lipophilic properties, (i.e. pesticides), necessitating sorbents that can effectively bind these chemicals in water and soil. Modifying clays with organic cations to enhance binding affinities for these chemicals has been widely studied (Groisman et al., 2004; Park et al., 2011; Shah et al., 2018). Clays such as montmorillonites are often used to synthesize these modified clays due to their cation exchange capacities, swelling abilities, and charge densities (Guégan, 2019). To create these modified clays, cationic compounds are intercalated with the inorganic cations in the clay interlayers through ion exchange, changing the chemical nature of the layers from hydrophilic to hydrophobic. These “organoclays” have large surface areas and hydrophobic behaviors and have been used as adsorbents of organic pollutants in water and soil. A study using montmorillonite clays modified with 6 different organic cations found that modified clays removed 95% of pesticides from water while unmodified clays removed 15% of pesticides (Celis et al., 2007). Soil amendments of montmorillonites modified with organic cations at inclusion levels up to 5% significantly enhanced pesticide retention in soil and reduced leaching through soil columns (Gámiz et al., 2010).

In this study, the parent CM clay was modified with L-carnitine (CM-carnitine) and choline (CM-choline). L-carnitine is an endogenous ammonium compound that is involved in energy metabolism, hormone action, adaptation to stress and detoxification (Oney-Birol, 2019). Choline is an essential nutrient that is important in phospholipid and trimethylglycine production as well as acetylcholine synthesis. CM-carnitine and CM-choline have been shown to tightly bind a variety of chemicals in aqueous conditions. These modified clays had significantly increased binding capacities and affinities for two lipophilic chemicals, benzo[*a*]pyrene (BaP) and aldicarb, compared to the parent CM clay (Wang et al., 2019a). CM-carnitine and CM-choline bound aflatoxin B_1 (AFB_1) with capacities of 0.39 and 0.42 mol kg^{-1}, respectively, and significantly protected *Hydra vulgaris* from AFB_1 toxicity (Wang et al., 2017). PFOA and PFOS were tightly adsorbed to interlayer surfaces of modified clays at pH 2, with sorbent capacities up to 0.65 mol kg^{-1} (Wang et al., 2021b). Results from molecular dynamics (MD) simulations suggested that hydrophobic and electrostatic interactions as well as hydrogen bonds were involved in PFAS sorption to modified clays. Additionally, modified clays showed enhanced binding of Bisphenols A (BPA) and S (BPS) through the formation of hydrogen bonds with the two compounds (Orr et al., 2020).

Not only does L-carnitine enhance the lipophilicity of clay surfaces, it has also been shown to be beneficial for plant growth and health. L-carnitine is involved in various physiological processes in animals and microbes, such as fatty acid metabolism. Specifically, L-carnitine is an essential cofactor of fatty acid intracellular trafficking. It is also important in cellular maintenance and oxidative stress regulation due to its antioxidant properties. Precursors of L-carnitine biosynthesis are similar between animal cells and plant cells. Although the role of L-carnitine in plants is not well understood, it is hypothesized that L-carnitine may be similarly important in cellular processes in plant tissues (Jacques et al., 2018). L-carnitine can be found in various plant parts such as leaves and germinating seeds (Bourdin et al., 2007). Importantly, one study found that the exogenous application of L-carnitine to barley seeds germinated and grown under salt stress conditions promoted plant growth and mitotic activity as well as protected plant cells from the genotoxic effects of salt (Oney-Birol, 2019). Inclusion of clays modified with L-carnitine to reduce chemical exposures could present an added benefit to plant health and growth.

1.4.4 Acid processed clays

Due to the diverse characteristics and properties of environmental chemicals, broad-acting sorbents are needed to bind a variety of chemicals that may be of concern at a particular site or region. Processing clays with high concentrations of acid has been shown to significantly increase pore volume and size as well as enhance surface area. Treatment with sulfuric acid (>4N) results in leaching of interlayer cations causing decreases in aluminum, magnesium, and ferric content, replacement with protons from the acid causing increases in the overall acidity of the clay, and destroying the clay structure to increase porosity and surface area (Kumar et al., 1995; Narayanan & Deshpande, 1998). The exchanges of aluminum, iron, and magnesium with H^+ ions result in modifications of the smectite crystalline structure (Tomic et al., 2011). The fraction of mesopores is augmented by increases in interlayer distance (Novikova et al., 2006). These changes in the octahedral sheet promote dispersion of clay layers and the formation of amorphous silica, resulting in a clay with lower cation exchange capacity (CEC) and higher surface area and porosity.

Acid-processed clays have been used as catalysts in esterification reactions and as sorbents to remove chemicals from water or immobilize chemicals in soil. Acid-treated montmorillonites have shown promise as catalysts for fatty acid esterification reactions, specifically in the production of biodiesels (Nascimento et al., 2015; Zatta et al., 2014). Enhanced sorption of heavy metals (zinc, copper, manganese, cadmium, lead, and nickel) from automobile effluent was seen with an acid-treated montmorillonite clay compared to the parent clay (Akpomie & Dawodu, 2016). Acid-treated clays have also been used to remove dyes like methylene blue and rhodamine 6g from water (Banat et al., 2007; Teng & Lin, 2006).

The acid processed montmorillonite clay (APM) used in this study was synthesized using calcium montmorillonite clay and sulfuric acid. APMs have shown to be highly effective sorbents of mycotoxins such as aflatoxin and zearalenone as well as pesticides such as glyphosate (Wang et al., 2019b). For example, APM had a capacity of 0.58 mol kg^{-1} for glyphosate compared to 0.34 mol kg^{-1} for the parent calcium montmorillonite clay. Additionally, APMs bound polychlorinated biphenyls (PCBs) and PCB mixtures with high capacities (up to 0.43 mol kg^{-1}), affinities (up to 2.0E+06), and enthalpies (up to -136 kJ mol^{-1}) and protected against PCB toxicity *in vivo* (Wang et al., 2019d). Based on isothermal analyses and *in vivo* results, APM was also a highly effective and high-capacity sorbent of lead, reducing toxicity by 75% (Wang et al., 2021a). These studies suggest that APMs are effective sorbents of a variety of chemicals in aqueous environments. However, the use of acid-processed montmorillonite clays as soil amendments to reduce chemical bioavailability has not been explored.

1.5 Research objectives

During natural and manmade disasters such as floods and chemical spills, hazardous chemicals can be mobilized in the environment and affect water and food sources. These events can exacerbate existing levels of contamination in the soil, water, and sediment. There are growing concerns about exposures to environmental chemicals through either contact with contaminated soil and water or consumption of contaminated food products. Hazardous chemicals including pesticides and PFAS accumulate in the environment over time and can be detected in high levels after disasters, negatively affecting human health. Practical and economically-feasible technologies are needed to reduce the bioavailability of these chemicals and the risk of potential exposures.

Clays are naturally-occurring in the environment and thus present safe and efficient strategies to immobilize chemicals in soil. Montmorillonite clays are natural components of many soil types and thus do not present any negative impacts on environmental health when added as soil amendments. Additionally, extensive research has been conducted on carbon-based sorbents in remediation of water and soil. Sorbents including clays and carbons have been used in water treatment and as soil amendments to immobilize contaminants and protect against exposures. However, the use of novel, broad-acting, processed/modified sorbents has not been as widely studied, especially as soil amendments. We propose that complementary studies focused on the characterization of binding reactions between sorbents and chemicals, bioassays to evaluate sorbent abilities to protect against chemical toxicity, and efficacy testing in soil and plant studies will result in enhanced soil amendment strategies to protect against exposures to hazardous chemicals. Application of broad-acting sorbents to soil can protect against human and animal exposures by sequestering hazardous chemicals in the soil. Additionally, using sorbents as soil amendments can reduce translocation of chemicals to plants, thus protecting food supplies and decreasing adverse health effects associated with chemical exposure. The data collected on these sorbent-chemical relationships can be used to predict the best sorbents for chemicals and select optimal sorbents or combinations of sorbents depending on the chemicals of concern. Sorbent mixtures can be fine-tuned and optimized based on the contaminants at specific sites. The contribution of this research includes: 1) well-characterized sorbent-chemical interactions, 2) potential binding mechanisms of the interactions of chemicals at active sorbent surfaces, and 3) the development of safe and effective broad-acting sorbents that can be applied as soil amendments to protect against harmful exposures to environmental chemicals.

The main objective of this study was to develop novel, broad-acting sorbents that can be applied as soil amendments to reduce the bioavailability of diverse environmental contaminants and reduce translocation of these contaminants to plants. We have characterized sorbent-chemical relationships and described optimal sorbents for specific chemicals and classes of chemicals. *In vitro* adsorption isotherms were used to describe the capacities and surface affinities of the sorbents for chemicals and the thermodynamics of the binding reactions. *In vivo* bioassays using *Hydra vulgaris* and *Lemna minor* were used to evaluate the safety of sorbents for living organisms and validate sorbent ability to protect against chemical toxicity. We then investigated the efficacy of the sorbents in a soil matrix and their ability to reduce plant uptake of hazardous chemicals. To accomplish these objectives, we:

1) Screened carbon- and clay-based sorbents for binding potential of diverse environmental chemicals. We selected chemicals of concern from the ATSDR SPL and emerging chemicals that present exposure risks based on prevalence and toxicity. Optimal binding was determined based on sorption parameters. *In vitro* adsorption isotherms provided information about the capacities (Q_{max}) and surface affinities (K_d) of the sorbents for chemicals and the enthalpy (ΔH) of the binding reactions. Analytical methods were validated for each specific chemical using high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (UPLC-MS/MS). These methods confirmed the structural identity and purity of each chemical and were used for sample analysis.

2) Conducted *in vivo* bioassays using *Hydra vulgaris* (freshwater *Cnidarian* polyp) and *Lemna minor* (freshwater aquatic plant) to evaluate the safety of sorbents for living organisms and sorbent ability to protect against chemical toxicity. *Hydra vulgaris* and *Lemna minor* are both highly sensitive to environmental contaminants. We evaluated the phenotype endpoints of both species over time as indicators of toxicity.

3) Applied optimal sorbents for specific chemicals to soil bioavailability and plant uptake models to validate sorbent efficacy to reduce chemical bioavailability and translocation to plants. Soil bioavailability studies provided insight into the efficacy of the sorbents in a soil matrix. Plant uptake studies were important in evaluating sorbent ability to reduce translocation of hazardous chemicals to plants and protect plants from chemical toxicity.

These studies were conducted for 4 chemicals of concern: dieldrin, AMPA, PFOA, and PFOS based on the diverse properties of these chemicals. Dieldrin is lipophilic (logP 5.4), AMPA is hydrophilic (logP -4.7), and PFOA and PFOS are amphiphilic with hydrophilic and lipophilic properties. The sorbents used in this study have unique structures, physical characteristics and binding sites, making them potential sorbents of these diverse chemicals. Thus, the specific aims of this study were to:

1) Evaluate parent CM and APM as sorbents of dieldrin and their abilities to reduce dieldrin bioavailability from soil. Adsorption isotherms were used to determine sorbent capacities and affinities as well as enthalpies and potential mechanisms of the binding reactions. A *Hydra vulgaris* assay was used to determine sorbent ability to protect against dieldrin toxicity, soil studies were used to determine sorbent ability to reduce dieldrin bioavailability, and dosimetry plots were used to extrapolate doses of sorbents that could protect against dieldrin exposure.

2) Evaluate AC and CM as sorbents of AMPA and abilities to reduce AMPA bioavailability from soil and translocation to GM-corn sprouts. Adsorption isotherms were used to determine sorbent capacities and affinities as well as enthalpies and potential mechanisms of binding reactions. A *Hydra vulgaris* assay was used to determine sorbent ability to protect against AMPA toxicity, soil studies were used to determine sorbent ability to reduce AMPA bioavailability, and plant studies were conducted to evaluate PMG and AMPA translocation to GM-corn sprouts, metabolism of PMG to AMPA, and reduction of AMPA translocation with sorbents.

3) Evaluate AC, parent CM, CM-carnitine, and CM-choline as sorbents of PFOA and PFOS and their abilities to reduce PFOA and PFOS bioavailability from soil and translocation to cucumber sprouts. Adsorption isotherms were used to determine sorbent capacities and affinities as well as enthalpies of binding reactions. A *Lemna minor* assay was used to determine sorbent ability to protect against PFAS toxicity, soil studies were used to

determine sorbent ability to reduce PFAS bioavailability, and plant studies were conducted to evaluate PFAS translocation to cucumber sprouts, and reduction of PFAS translocation with sorbents. Additionally, molecular dynamics (MD) simulations were conducted to validate binding percentages and binding modes.

2. STRONG ADSORPTION OF DIELDRIN BY PARENT AND PROCESSED MONTMORILLONITE CLAYS*

2.1 Introduction

The widespread use of pesticides has resulted in the accumulation of pesticide residues in the environment due to their persistence and stability. Dieldrin, an organochlorine insecticide, was used extensively in agricultural and commercial settings until the 1970s, when many countries began to prohibit its use due to toxicity concerns (Agency for Toxic Substances and Disease Registry [ATSDR], 2002). However, dieldrin and other persistent organic pollutants can still be found throughout the environment, posing significant contamination threats. High levels of dieldrin have been detected in soil from vegetable farms and other agricultural fields years after discontinuation of use (Getenga et al., 2004; Harner et al., 1999; Hashimoto, 2005; Zhang et al., 2012). The high affinity of dieldrin for organic matter such as animal fat and plant wax can result in its bioaccumulation (US Environmental Protection Agency [USEPA], 2003). Reports of dieldrin uptake by plants such as root crops indicate the potential for exposure to dieldrin through consumption of contaminated produce (Doong et al., 1999; Donnarumma et al., 2009; Otani et al., 2007; Saito et al., 2012). Dieldrin uptake by grasses and other herbaceous plants is of concern for livestock and domestic animals (Paton & Petterson, 1997). Moreover, exposure to dieldrin has been associated with neurotoxic, reproductive, and carcinogenic effects (Abramovich et al., 2007; Kanthasamy et al., 2005; Stern, 2014).

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Exposure to pesticides and other chemicals can be enhanced during natural and man-made disasters such as floods and chemical spills. Chemicals can be mobilized and redistributed throughout the environment following these disasters and can further contaminate food and water sources, increasing the risk of harmful exposures. Humans and animals can be exposed to these chemicals through contaminated food and water or hand-to-mouth exposures resulting from contact with contaminated soil. Pesticides such as dieldrin are of particular concern due to their widespread use and persistence in the environment. Protecting humans and animals from harmful exposures to these environmental contaminants is an emerging issue in public health.

Natural clay materials can be used as sorbents to reduce the bioavailability of hazardous chemicals. Montmorillonite clays have a 2:1 aluminosilicate layered structure with high surface areas that contribute to their abilities to bind various chemicals in pores and on active interlayer surfaces. Our laboratory has shown that calcium montmorillonite (CM) clays are exceptional sorbents that are safe for human and animal consumption on a short-term basis and can tightly bind aflatoxin in the gastrointestinal tract, reducing its bioavailability (Phillips et al., 2008, 2019). Previous studies indicate that acid treatment of clays increases their sorption capacities. Acid processing induces structural changes such as exchange of extralattice cations and dissociation of octahedral and tetrahedral sheets, resulting in the formation of amorphous silicon and increased surface area and pore volume (Kumar et al., 1995; Narayanan & Deshpande, 1998; Tomić et al., 2011). For example, treatment of CM with 17% sulfuric acid can increase montmorillonite surface area by a factor of 2 (Novikova et al., 2006). Recent studies in our laboratory have shown that acid-processed calcium montmorillonite clays (APMs) can have increased capacities for mycotoxins and organophosphate pesticides compared with the parent CM clay (Wang et al., 2019b). These novel, broad-acting sorbents can bind a variety of environmental chemicals and could be included in diets on a short-term basis to reduce human and animal exposures to chemicals in water or food.

Previous studies have evaluated the potential of various natural and amended clay minerals as sorbents for pesticides. A natural kaolinite/montmorillonite clay was able to reduce levels of methomyl in aqueous solutions by 27 to 33% (El-Geundi et al., 2012). In addition, clays modified with cationic surfactants show increased binding potentials for pesticides compared with natural clays. Montmorillonite clay modified with octadecyltrimethylammonium bromide was 100-fold more effective than the natural montmorillonite in binding the hydrophobic pesticides penconazole and metalaxyl, and could be used to decrease the mobility of these pesticides in soil (Sanchez-Martin et al., 2006). Organosmectites reduced bentazone availability in soil almost instantaneously from 124 $\mu g\ g^{-1}$ to 1 $\mu g\ g^{-1}$ (Carrizosa et al., 2000).

There are currently no reports of acid-modified clay-based sorbents that can be used as enterosorbents to reduce the bioavailability (bioaccessibility) of dieldrin. The present study was designed to determine the effectiveness of CM and APMs as sorbents for dieldrin using in vitro isothermal analyses (including thermodynamics and binding mechanisms), dosimetry studies, and the hydra assay. In addition, CM and APMs were evaluated for their ability to bind dieldrin in soil and reduce potential exposures.

2.2 Materials and methods

2.2.1 Reagents

High-pressure liquid chromatography (HPLC)-grade acetonitrile and pH buffers (4.0, 7.0, and 10.0) were purchased from VWR. Both HPLC-grade hexane and dieldrin analytical standard were purchased from Sigma-Aldrich, and HPLC-grade acetone was purchased from Fisher Scientific. The CM was obtained from Engelhard, and it had an average total surface area of 850 $m^2\ g^{-1}$, an external surface area of approximately 70 $m^2\ g^{-1}$, and a cation exchange capacity of 97 $cmol\ kg^{-1}$ (Grant & Phillips, 1998). The general formula for CM is $(Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$, and the compound contains some quartz, mica, calcite, orthoclase feldspars, and sanidine as impurities (Marroquín-Cardona et al., 2011). Deionized water (18.2 MΩ) was generated in the laboratory using an Elga™ automated filtration system and was used in all experiments.

2.2.2 Synthesis of sorbents

The APMs were synthesized following methods previously described (Wang et al., 2019b). Briefly, CM was treated with sulfuric acid to produce sorbents with high surface areas and greater porosities than the parent clay. The CM clay suspensions (6% w/w) were treated with 18 N or 12 N (APM1 or APM2) sulfuric acid. The solutions were stirred vigorously in an oven at 60 °C overnight. The slurry was cooled, centrifuged at 2000 g for 20 min, and washed thoroughly with distilled water. This centrifugation/washing process was repeated until the pH was constant. Samples were oven-dried overnight at 110 °C, ground, and sieved through a 125-µm screen. Grinding and sieving were repeated until uniform particle sizes were obtained.

To determine the importance of clay interlayers in binding dieldrin, clays were heat-collapsed to significantly reduce interlayer surfaces. Collapsed CM and APM1 clays were prepared by heating at 200 °C for 30 min, and then at 800 °C for 1 hr (Grant and Phillips 1998).

2.2.3 In vitro analysis: isothermal adsorption

The dieldrin stock solution was prepared by dissolving pure powder into the analytical mobile phase (66:34 acetonitrile:water). Sorbents were added at 0.002% w/v to dieldrin solutions with an increasing dieldrin concentration gradient. The 0.002% inclusion level was achieved by adding 40 µL of 0.5 mg ml^{-1} clay suspension in the mobile phase, which was mixed vigorously during transfer to ensure equal distribution of clay to each sample. The concentration gradients of dieldrin solutions were achieved by adding a calculated amount of dieldrin stock solution to a complementary volume of mobile phase in 1.5-mL centrifuge tubes to a total volume of 1 mL. In addition to these samples, 3 controls were tested (mobile phase, dieldrin solution, and 0.002% clay solution). All samples were agitated on an orbital shaker at 1000 g for 2 hrs at ambient temperature (25 °C) and high temperature (37 °C) for thermodynamics experiments. Samples were then centrifuged at 2000 g for 20 min to separate clay/dieldrin complexes from the solution.

To measure the amount of free dieldrin in the supernatant, HPLC was used (Pantaleoni et al., 1989). Chromatography was conducted on a Waters HPLC device equipped with a 717plus Autosampler, model 1525 binary pumps, model 2487 Duel Absorbance Detector, and a Symmetry® C18 5-µm (4.6 × 150 mm) column. Chemical separation was achieved by a mobile phase of 66% acetonitrile and 34% water at a flow rate of 1.5 mL min^{-1} and injection volume of 20 µL. The ultraviolet light detector was set at 215 and 254 nm. Breeze® software was used to control the HPLC system and collect the data. The standard curve for dieldrin was linear, with $r^2 > 0.99$.

2.2.4 Data calculations and curve fitting

Dieldrin was detected by HPLC and quantified using standard calibration curves. Dieldrin concentrations in solution were calculated from peak area at the dieldrin retention time (5.1 min). The amount adsorbed for each data point was calculated from the concentration difference between test and control groups. The resulting data were input into a Microsoft Excel program and plotted using TableCurve 2D (Systat Software) 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,

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

where q is the toxin adsorbed (mol kg^{-1}), $Q_{\max}$ is the maximum capacity (mol kg^{-1}), K_d is the distribution constant, and C_w is the 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:

$$K_d = \frac{q}{(Qmax-q)Cw}$$

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 2 temperatures (25 and 37 °C):

$$\Delta H = \frac{-R\ln\left(\frac{Kd2}{Kd1}\right)}{\left(\frac{1}{T2}\right)-\left(\frac{1}{T1}\right)}$$

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

2.2.5 Dosimetry extrapolations

Dosimetry studies were conducted to estimate the doses of sorbents necessary to maintain threshold limits of dieldrin. The dieldrin stock solution was diluted with mobile phase to produce 0.2 ppm dieldrin, which is twice its threshold limit. The regulatory limit of dieldrin in food is 0.1 ppm (ATSDR, 2002). An increasing gradient of sorbent (0.0005, 0.002, 0.005, and 0.2% w/v) was added to the dieldrin solution to make 1 mL. Control groups included mobile phase, dieldrin solution, and clay solution. Control and test groups in 1.5-mL centrifuge tubes were agitated at 1000 rpm for 2 hrs and then centrifuged at 2000 g for 20 min. Aliquots of dieldrin were measured by HPLC, and concentrations were calculated from the signal peak area. The toxicant sorption percentage for each group was calculated from the difference between control and test groups. The sorbent inclusion levels were plotted against % dieldrin sorption, and the values were fit to a natural log trend to extrapolate the sorbent inclusion levels that would be required to reduce high exposures of dieldrin from food to below the threshold limit.

2.2.6 In vivo analysis: hydra assay

Hydra vulgaris were obtained from Environment Canada (Montreal, QC, Canada) and maintained at 18 °C. The original hydra classification method (Wilby et al., 1990) was used with modifications to rate morphology of the adult hydra as an indicator of solution toxicity. Following our modifications (Brown et al., 2014), the morphological scoring of hydra in this assay was objective and repeatable. A score of 0 to 10 was assigned individually to 3 hydra in each experimental group. Scores of 7 to 10 indicated minor toxicity, 4 to 6 moderate toxicity, and 0 to 3 severe toxicity. The modified assay included monitoring at shorter time intervals during the first 2 d (0, 4, 20, and 28 h) and at 24-hr intervals during the last 3 d (44, 68, and 92 h). Solutions were not changed during the testing period. Control groups included hydra media (18.2 MΩ water, 4 mg L^{-1} ethylenediamine tetraacetic acid, 115 mg L^{-1} *N*-tris[hydroxymethyl]methyl-2- aminoethanesulfonic acid, and 147 mg L^{-1} $CaCl_2$ adjusted to pH 6.9–7.0). Toxin treatment groups included 30 ppm dieldrin in hydra media based on the minimum effective dose that caused 100% mortality of the hydra in 92 h. Sorbent inclusion levels were equal to 0.5% w/v. All test groups in disposable culture tubes were capped and agitated at 1000 g for 2 hrs 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 score or average score for each group was used to determine the average toxicity rating at each time point. Each group contained 3 adult hydra in 4 mL of test solution at 18 °C.

2.2.7 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 Scotts Miracle-Gro. Soil was air-dried and sieved through a 1-mm screen before use, and then 1-g aliquots of soil were added to disposable culture tubes. Each soil sample was spiked with 2 mL of dieldrin/acetone solution and mixed to ensure even distribution of dieldrin. Samples were left uncapped in a fume hood until the acetone had completely evaporated (48 h). Sorbents were added at 1% w/w to a gradient of dieldrin concentrations and mixed. The samples were hydrated by adding 4 mL of distilled water and then slowly agitated at 200 rpm for 24 h. Soil extraction methods (Fish & Revesz, 1996; Correia-Sá et al., 2012) with modifications were used to extract dieldrin from soil samples. Briefly, 4 mL of 40:60 hexane:acetone was added to each sample before agitation at 1000 rpm for 1 h. Samples were centrifuged at 2000 g for 20 min, and the supernatants were transferred to new culture tubes. The samples were extracted twice. The supernatants were evaporated with nitrogen gas and then made up with 500 µL of the mobile phase. All samples were analyzed using HPLC. Calibration curves were conducted for each group of dieldrin extracts to ensure linearity of peak concentrations and consistency of the extraction method. Water and soil blanks and controls (spiked with different levels of dieldrin standard) were included in each experiment to validate the accuracy of our method and to determine the % recovery of dieldrin from the soil matrix. Peak areas of dieldrin from samples that included sorbents were compared with the dieldrin control samples to determine the percentage reduction in dieldrin bioavailability from soil. Results were reproducible and based on at least 3 independent replicates.

2.2.8 Statistical analysis

A 2-way t test was used to determine statistical significance. Each experiment was conducted in triplicate to derive means and standard deviations. The Q_{max} and K_d values from isothermal analyses, toxicity scores from the hydra assay, and peak areas of dieldrin from soil studies were used to calculate t values. The t values and degrees of freedom were used to determine the p value. Results were considered significant at $p \leq 0.05$.

2.3 Results

Adsorption isotherms for CM, APM1, and APM2 were generated using a Microsoft Excel program developed in our laboratory and plotted with TableCurve 2D to derive sorbent–dieldrin binding parameters including Q_{max} (capacity), K_d (affinity), and ΔH (enthalpy). Figure 5 shows the isothermal plots for dieldrin on the parent CM clay and the modified APM1 and APM2 clays. All 3 plots fit a Langmuir model with r^2 values >0.93, indicating tight binding of dieldrin. The curved shape of the Langmuir trend shows that binding of dieldrin onto active surfaces of the 3 sorbents was saturable. Both APM1 and APM2 had significantly higher binding capacities compared with the parent CM clay, with Q_{max} values of 0.45 and 0.4 mol/kg, respectively, compared with 0.32 mol/kg for CM ($p \leq 0.01$).

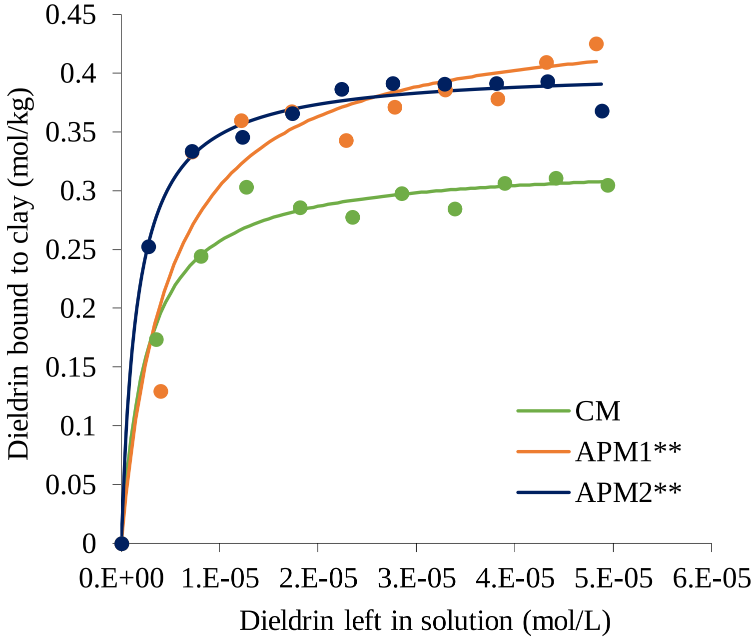
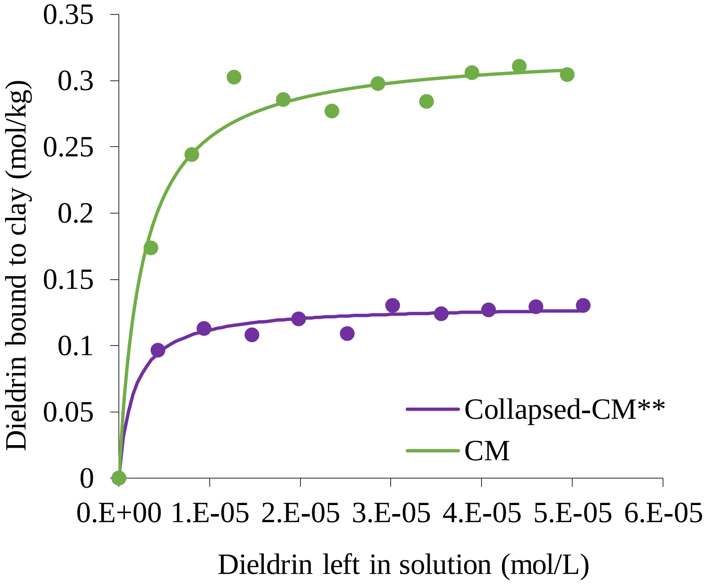


Figure 5. Langmuir plots of binding of dieldrin to calcium montmorillonite clay (CM), acid-processed calcium montmorillonite clay (APM)1, and AMP2 indicating tight binding of dieldrin by all 3 sorbents (CM: $Q_{max} = 0.324$ mol kg^{-1}, $K_d = 3.82E + 05$; APM2: $Q_{max} = 0.403$ mol kg^{-1}, $K_d = 6.21E + 05$; APM1: $Q_{max} = 0.450$ mol kg^{-1}, $K_d = 2.07E + 05$). Both APM1 and APM2 showed significantly higher Q_{max} values than the parent clay ($^{**}p \leq 0.01$).

Both CM and APM1 were heat treated to determine the importance of interlayer surfaces in binding of dieldrin. Heating clays at 200 and 800 °C has been shown to considerably dehydroxylate and collapse interlayer surfaces, thus reducing the interlayer binding sites available for dieldrin. The isothermal plots in Figure 6 show Langmuir trends for heat-collapsed CM and APM1 clays. The $Q_{\max}$ values were significantly decreased by 60 and 66% for the collapsed CM and APM1 clays, respectively, compared with the intact parent CM and APM1 clays ($p \leq 0.01$). Based on the extent of collapse in these experiments, the interlayer surfaces of both clays are clearly important for binding dieldrin.

(A)



(B)

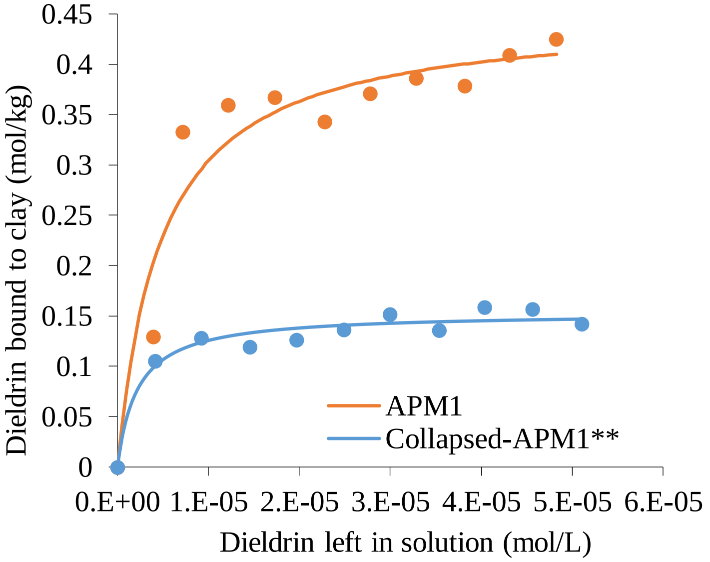


Figure 6. Langmuir plots of binding of dieldrin to calcium montmorillonite clay (CM; **A**) and acid-processed calcium montmorillonite clay (APM1; **B**) compared with heat-collapsed CM and APM1. The Q_{max} values indicated significantly reduced binding capacities of dieldrin with the collapsed clays (collapsed CM: $Q_{max} = 0.130$; collapsed APM1: $Q_{max} = 0.153$; $^*p \leq 0.01$).

To determine the enthalpy values for dieldrin–sorbent binding reactions, equilibrium isotherms were conducted at 25 and 37 °C. Isothermal plots for CM and APM1 at 37 °C are shown in Figure 7. The K_d values for these plots were compared with the K_d values for the plots determined at ambient temperature (25 °C), to calculate the enthalpies (ΔH) of the binding reactions using the van't Hoff equation. Enthalpy values for CM and APM1 were –55.7 and –61.4 kJ mol^{-1}, respectively.

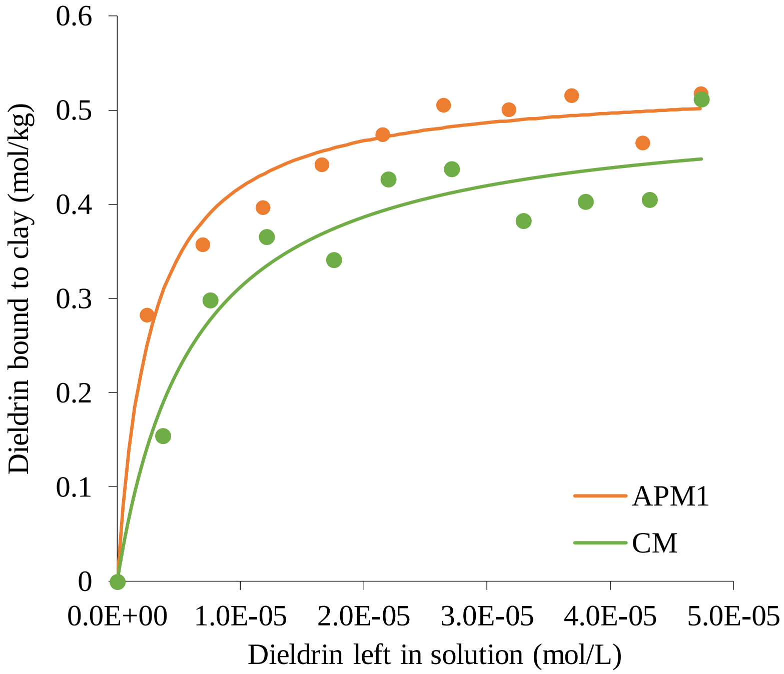


Figure 7. Langmuir plot of binding of dieldrin to calcium montmorillonite clay (CM) and acid-processed calcium montmorillonite clay (APM)1 at 37 °C (CM: $K_d = 1.60E+05$; APM1: $K_d = 4.33E+05$). The K_d values for these plots were compared with experiments conducted at 25 °C to determine the enthalpy of the binding reactions.

Figure 8 shows results of the hydra assay for CM and APM1 and the efficacy of these sorbents in protecting a living organism from dieldrin toxicity. Hydra groups were exposed to 30 ppm dieldrin solution, based on the minimum effective dose that caused 100% mortality of the hydra in 92 h. The control group received no sorbent, and the treatment groups were dosed with sorbent (0.5% w/v). Inclusion of 0.5% sorbent provided significant reduction in mortality of the hydra, with 60 and 70% protection for CM and APM1, respectively ($p \leq 0.01$).

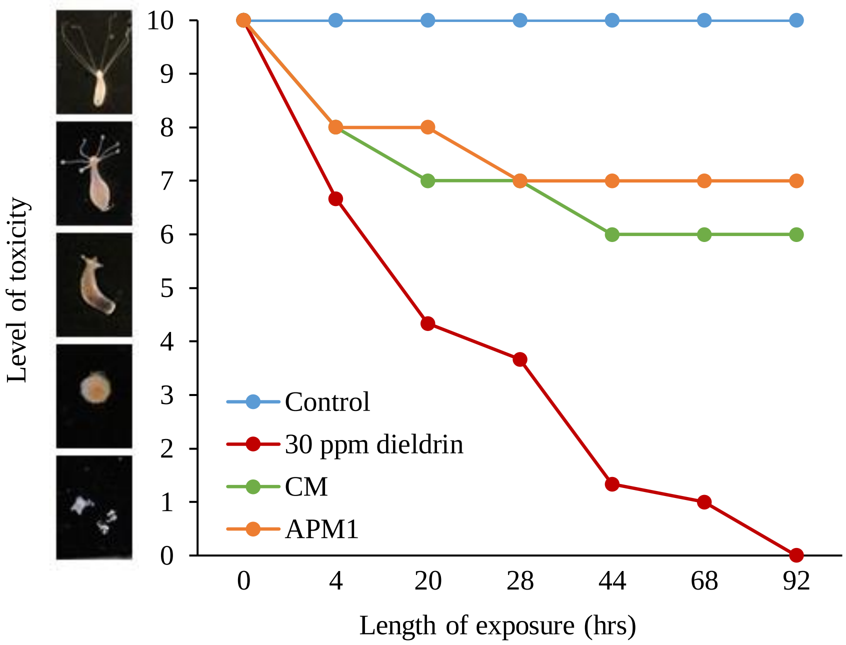


Figure 8. Hydra assay showing dieldrin toxicity at 30 ppm and protection of hydra with inclusion of 0.5% w/v calcium montmorillonite clay (CM) and acid-processed calcium montmorillonite clay (APM)1. Control groups with no dieldrin (hydra media and sorbent controls) are included for comparison and show scores of 10 throughout the test period. Sorbent inclusion at 0.5% was able to significantly protect the hydra from dieldrin toxicity ($^{**}p \leq 0.01$).

Dosimetry studies were conducted to determine the amounts of CM and APM1 that would be necessary to reduce exposures to high levels of dieldrin. The exposure level was set to 0.2 ppm, which is twice the regulatory limit in food (0.1 ppm; US Environmental

Protection Agency 2003). The sorbent inclusion levels (g kg^{-1}) were plotted against % toxicant sorption, and the data were fit to a log function, with CM being described by the equation $y = 0.0822 \ \ln(x) + 0.6206$ and APM1 by the equation $y = 0.067 \ \ln(x) \ 0.6055$ (Figure 9). The sorbent inclusion levels needed to reduce dieldrin exposure of 0.2 ppm to below threshold limits were calculated to be 0.230 g kg^{-1} for CM and 0.207 g kg^{-1} for APM1.

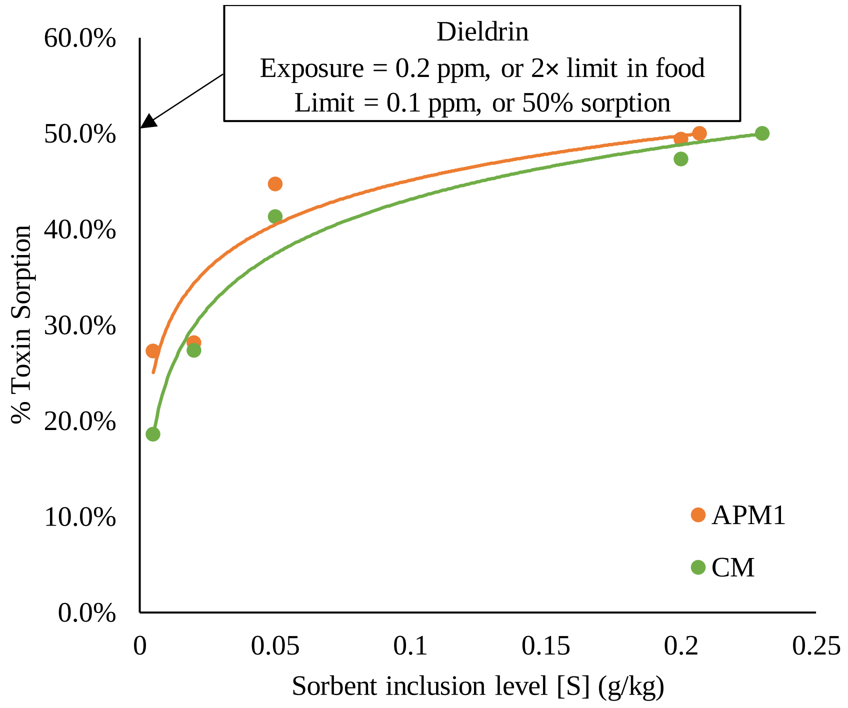
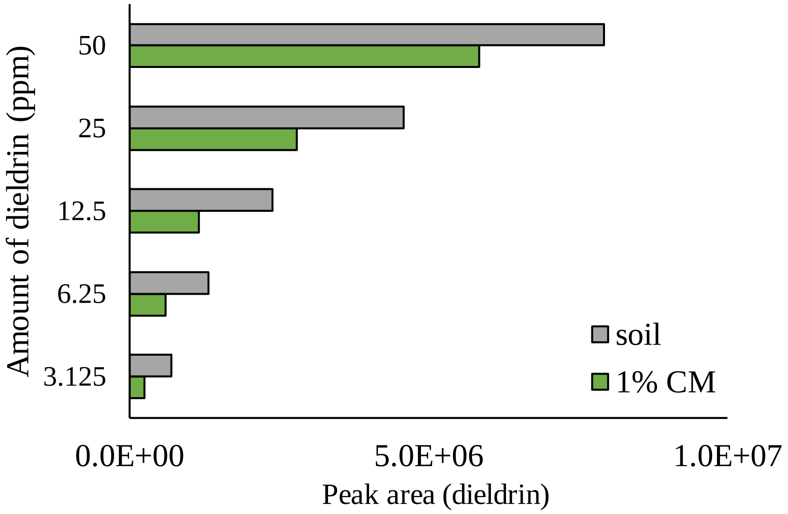


Figure 9. Dosimetry plots showing extrapolation of sorbent doses that can protect against dieldrin exposure. Calcium montmorillonite clay (CM) and acid-processed calcium montmorillonite clay (APM)1 trends are described by the equations $y = 0.082 \ln(x) + 0.6206$ and $y = 0.067 \ln(x) + 0.6055$, respectively.

Soil studies were conducted to determine the potential of CM and APM1 to reduce the bioavailability of dieldrin from soil. Soil samples (1.0 g) were spiked with a gradient of dieldrin ranging from approximately 3 to 50 ppm. Recovery of dieldrin from soil samples ranged from 74 to 82%. Figure 10 shows reduced recovery of dieldrin from spiked soil with

CM and APM1 treatment at 1% w/w compared with recovery without treatment. Inclusion of 1% CM reduced the amount of dieldrin extracted from soil in a dose-dependent manner ranging from 26.4 to 65.1%. Inclusion of 1% APM1 also reduced the amount of dieldrin extracted from soil in a dose-dependent manner ranging from 47.9 to 63.4%. These results indicate that CM and APM1 at low inclusion levels (1%) can significantly reduce the bioavailability of dieldrin from soil ($p \leq 0.01$).

(A)



(B)

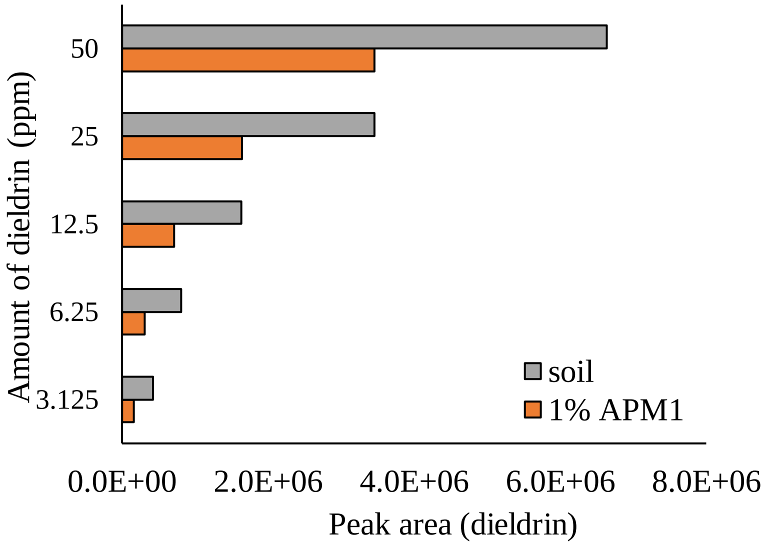


Figure 10. Soil studies showing significant reduction of dieldrin bioavailability with inclusion of (**A**) 1% calcium montmorillonite clay (CM) and (**B**) 1% acid-processed calcium montmorillonite clay (APM1; $*p \leq 0.01$). Inclusion of 1% CM and 1% APM1 provided 26.4 to 65.1% and 47.9 to 63.4% reduction in dieldrin bioavailability, respectively.

2.4 Discussion

Dieldrin is considered a persistent organic pollutant and has been shown to be toxic to humans and animals. Exposure to dieldrin has been associated with neurological, developmental, and carcinogenic effects in animal models (Bachowski et al., 1998; Nebeker et al., 1992; Richardson et al., 2006). Dieldrin residues have been detected in adipose tissue of sheep, ducks, cows, and swine (Corrigan & Seneviratna, 1990; Falandysz & Kannan, 1992; Paton & Petterson, 1997). In addition, dieldrin is the active product of aldrin, another organochlorine insecticide that was widely used to treat seed and soil. Because of the extensive use of these 2 chemicals and their abilities to bioaccumulate, high levels can be found throughout the environment (Jorgenson, 2001). Dieldrin and aldrin are ranked #18 and #25, respectively, on the ATSDR Substance Priority List of chemicals that are commonly found in contaminated facilities and pose a significant risk to human health due to their potential toxicities (ATSDR, 2017). Thus strategies are needed to reduce the risk of exposures to dieldrin and protect humans and animals from the adverse health effects associated with dieldrin exposure. Previous studies have investigated removal of dieldrin from environmental media with strategies such as magnetic nanoparticles, activated carbon sorbents, and bioremediation strategies (Hilber et al., 2009; Matsumoto et al., 2009; Shrivas et al., 2017). However, no studies have looked at the safety and efficacy of clays to mitigate unintended exposures to dieldrin in the environment. To develop an effective enterosorbent for dieldrin, we have characterized the sorption of the toxicant onto active interlayer surfaces of CM and APMs.

Various sorbents have been evaluated for binding potential with dieldrin in aqueous environments. Activated carbons from bamboo and coconut shell reduced 100 μg L^{-1} of dieldrin in drinking water sources by 68 and 71%, respectively (Thuy et al., 2012). A triolen-embedded activated carbon composite sorbent decreased dieldrin concentrations in an aqueous solution from 10 to 1.94 μg L^{-1} (Ru et al., 2007). Adsorption curves for silica aerogel and dieldrin showed 92% removal of dieldrin in 3 h (Liu et al., 2009). Bromopropyl functionalized silica nanofibers had a removal rate of 91% for dieldrin residues in water (Yue et al., 2012). All of these sorbents for dieldrin resulted in Freundlich trends, suggesting partitioning of dieldrin on and off sorbent surfaces and potentially weak bonding.

Equilibrium isothermal analyses were conducted for CM, APM1, and APM2. All 3 isotherms fit Langmuir models with high Q_{max} and K_d values, indicating efficient binding of dieldrin with saturable binding sites and high capacities and affinities for dieldrin. The APM1 and APM2 modified clays had significantly increased binding capacities for dieldrin compared with the CM clay. These findings are consistent with previous studies indicating that acid-processed clays have increased surface areas and toxin-binding capacities compared with parent clays. We found that APM1 had a higher capacity for dieldrin than APM2, so it was chosen for use in subsequent studies along with the parent CM clay.

The plateau surface density of CM was also calculated to determine the clay surface area available for dieldrin binding at saturation. The surface area of CM interlamellar binding sites is equal to $850 - 70 = 780\ m^2\ g^{-1} = 7.8 \times 10^{22}\ Å^2\ g^{-1}$. Using the Q_{max} value (maximum binding capacity) for dieldrin binding to CM, the number of dieldrin molecules bound (plateau surface density) is equal to $0.32\ \mathrm{mol\,kg^{-1}} \times 6.02 \times 10^{23}$ molecules $\mathrm{mol^{-1}} = 1.9 \times 10^{20}$ molecules $\mathrm{g^{-1}}$. The topological polar surface area of one dieldrin molecule is 12.5 Å^2, so the total binding area for dieldrin is equal to 1.9×10^{20} molecules $\mathrm{g^{-1}} \times 12.5$ Å$^2 = 2.4 \times 10^{21}$ Å^2 $\mathrm{g^{-1}}$ (smaller than the available CM surface area of 7.8×10^{22} Å^2 $\mathrm{g^{-1}}$). The high binding capacity of CM for dieldrin along with the plateau surface density indicates that CM contains more than enough binding sites for monolayer sorption, which is consistent with the curved shape and homogeneous binding site requirements of the Langmuir plots shown in Figure 5.

To investigate binding sites of dieldrin on both CM and APM1, the sorbents were heat-collapsed to reduce interlayer surfaces. Heating CM and APM1 at 800 °C significantly collapsed the interlayers. The results in Figure 6 show a 60% decrease in Q_{max} of collapsed CM compared with the intact CM (40% binding capacity remaining), and a 66% decrease in Q_{max} of collapsed APM1 compared with the intact APM1 (34% binding capacity remaining). This finding suggests that the interlayer surfaces of the clays are important in dieldrin binding to CM and APM1. After further heating, we would expect to see a greater degree of collapsed interlayer surfaces and a greater decrease in Q_{max} for both CM and APM1.

Enthalpy is another important parameter to consider in terms of sorbent–chemical interactions. Equilibrium isotherms were conducted at room temperature (25 °C) and high temperature (37 °C), and the K_{d} values were compared using the van't Hoff equation. The negative enthalpy values of CM and APM1 (–55.7 and –61.4 kJ $\mathrm{mol^{-1}}$, respectively) indicate a high heat of sorption for dieldrin binding to clay surfaces. The high absolute values of ΔH for both sorbents (> -20 kJ $\mathrm{mol^{-1}}$) indicate chemisorption (instead of physisorption) of dieldrin. The results suggest that the dieldrin–sorbent complex is stable and that dieldrin would not be readily dissociated from either sorbent.

The hydra assay predicted safety and efficacy of the sorbents in protecting an aquatic species against dieldrin toxicity. Both sorbents were not toxic to the hydra with inclusion levels as high as 0.5% w/v. The results indicate that CM and APM1 were able to significantly protect the hydra from dieldrin toxicity (up to 30 ppm). Inclusion of 0.5% of CM and APM1 reduced the toxicity of dieldrin from severe toxicity (score of 0) to moderate toxicity (score of 6) and to minor toxicity (score of 7), respectively. These results agree with the isothermal analyses, further indicating that CM and APM1 are both effective sorbents for dieldrin, with APM1 having a higher capacity. The in vivo results of our study were limited to an aquatic testing species (*H. vulgaris*) and toxin binding in an aqueous environment. Further work to investigate any potential differences in nonaqueous environments is warranted.

Dosimetry studies were conducted to determine the doses of CM and APM1 that would be necessary to protect humans and animals from high levels of dieldrin exposure. Based on the assumption that humans consume 1 kg of food at each meal, the low inclusion levels of sorbent (0.230 g kg^{-1} for CM and 0.207 g kg^{-1} for APM1) would equate to a small amount of sorbent taken 3 times a day (at least 2 h before or after medications to prevent any potential interactions).

Based on these in vitro and aquatic toxicity studies, CM and APM1 were found to be effective sorbents of dieldrin. After further investigation, it is possible that CM and APM1 could be delivered in tablets, capsules, condiments, snacks, and flavored water at low levels on a short-term basis to reduce harmful exposures to dieldrin. Including these sorbents could be important for vulnerable populations such as first responders and people living near contaminated sites or working in settings susceptible to spills or disasters. Further studies are necessary to determine the efficacy of these materials as sorbents of dieldrin in humans and animals, as various processes may alter their effectiveness in more complex biological systems.

Extensive research has been conducted on the use of activated charcoals as soil amendments to sequester environmental contaminants in soil (Sarmah et al., 2010; Yang et al., 2010). Reports of charcoals (i.e., biochars) used as soil amendments to reduce bioavailability and plant uptake of dieldrin support their effective sorption capacities for dieldrin and their potential to protect against harmful exposures (Donnarumma et al., 2009; Hilber et al., 2009). However, potential safety and adverse environmental impacts (e.g., changes in soil chemistry, nutrient availability, and metal contamination) associated with activated charcoals warrant the development of alternative materials with similar sorption abilities (Kookana et al., 2011). According to the isothermal and dosimetry results from our study, CMs and APMs are highly effective sorbents of dieldrin. In particular, APM1 has a very high capacity for dieldrin ($Q_{max} = 0.45$ kJ mol^{-1}), so it could be an effective sorbent for high concentrations of dieldrin that might occur as a result of a disaster or spill. The APM1 form was approximately twice as effective at reducing high levels (50 ppm) of dieldrin versus CM (47.9 vs 26.4% reduction). The CM form had a slightly lower capacity for dieldrin than APM1 ($Q_{max} = 0.324$ kJ mol^{-1}). At 1% inclusion, it reduced dieldrin's bioavailability by 65% at a concentration of 3 ppm. Importantly, CM has been shown to be safe for use in animals and humans in earlier animal studies and clinical trials (Phillips et al., 2019). Because environmental levels of dieldrin are relatively low (<3 ppm), inclusion of CM as a soil amendment at inclusion rates as low as 1% could be used routinely to reduce dieldrin exposures from soil (Hashimoto, 2005; Otani et al., 2007). In the case of a disaster or chemical spill, the more active APM1 could be added to protect against higher level exposures. Thermodynamic studies suggested that the clay–dieldrin complex is not easily dissociated, indicating that soil-based clay–dieldrin complexes would be stable in a soil environment. Montmorillonites and other clay materials are natural components of many soil types, so minimal adverse environmental impacts are expected (Ito & Wagai, 2017).

2.5 Conclusions

Our results collectively indicate that CM and APM clays are effective sorbents of dieldrin. Acid-processed CM clay has significantly increased binding capacity and affinity for dieldrin compared with the parent clay. These results are in agreement with previous studies indicating that acid-processed clays have increased surface areas and capacities. Future studies should be conducted to evaluate the safety and efficacy of APMs in reducing the bioavailability of environmental chemicals in animals and humans to determine whether APMs could be used as enterosorbents on a short-term basis to protect against harmful exposures.

The use of clay materials such as montmorillonites as toxicant enterosorbents may be effective in reducing the bioavailability of chemicals that have been ingested by humans and animals and from contaminated soil. Further studies will investigate APMs as broad-acting sorbents that can mitigate exposures to environmentally relevant mixtures of chemicals.

3. DECREASED BIOAVAILABILITY OF AMINOMETHYLPHOSPHONIC ACID (AMPA) IN GENETICALLY MODIFIED CORN WITH ACTIVATED CARBON OR CALCIUM MONTMORILLONITE CLAY INCLUSION IN SOIL*

3.1 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, 2014). 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.

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Glyphosate (phosphonomethylgycine; 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., 2020). 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.

3.2 Materials and methods

3.2.1 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 m^2/g, an external surface area of approximately 70 m^2/g, and a cation exchange capacity of 97 cmol/kg (Grant and Phillips, 1998). CM has a general formula of $(Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$ (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.

3.2.2 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.

3.2.3 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,

Langmuir model (LM) $q = Q_{max}\left(\frac{KdCw}{1+KdCw}\right)$

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:

$$K_d = \frac{q}{(Qmax-q)Cw}$$

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):

$$\Delta \mathrm{H} = \frac{-R\ln\left(\frac{Kd2}{Kd1}\right)}{\left(\frac{1}{T2}\right)-\left(\frac{1}{T1}\right)}$$

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

3.2.4 In vivo studies: hydra assay

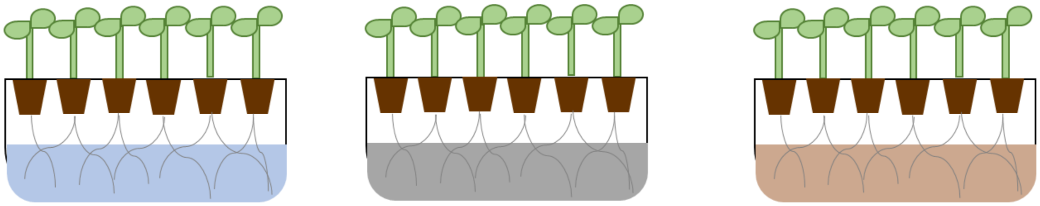
Hydra vulgaris were obtained from Environment Canada (Montreal, QC) and maintained at 18 °C. Using a hydra classification method, morphology of the hydra was rated over time as an indicator of solution toxicity. The morphological scoring of hydra in this assay is objective and repeatable (Brown et al., 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 $CaCl_2$ 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.

3.2.5 Soil studies

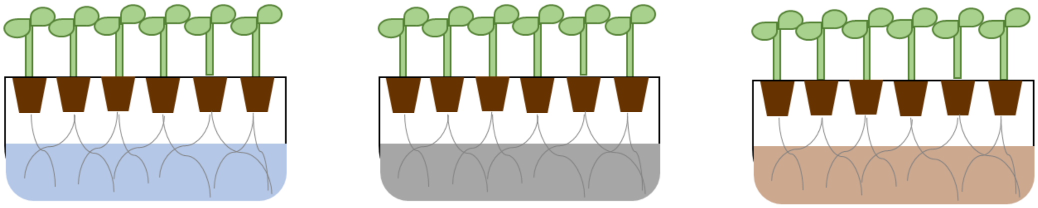
Garden soil (with a composition including compost, processed forest products, sphagnum peat moss, a wetting agent, and fertilizer containing 0.09% total nitrogen, 0.05% available phosphate, and 0.07% soluble potash) was obtained from The Scotts Miracle-Gro Company (Marysville, OH). Soil was air-dried 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 Na_2-EDTA (572 µL acetic acid and 0.74 g Na_2-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.

3.2.6 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 Foxfarm 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 (Figure 11). 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.



Blank (Soil/Compost Control)   Sorbent 1 (AC) Control   Sorbent 2 (CM) Control



Chemical (PMG) Control   PMG + AC (Treatment)   PMG + CM (Treatment)

Figure 11. 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.

Extraction methods described by Chamkasem and Harmon (2016) were followed with modification. Briefly, 5 mL of 50 mmol/L acetic acid/10 mmol/L Na_2-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 2000 × g for

20 min. The supernatants were passed through Strata C18-E (55 um, 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.

3.2.7 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.

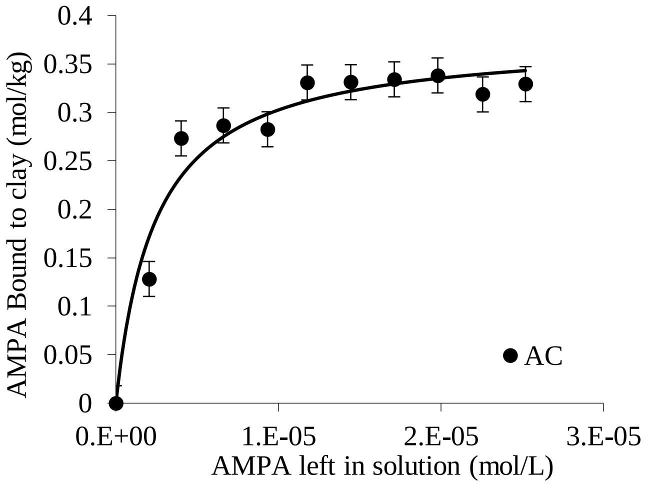
3.2.8 Statistical analysis

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 $p \leq 0.05$.

3.3 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 (Figure 12A 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 (Figure 12B). 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.

(A)



(B)

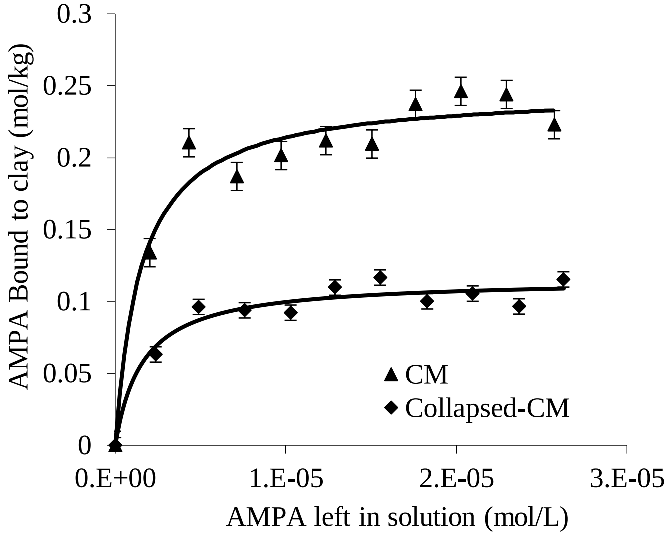


Figure 12. (A) Langmuir plots of AMPA on AC at 25 °C indicating binding of AMPA. (B) Langmuir plots of AMPA binding to CM and collapsed CM at 25 °C. Q_{max} value was significantly decreased with collapsed CM clay, indicating interlayer surfaces are important in AMPA binding to CM clay.

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 (Figure 13). 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.

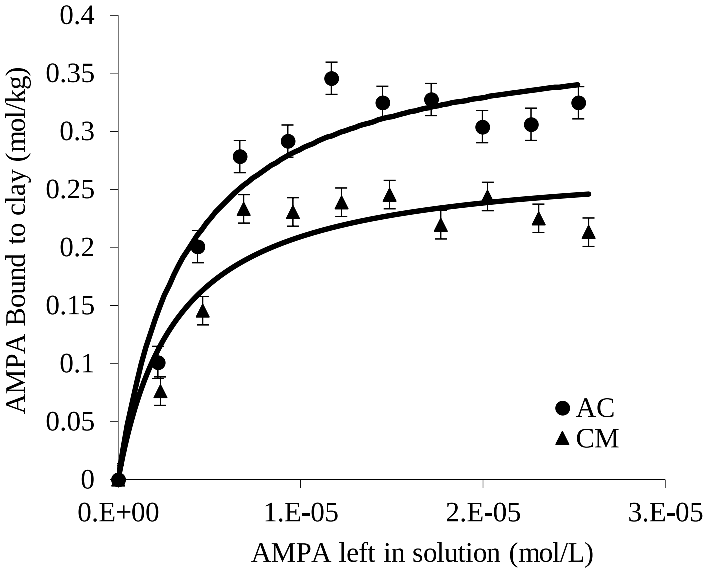


Figure 13. Langmuir plots of AMPA on CM and AC at 37 °C (CM: K_d = 3.10E+05; AC: K_d = 2.70E+05). K_d values for these plots were compared to those conducted at 25 °C to determine the enthalpy (ΔH) of the binding reactions. ΔH values for CM and AC were -48.2 and -26.6 kJ/mol respectively.

The hydra assay in Figure 14A 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 Figure 14B shows toxicity of 20 µg/mL PMG over the 92 hr exposure period.

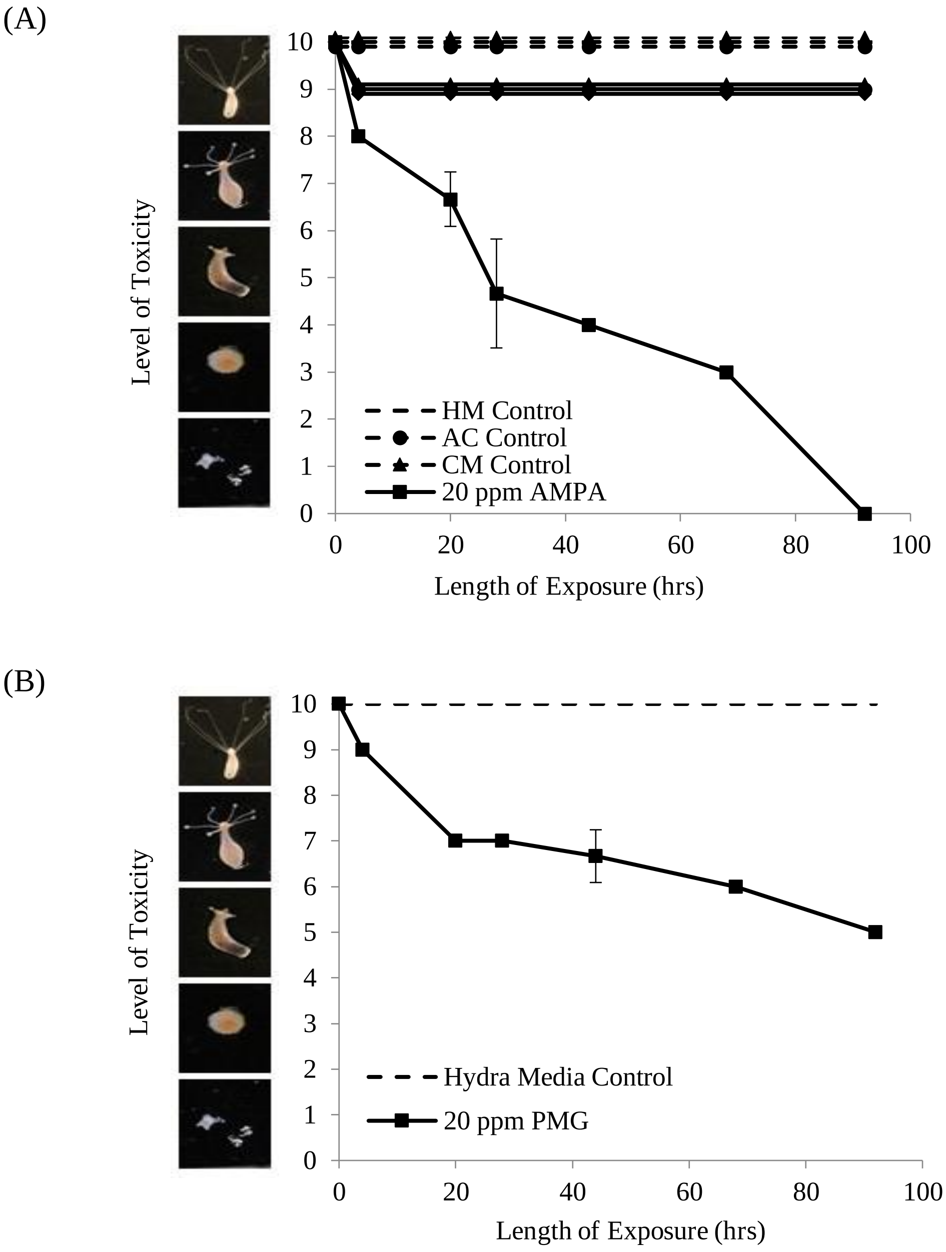


Figure 14. (A) Hydra assay showing AMPA toxicity at 20 µg/mL and the protection of hydra with inclusion of 1.0% *W/V* AC, CM, and 50:50 AC:CM. Control groups with no AMPA (hydra media and sorbent controls) are included for comparison and show scores of 10 throughout the test period. Sorbent inclusion 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.

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 Figure 15 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 ($p \leq 0.01$).

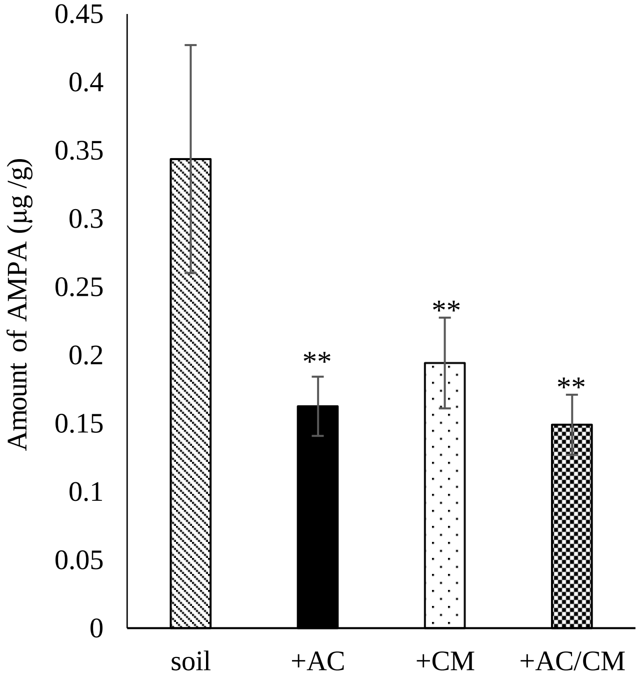
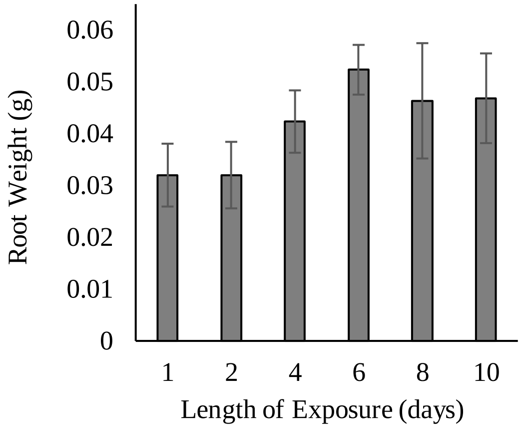


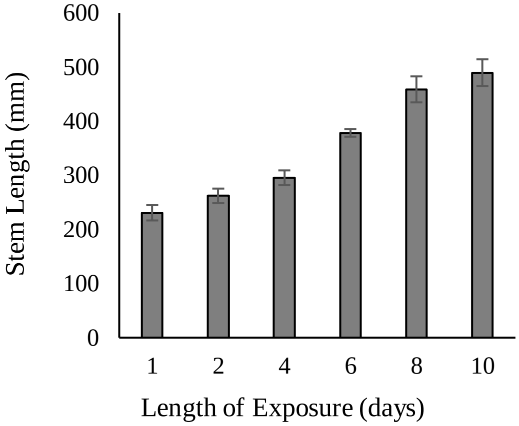
Figure 15. Soil studies showing significant reduction of AMPA bioavailability with inclusion of 1% AC, CM, and 50:50 AC:CM at 52.7%, 43.5%, and 56.6%, respectively. (**$p \leq 0.01$)

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 (Figure 16). 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 (Figure 16A), 45.6 mg/day (Figure 16B), and 29 mm/day (Figure 16C). 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 (Figure 16D 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 (Figure 16F and G).

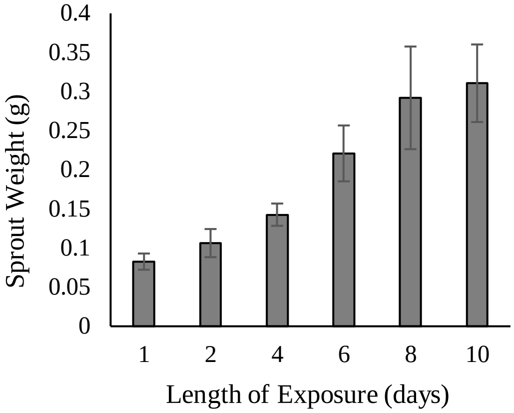
(A)



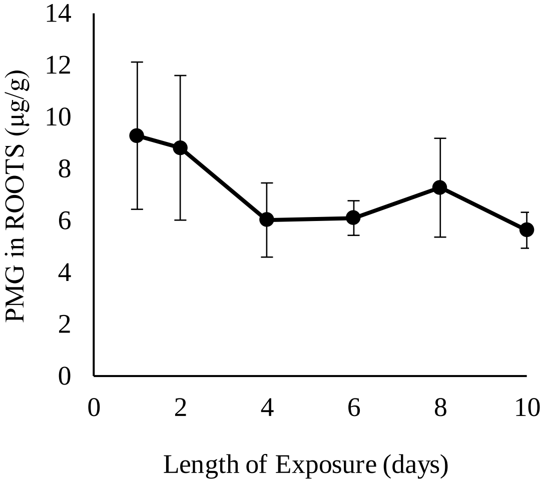
(B)



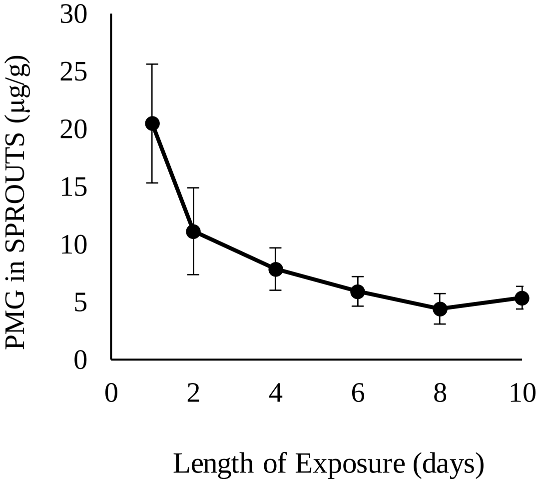
(C)


0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Sprout Weight (g)
1
2
4
6
8
10
Length of Exposure (days)

(D)


14
12
10
8
6
4
2
0
PMG in ROOTS (µg/g)
0
2
4
6
8
10
Length of Exposure (days)

(E)


30
25
20
15
10
5
0
PMG in SPROUTS (µg/g)
0
2
4
6
8
10
Length of Exposure (days)

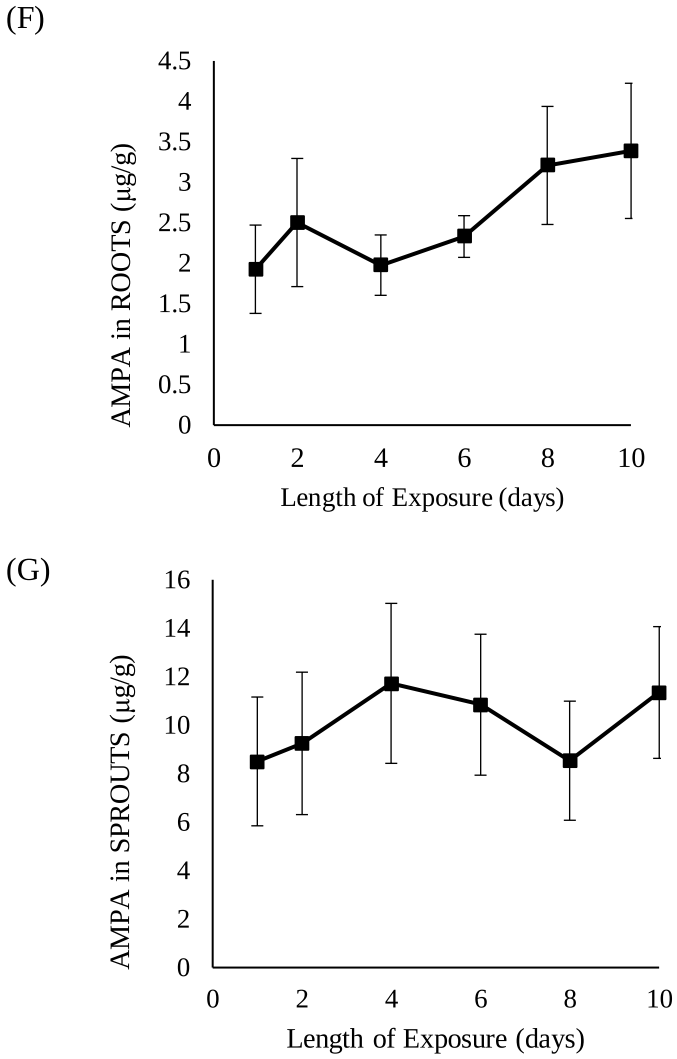
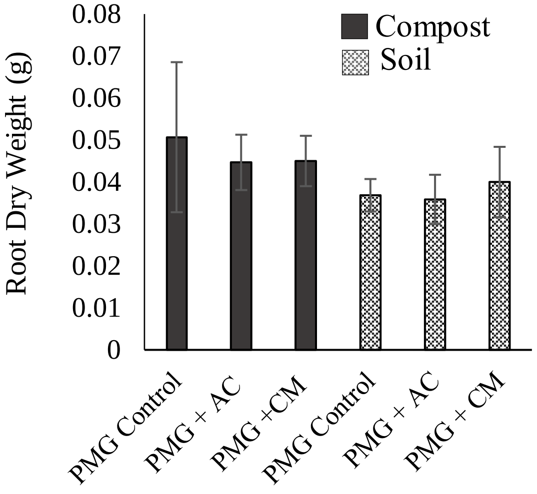


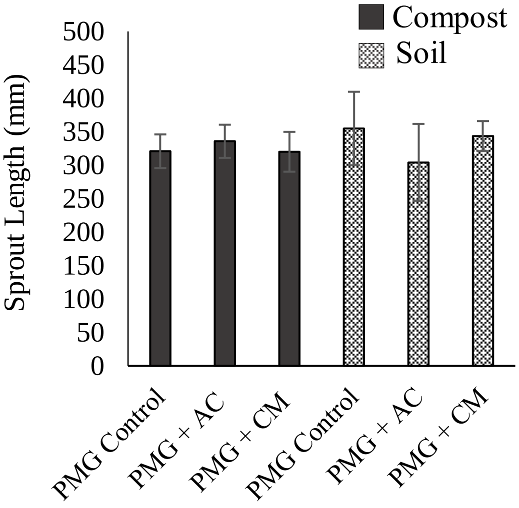
Figure 16. Time course plant study showing uptake of PMG and conversion to the metabolite AMPA. Root dry weight (A), sprout dry weight (B) and total sprout length (C) were measured over the duration of the 10-day exposure period. PMG and AMPA concentrations were measured in both the roots (D, F) and sprouts (E, G).

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 (Figure 17). 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 (*$p \leq 0.05$) (Figure 17A 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 (**$p \leq 0.01$) (Figure 17E). 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 (**$p \leq 0.01$) (Figure 17D). 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.

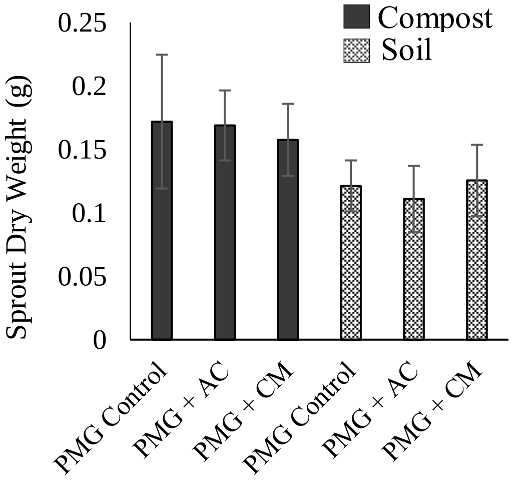
(A)


Compost
Soil
Root Dry Weight (g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
PMG Control
PMG + AC
PMG +CM
PMG Control
PMG + AC
PMG + CM

(B)


Compost
Soil
Sprout Length (mm)
500
450
400
350
300
250
200
150
100
50
0
PMG Control
PMG + AC
PMG + CM
PMG Control
PMG + AC
PMG + CM

(C)


Compost
Soil
Sprout Dry Weight (g)
0.25
0.2
0.15
0.1
0.05
0
PMG Control
PMG + AC
PMG + CM
PMG Control
PMG + AC
PMG + CM

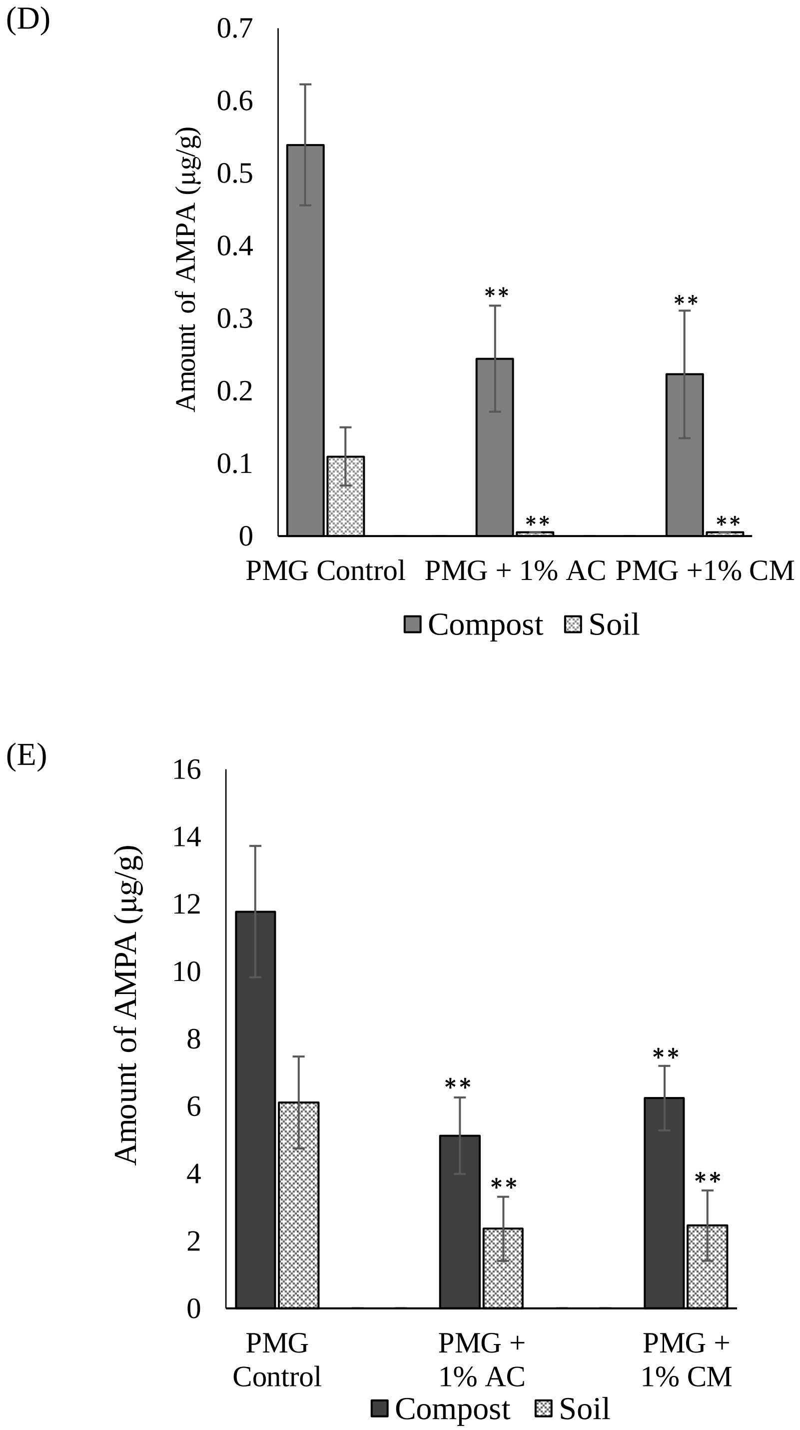


Figure 17. Plant uptake study showing AMPA residues in corn roots and sprouts for sprouts grown in both compost and soil. No significant differences were seen between groups for root dry weight (a), sprout dry weight (b), and total sprout length (c). 1% AC and CM were both able to significantly reduce AMPA residues in corn roots (d) and sprouts (e) in both the compost and soil studies (**$p \leq 0.01$)

3.4 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 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 (4.09E+05 and 6.05E+05 for AC and CM, respectively) indicated high affinities of the sorbents for AMPA (Table 1).

		$\boldsymbol{Q}_{\mathbf{max}}$	$\boldsymbol{K}_{\mathbf{d}}$
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

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).

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

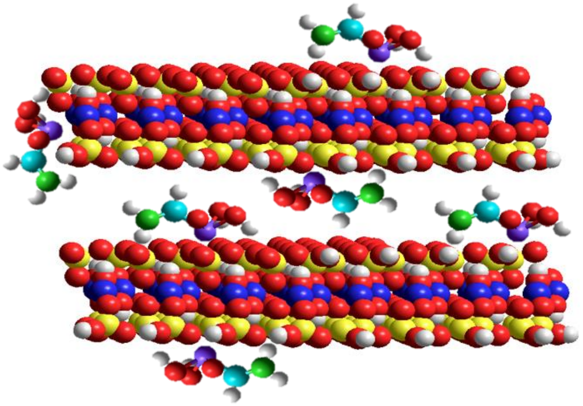


Figure 18. Molecular model of geometrically optimized CM clay layers and AMPA molecules showing predicted binding of AMPA onto CM surfaces. Binding sites may include interlayer surfaces, basal surfaces, and edge sites.

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 involved chemisorption mechanisms. This result agrees with a previous study where AMPA predominantly bound to CM via covalent interlayer surface complexes through the phosphonate moiety (Rennig 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 Figure 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 & Wagai, 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.

3.5 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.

4. REDUCTION OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) BIOAVAILABILITY FROM SOIL AND TRANSLOCATION TO PLANTS WITH MONTMORILLONITE CLAY-BASED SORBENTS

4.1 Introduction

Per- and polyfluoroalkyl substances (PFAS) are of growing concern due to the adverse effects they pose on the environment and human health. PFAS have been used in food packaging and household products like stain repellants, nonstick cookware, lubricants, and textile treatments (Trudel et al., 2008). They have also been released to the environment during fluoropolymer manufacturing or processing disposal, use of aqueous firefighting foam (AFFF), biosolid application, and landfill leachate (Teaf et al., 2019). PFAS are often referred to as “forever chemicals” as they do not easily break down in the environment. According to the National Health and Nutrition Examination Survey (NHANES), PFAS were detected in nearly all serum samples from the general population, indicating widespread exposure in the US (CDC, 2009). Adverse health effects associated with PFAS exposure include immune dysfunction, reproductive issues, liver toxicity, and various cancers (DeWitt et al., 2019; Saikat et al., 2013; Shearer et al., 2020). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are two of the most commonly detected PFAS in the environment and the representative compounds used in this study.

Although PFAS such as PFOA and PFOS have been voluntary phased out by most industries, they are persistent and still widely distributed in the environment. PFAS detection in water and soil has been well-documented in the literature (Eschauzier et al., 2013; Strynar et al., 2012). PFAS and PFAS precursors have been detected in levels typically < 100 ng L^{-}

^{1} in groundwater, surface water, and drinking water and > 1000 ng L^{-1} in areas directly affected by PFAS sources (Vo et al., 2020). PFAS was detected in 100% of soil samples from 62 locations on 7 continents, with PFOA and PFOS being the most commonly detected (up to 2.7 and 3.1 µg kg^{-1}, respectively) (Rankin et al., 2016). PFOA and PFOS have been measured at levels up to 50,000 and 373,000 µg kg^{-1} in surface soil from U.S. Air Force AFFF-contaminated sites (Brusseau et al., 2020). Importantly, studies have shown that PFAS tend to accumulate in soil at much higher rates than in water sources. Zareitalabad et al. (2013) concluded that field-based dissociation constants suggest longer residence times of PFAS in soil than in water, which can result in increased transfer of PFAS from soil to produce. This presents an exposure risk to humans who consume produce contaminated with PFAS (Brown et al., 2020; Domingo et al., 2012; Vestergren et al., 2012). Bioavailable fractions of PFOA and PFOS in water and soil can translocate to plants such as vegetables and produce, resulting in unintended exposures to humans and animals. Lal et al. (2020) demonstrated PFOS uptake by lettuce, carrots, and tomatoes and concluded that this translocation presents a risk of PFOS exposure to humans and animals via the food chain. PFOA and PFOS have also been shown to translocate to wheat, oat, maize, potato, and ryegrass plant compartments (Stahl et al., 2009).

Practical strategies are needed to reduce PFAS contamination, and as most biological and chemical destruction techniques have limited efficacies (Vu & Wu, 2020), immobilization with carbon- and clay-based sorbents is a promising remediation strategy. Activated carbon (powdered and granular) is popular due to its low cost and wide applicability, and has been shown to be effective in removing PFOA and PFOS from aqueous environments (Carter & Farrell, 2010; Rattanaoudom et al., 2012; Yu et al., 2009a).

Activated carbon (AC) and “biochars” have also been investigated for PFAS remediation of soil (Askeland et al., 2020; Silvani et al., 2019; Sørmo et al., 2021). However, the use of clay-based sorbents to reduce PFAS bioavailability from soil and translocation to plants has not been as widely studied (Das et al., 2013; Hale et al., 2017).

Montmorillonite clay has been shown to effectively bind a variety of environmental chemicals and reduce their bioavailability from soil (Hearon et al., 2020, 2021; Sun et al., 2014; Yang et al., 2017). CM clay can tightly bind chemicals in active interlayer surfaces as well as basal surfaces and edge sites (Gu et al., 2010; Orr et al., 2020 Wang et al., 2019c). Importantly, multiple clinical trials and intervention studies have demonstrated the safety of calcium montmorillonite (CM) clay for consumption by humans and animals (Phillips et al., 2019). Our laboratory has modified CM clays with the natural nutrients L-carnitine (CM-carnitine) and choline (CM-choline) to increase the potential to bind more lipophilic chemicals. These nutrient-modified clays have been shown to effectively bind PFOA and PFOS in an aqueous environment (Wang et al., 2021b).

The objective of this study was to evaluate binding of PFAS by carbon- and clay-based sorbents and the applicability of these sorbents in PFAS remediation of soil. This study was designed to determine the effectiveness of AC, CM, CM-carnitine and CM-choline in binding PFOA and PFOS using 1) *in vitro* isothermal analyses (including thermodynamics and binding mechanisms), 2) *in silico* molecular dynamics (MD) simulations to validate binding percentages and binding modes, and 3) *in vivo* lemna assay, soil bioavailability and plant uptake studies to validate the potential application of these sorbents.

4.2 Materials and methods

4.2.1 Reagents

High pressure liquid chromatography (HPLC) grade acetonitrile and pH buffers (4.0, 7.0 and 10.0) were purchased from VWR (Atlanta, GA). PFOA and PFOS standards were purchased from Sigma-Aldrich (Saint Louis, MO). HPLC grade acetone, methanol, and water + 0.1% formic acid were purchased from Fisher Scientific (Hampton, NH). Deionized water (18.2 MΩ) was generated in the laboratory using an Elga™ automated filtration system (Woodridge, IL) and was used in all experiments.

Activated carbon (AC) derived from coconut shell (mesh size: 100 – 325; Iodine number: 1100 mg g-1; 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-1, an external surface area of approximately 70 m2 g-1, and a cation exchange capacity of 97 cmol kg-1 (Grant & Phillips, 1998). CM has a general formula of (Ca)0.3(Al,Mg)2Si4O10(OH)2·nH2O (Marroquin-Cardona et al., 2011). Montmorillonite clays were modified with L-carnitine and choline at 100% cation exchange capacity (CEC = 97 cmol kg–1) as previously described (Wang et al., 2017). Briefly, calculated amounts of cations and 25 g of parent materials were added in 500 mL of 1 mM HNO3. The suspensions were mixed and stirred for 24 hrs at ambient temperature, then centrifuged at 2000g for 20 min and washed with 100 mL of distilled water. The centrifugation–washing process was repeated three times. All samples were dried in a desiccator before grinding and passing through a 125 µm sieve.

4.2.2 In vitro studies: Isothermal adsorption analyses

PFAS stock solutions were prepared by dissolving pure crystals in deionized water (pH 7) to yield 1000 µg mL^{-1}. AC, CM, CM-choline, and CM-carnitine were used as sorbents for adsorption isotherms. Sorbents were added at 0.0005% w/vol to PFAS solutions with an increasing concentration gradient (1 to 10 µg mL^{-1}). To achieve the 0.0005% inclusion level, 10 µL of 0.5 mg mL^{-1} clay suspension in pH 7 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 PFAS solutions were achieved by adding a calculated amount of PFAS stock solution to a complementary volume of pH 7 water in 1.5 mL centrifuge tubes to a total volume of 1 mL. Additionally, 3 controls were tested (mobile phase, PFAS solution, and 0.0005% sorbent solution). All samples were agitated at 1000 g for 2 hrs at ambient temperature (25°C) and high temperature (37°C) to investigate the thermodynamics of the reaction. Samples were then centrifuged at 2000g for 20 minutes to separate sorbent/PFAS complexes from the solution. The supernatants were collected for analysis.

PFAS 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 methods previously described (Liang and Chang, 2019; Roberts et al., 2017). Briefly, a gradient elution of 20 mM ammonium acetate (eluent A) and acetonitrile (eluent B) was carried out at a flow rate of 0.6 mL/min. The gradient for elution was 10% eluent B (initial), 10%-55% (0 to 0.1 min), 55%-99% (0.1 to 4.5 min), 99% (4.5 to 5 min), and 99%-10% (5 to 6.5 min). The injection volume was 10 µL for each sample. The LC/MS-MS was operated with an electrospray ionization interface

(ESI) operated in negative ion mode. The spray voltage was maintained at 4.5 kV and the source temperature was kept at 450°C. The cone voltage (kV) for PFOA and PFOS was 25 and 40, respectively. The monitored precursor and product ions (m/z) for PFOA and PFOS were 413 to 369 and 499 to 80, respectively. The LC/MS-MS was operated under multiple reaction monitoring (MRM) mode. Nitrogen gas was used as the collision and curtain gas, and argon gas was used as the nebulizer and heater gas. Empower analyst software was used to control the LC/MS-MS system and acquire the data.

4.2.3 Data calculations and curve fitting

PFAS were detected by LC/MS-MS and quantified using standard calibration curves. PFAS concentrations in solutions were calculated from peak area at the retention times. 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,

Langmuir model (LM) $q = Q_{max}\left(\frac{KdCw}{1+KdCw}\right)$

where q = toxin adsorbed (mol kg^{-1}), Q_{max} = maximum capacity (mol kg^{-1}), K_d = distribution constant, and C_w = 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:

$$K_d = \frac{q}{(Qmax - q)Cw}$$

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

$$\Delta H = \frac{-R \ln\left(\frac{Kd2}{Kd1}\right)}{\left(\frac{1}{T2}\right) - \left(\frac{1}{T1}\right)}$$

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

4.2.4 Molecular Modeling and Molecular Dynamics Simulations

Parent CM, carnitine-modified CM, and choline-modified CM in the presence of PFOA and PFOS, independently, at neutral pH (pH 7) were computationally investigated through quintet 100 ns MD simulations. Each of the six systems (parent CM : PFOA, parent CM : PFOS, carnitine-modified CM : PFOA, carnitine-modified CM : PFOS, choline-modified CM : PFOA, and choline-modified CM : PFOS) was built and simulated analogously to our previous study investigating CM in the presence of PFAS in acidic conditions (pH 2) (Wang et al., 2021b). The initial structure of the parent CM corresponded to the two-layered montmorillonite clay model at pH 7 reported in our previous study (Wang et al., 2019c). This model was built using structural data extracted from the INTERFACE MD model database (Heinz et al., 2005, 2013). The d_{001} spacing between the two layers was set to 21 Å (Wang et al. 2017, 2021). The two layered CM model was subsequently solvated in a 90 × 90 × 54 $Å^3$ water box such that, with application of periodic boundary conditions in the MD simulations, the modeled system corresponded to an infinite number of CM layers with a d_{001} spacing of 21 Å (Wang et al., 2017, 2021). The initial structure of the carnitine- and choline-modified CM was generated through a short 10 ns simulation equilibrating the modeled parent CM in the presence of carnitine or choline molecules as described in our previous study (Wang et al., 2021b). Subsequently, for the parent, carnitine-modified, and choline-modified CM in the presence of PFAS, 16 copies of PFOS or PFOA, independently, were initially dispersed around the parent clay within the 90 × 90 × 54 $Å^3$ water box in random configurations and orientations. The initial random configurations of all molecules for the parent and modified CM were generated from short 1 ns simulations of single molecules at infinite dilution (Wang et al., 2021b). Within the simulations, the concentration of PFAS (0.055 M) was higher than experimental conditions. A higher concentration was used to enhance the statistical sampling and accelerate the potential sorption of PFAS to the clay surfaces (Orr et al., 2020; Wang et al., 2019c, 2021).

Subsequently, each simulation system initially underwent energetic minimizations and a constrained 1 ns equilibration stage prior to each 100 ns MD simulation run as in our previous studies (Orr et al., 2020; Wang et al., 2019c, 2021). Throughout the energy minimizations and 1 ns equilibration stage, all atoms excluding hydrogens of the clay, PFAS, and modifying molecules were constrained under 1.0 kcal mol^{-1} $Å^{-1}$ harmonic constraints. Following energy minimization and equilibration, the simulation systems entered a 100 ns production stage in which all constraints were released with the exception of light 0.1 kcal mol^{-1} $Å^{-1}$ harmonic constraints on aluminum atoms of the clay layers.

All MD simulations and setup were conducted in CHARMM (Brooks et al., 2009). Parameters and topologies for CM were extracted from the INTERFACE force field (Heinz et al., 2005, 2013). The PFAS and modifying molecules were all parameterized using CGENFF (Vanommeslaeghe et al., 2010; Yu et al., 2012). The CHARMM36 force field was used to model water (Jorgensen et al., 1983) and counter ions (Marchand & Roux, 1998).

The temperature and pressure of the simulation systems were set to 300 K and 1 atm. Simulation snapshots were extracted in 100 ps intervals for subsequent analysis. Five 100-ns replicates of MD simulations with different initial velocities were performed for each system for an aggregate simulation time of 500 ns for each of the 6 simulation systems.

4.2.5 Structural Analysis of PFAS Sorption to Montmorillonite Clays within Molecular Dynamics Simulations

In-house FORTRAN programs were used to identify interactions between the PFAS and the parent CM, carnitine-modified CM, or choline-modified CM. A PFAS molecule was defined as interacting with the clay if the distance between any pair of atoms between the PFAS molecule and the clay was within 3.5 Å; such interactions were considered as "self-binding" interactions, in all sets of simulation systems comprising CM, carnitine-modified CM, and choline-modified CM. A carnitine or choline molecule was considered to be a modifying carnitine or choline if the distance between any pair of atoms between the carnitine or choline and the clay surface was within 3.5 Å. A PFAS molecule was defined as interacting with a modifying carnitine or choline, if the distance between any pair of atoms between the PFAS molecule and the modifying carnitine or choline was within 3.5 Å; such interactions were considered as "assisted-binding" interactions. It is worth noting that a PFAS molecule could be bound to the clay through both self- and assisted-binding interactions simultaneously within the simulation systems of the carnitine- or choline-modified CM such that a PFAS molecule interacts concurrently both with the clay and a modifying molecule; such interactions were considered as assisted-binding interactions only (to avoid double counting and to also highlight the importance of carnitine and choline in the modified CM).

To identify the predominant binding modes of the PFAS binding to parent, carnitine-modified, and choline-modified CM, we performed a statistical analysis on the interactions between the PFAS and the clay layers within their respective simulations. Binding modes of the PFAS and the modifying molecules were classified based on decomposition of the molecules into functional groups. Self-binding interactions with unmodified or modified clay were clustered into different binding modes depending on which functional groups of the toxic compounds, independently or in combinations, were binding to the clay layer. Assisted-binding interactions with modified CMs were classified into assisted-binding modes based on the functional groups of the modifying molecule, independently or in combinations, binding to the clay layer as well as the functional groups of PFAS and modifying molecules, independently or in combinations, interacting with each other based on the 3.5 Å cutoff.

4.2.6 In vivo studies: Lemna minor assay

The efficacy and safety of sorbents was predicted for a living organism using the freshwater aquatic plant (*Lemna minor*). This well-established toxicity assay was used to screen and validate sorbent ability to protect against PFAS toxicity before more extensive soil and plant studies were conducted. *L. minor* were obtained from Aqua L'Amour (Elk Grove, CA). *L. minor* were kept under a growth light (8:16 light: dark cycle) in ambient temperature using Steinberg nutrient medium (3.46 mM KNO_3, 1.25 mM $Ca(NO_3)_2$, 0.66 mM KH_2PO_4, 0.072 mM K_2HPO_4, 0.41 mM $MgSO_4$, 1.94 µM H_3BO_3, 0.63 µM $ZnSO_4$, 0.18 µM Na_2MoO_4, 0.91 µM $MnCl_2$, 2.81 µM $FeCl_3$, 4.03 µM EDTA; pH 5.5 ± 0.2) (Drost et al. 2007).

L. minor studies were conducted based on the OECD Test Guidelines for Testing Chemicals, Lemna sp. Growth Inhibition Test (OECD, 2002). Experiments were conducted in 24-well plates with 2 mL of test solution and three 2-frond *L. minor*. Plants were monitored and photographed over the 7-day exposure period. Frond numbers were recorded and surface areas were calculated using ImageJ (Abramoff et al., 2003). Frond surface areas were used to calculate the growth rate (μ):

$$\mu = \frac{\ln(At2) - \ln(At1)}{t2 - t1}$$

where A_{t1} and A_{t2} are the surface areas (mm^2) calculated at day t_1 and day t_2, respectively. The growth rate inhibition was calculated by comparing the growth rates of samples and controls:

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\mu sample}{\mu control}\right)$$

(Drost et al., 2007). *L. minor* were homogenized in 90% acetone using a 150 Homogenizer and 7 mm Generator Probe (Fisher Scientific, Waltham, MA) and placed in a freezer for 72 hrs to extract chlorophyll (Su et al., 2010; Taraldsen & Norberg-King, 1990). Chlorophyll a and b concentrations were measured using a UV-Visible spectrophotometer at wavelengths of 663 and 644 nm, respectively. Total chlorophyll (a and b) was calculated using Beer's Law:

$$C_{a+b} = \left(\frac{A_a}{\varepsilon_a \times b}\right) + \left(\frac{A_b}{\varepsilon_b \times b}\right)$$

Where C is the total chlorophyll concentration (chlorophyll a + chlorophyll b), A_a and A_b are the absorbance values measured at 663 nm and 644 nm, respectively, ε_a and ε_b are the absorption coefficients for chlorophyll a and chlorophyll b (87.67 L g cm^{-1} and 51.36 L g cm^{-1}, respectively) and b is the length of the light path (Jeffrey & Humphrey, 1975).

4.2.7 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) and 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 Foxfarm Soil & Fertilizer Company (Humboldt County, CA). Soil and compost were air-dried and sieved through a 1 mm screen before use. Each 1 g sample in a disposable culture tube was spiked with 2 mL of 1.0 µg mL^{-1} PFAS/acetone solution and thoroughly mixed to ensure even distribution of PFAS. Samples were left uncapped in a fume hood for 3 days allowing evaporation of the acetone. Sorbents were added at 2% w/w to soil and compost samples and thoroughly mixed. Samples were hydrated by adding 4 mL of distilled water and then slowly agitating at 200 rpm for 24 hrs. A soil extraction method (Huset & Barry, 2018) with modification was used to extract PFOA and PFOS from soil/compost samples. Briefly, 4 mL of methanol + 1% NH_4OH was added to each sample before agitating at 1000 rpm for 1 hr. Samples were centrifuged at 2000*g* for 20 minutes 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 PFAS 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 PFAS, and soil with sorbent. Peak areas of PFAS from samples that included sorbents were compared to the PFAS control samples to determine the percent reduction in PFAS bioavailability from soil.

4.2.8 Plant uptake studies

Organic cucumber seeds were obtained from Ferry Morse (Norton MA). Time course and sorbent inclusion studies were conducted using a 1:1 mixture of the soil and compost used in soil bioavailability studies. Each planter contained 10 g of soil/compost hydrated with 20 mL water. Cucumber 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 2 L nutrient solution (potassium nitrate, calcium nitrate, monopotassium phosphate, potash, magnesium sulfate, and trace elements at 2.9 – 3.2 EC and 6.3 pH) on day 7. 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. Cucumber roots had significantly grown into the nutrient solutions after 7 days. For each study, ten experimental groups (soil/compost control, chemical control with 1 µg mL^{-1} PFAS, 4 sorbent controls at 2% in base media, and 4 treatment groups of 2% sorbent and 1 µg mL^{-1} PFAS) were set up with six planters in each group. Experimental groups were administered 500 mL of nutrient solution containing 1 µg mL^{-1} for chemical controls, 2% sorbent for sorbent controls and 1 µg mL^{-1} plus 2% sorbent for treatment groups. After a 7-day exposure period, planters were removed from hydroponic systems. Roots and sprouts were separated, washed thoroughly with DI water to remove soil, and air-dried before dry weights were measured. Samples were then ground to a powder-like texture before extraction.

Extraction methods described by Huset and Barry (2018) were followed with modification. Briefly, 5 mL of methanol + 1% NH_4OH were 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 2000g for 20 min. The supernatants were passed through Strata C18-E (55 um, 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.

4.2.9 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) growth parameters from the lemna assay, 3) concentrations of PFAS 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 $p \leq 0.05$.

3. Results and discussion

PFAS such as PFOA and PFOS have unique amphiphilic properties (hydrophobic fluoroalkyl chain and hydrophilic ionizable functional group), resulting in complex environmental behavior and difficulties in PFAS remediation of soil. Various environmental factors influence PFAS sorption in soil, including organic matter (OM) content, clay content, soil pH, salinity, and soil solution ionic strength (Sorengard et al., 2019; Tang et al., 2010;

Zhao et al., 2013). Practical strategies are needed to reduce the risk of environmental exposures, such as immobilization with sorbents. In this study, CM-carnitine and CM-choline clays were tested for their abilities to sorb PFOA and PFOS. Natural clay minerals like the parent CM clay have hydrophilic interlayer surfaces that are intrinsically negatively charged (Bolan et al., 2021). The L-carnitine and choline modifications change CM surfaces from hydrophilic to hydrophobic, enhancing the sorption of PFAS compounds (Darlington et al., 2018). Previous studies in our laboratory confirmed the efficacy of carnitine and choline modified CM clay in binding PFOA and PFOS at pH 2 (Wang et al., 2021b). These studies were important in determining the efficacy of these sorbents at pH 7 and in a soil matrix.

4.3.1 Adsorption isotherms

Adsorption isotherms for PFOA and PFOS were conducted at pH 7 to simulate environmentally-relevant conditions (Figure 19). Isothermal data was generated with TableCurve 2D software to derive sorbent-PFAS binding parameters. All isotherms fit the Langmuir equation ($r^2 > 0.95$), which was used to determine $Q_{\max}$ (capacity) and K_d (affinity) values for PFOA and PFOS binding to AC, CM, CM-carnitine and CM-choline (Table 2). The resulting $Q_{\max}$ and K_d values along with the curved shape of the Langmuir plot indicated saturable binding of PFOA and PFOS to active surfaces of all tested sorbents. Importantly, the modified CM-carnitine and CM-choline clays had significantly higher capacities for both PFOA and PFOS than AC and CM (**$p < 0.01$). The L-carnitine and choline modifications change the active surfaces of the parent montmorillonite clay from hydrophilic to hydrophobic, which allows them to attract more lipophilic moieties like the PFOA carboxylic functional group and the PFOS sulfonic functional group.

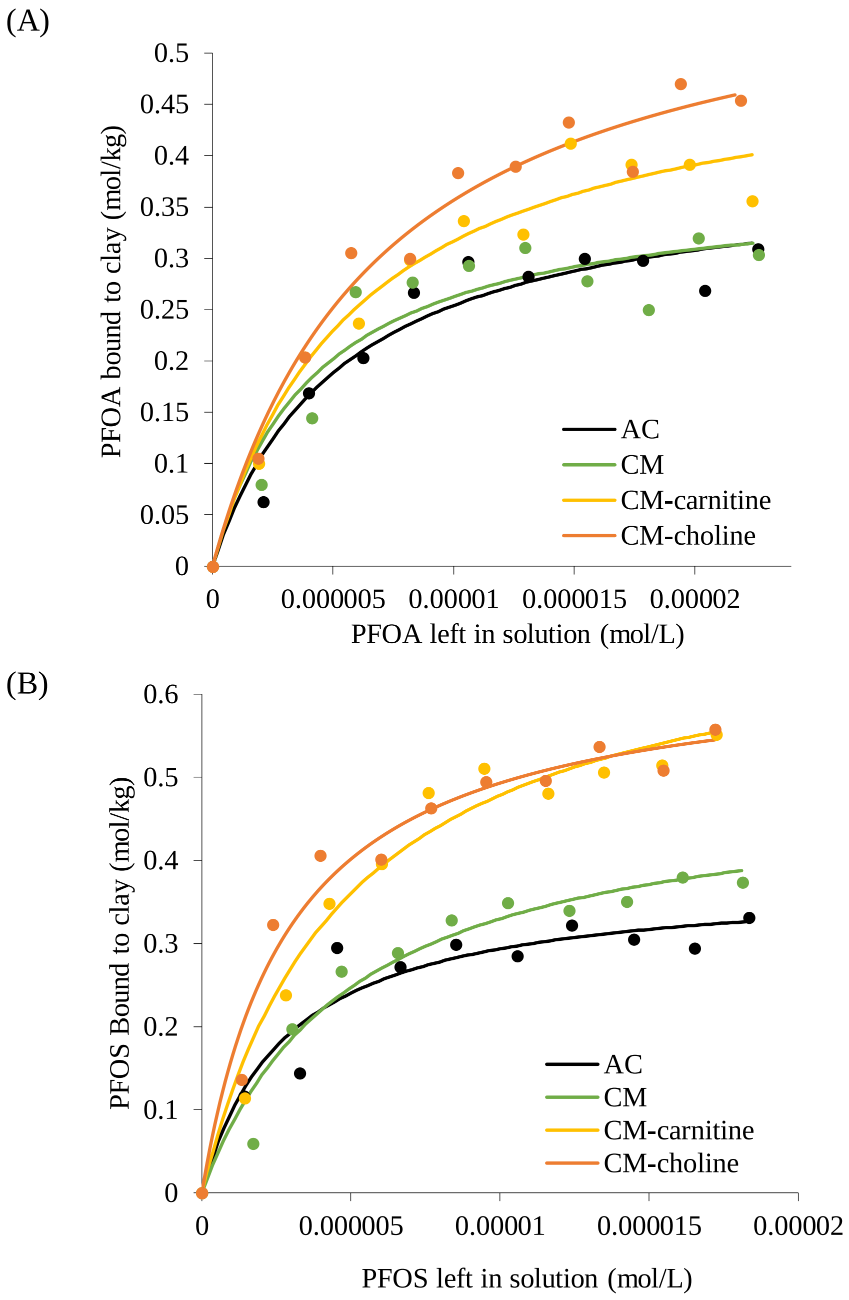


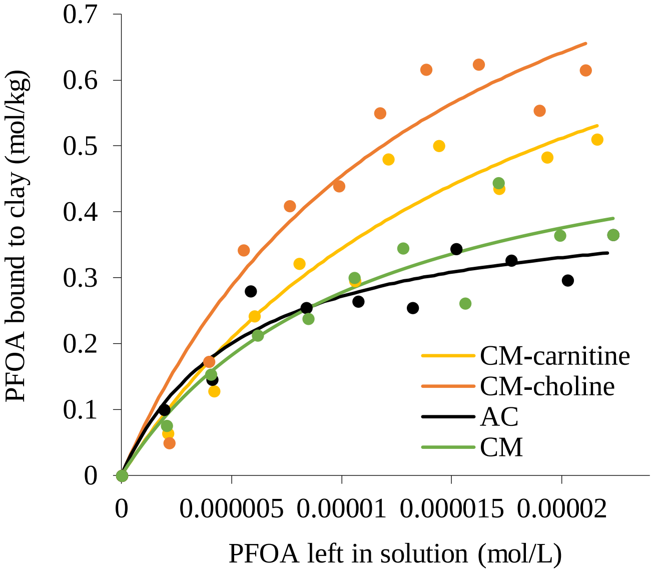
Figure 19. Langmuir plots of PFOA (A) and PFOS (B) on AC, CM, CM-carnitine, and CM-choline at 25°C indicating tight binding of both chemicals.

	Sorbent	**Q_{max} (25⁰C)**	**Q_{max} (37⁰C)**	**K_d (25⁰C)**	**K_d (37⁰C)**	**ΔH**
PFOA	AC	0.39	0.42	1.86E+05	1.80E+05	-2.06
	CM	0.37	0.58	2.34E+05	9.12E+04	-60.23
	CM-carnitine	0.51	0.99	1.63E+05	5.28E+04	-72.24
	CM-choline	0.61	1.09	1.41E+05	7.14E+04	-43.52
PFOS	AC	0.38	0.47	3.51E+05	2.67E+05	-36.68
	CM	0.49	0.59	1.99E+05	9.34E+04	-48.47
	CM-carnitine	0.71	0.98	2.05E+05	8.56E+04	-55.79
	CM-choline	0.63	0.93	3.40E+05	1.06E+05	-74.18

Table 2. Qmax and Kd values for all four sorbents binding PFOA and PFOS derived from isotherms conducted at 25°C and 37°C.

To determine the enthalpies of the binding reactions between PFAS and sorbents, isotherms were also conducted at 37⁰C (Figure 20). The Kd values for isotherms conducted at 25⁰C and 37⁰C were compared using the Van't Hoff equation to calculate enthalpies (ΔH). Determining the ΔH of sorbent-chemical binding can provide insight into the strength of reactions and potential mechanisms (i.e. chemisorption or physisorption). The lower ΔH values for PFOA and PFOS binding to AC surfaces may indicate weaker bonds such as hydrogen bonds, dipole-dipole interactions, and Van der Waals attractions (i.e., physisorption). The negative enthalpy values (Table 1) indicated exothermic reactions for PFAS binding to clay-based sorbents, and the high absolute values of ΔH (more than 20 kJ mol^{-1}) indicated chemisorption mechanisms (tight binding) of PFOA and PFOS. These results suggest that the PFAS-sorbent complexes are stable and PFOA/PFOS would not be readily dissociated from clay sorbent surfaces.

(A)



(B)

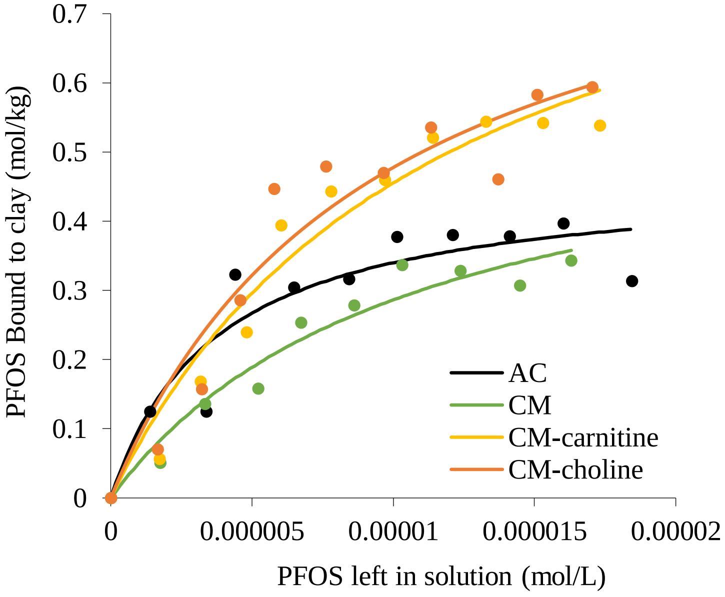


Figure 20. Langmuir plots of PFOA (A) and PFOS (B) on AC, CM, CM-carnitine, and CM-choline at 37°C. Kd values for these plots were compared to those conducted at 25°C to determine the enthalpy (ΔH) of the binding reactions.

4.3.2 Major Binding Sites & Mechanisms

The binding propensities of PFOA and PFOS to CM, CM-carnitine, and CM-choline were independently computationally assessed through calculations within their respective simulations. Our computational analysis showed that both PFOA and PFOS frequently bound to CM and that both carnitine and choline modifications improved the binding of both PFAS. Additionally, CM-carnitine appeared to be more effective at binding PFOS while CM-choline appeared to be more effective at binding PFOA.

For CM, the binding percentages of PFOA and PFOS onto parent CM were 25±1% and 27±3%, respectively (Figure 21). Both PFOA and PFOS predominantly bound to the parent CM interlayer through hydrophobic interactions with the fluorinated carbon chains. Specifically, in 62% and 51% of binding instances, PFOA and PFOS interacted with parent CM at the terminal end of their fluorinated carbon chains, respectively (Figure 22A & 22B). In 24% and 40% of binding instances, PFOA and PFOS interacted with parent CM through their entire fluorinated carbon chain, respectively (Figure 22A &22B). The predominance of PFOA and PFOS binding to parent CM through their hydrophobic tails suggests that hydrophobic interactions are key components of the binding mechanism for both PFAS to CM, which agrees with previous studies (Zhang et al., 2019).

Both carnitine and choline improved the binding of PFAS to CM (Figure 21). For CM-carnitine, the overall binding percentages of PFOA and PFOS onto CM-carnitine were 36±2% and 39±2%, respectively. For CM-choline, the binding percentages of PFOA and PFOS onto CM-choline were 38±2% and 34±2%, respectively. The higher binding percentages of the PFAS onto CM-carnitine and CM-choline compared to parent CM were attributed to the PFAS binding to modifying carnitine or choline through assisted-binding (Figure 21, green and blue bars).

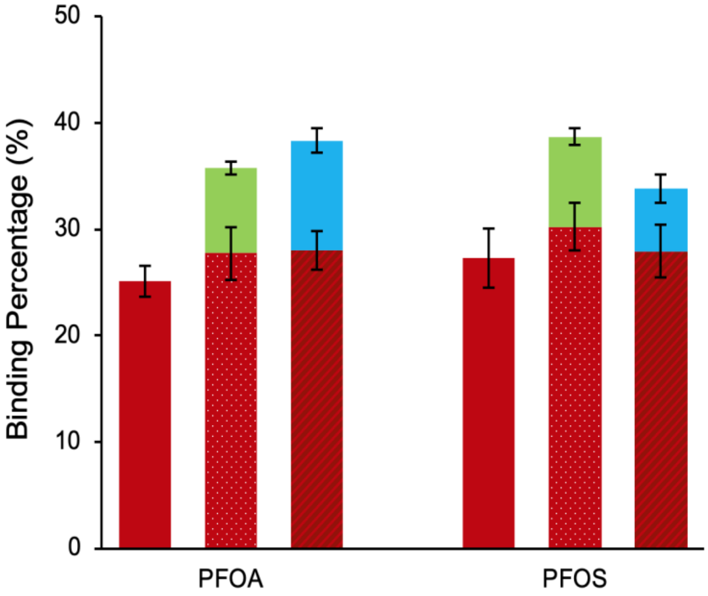


Figure 21. Overall binding percentages of CM (solid red bars), CM-carnitine (dotted red bars and green bars), and CM-choline (striped red bars and blue bars) for PFOA and PFOS. Red bars correspond to self-binding instances in which the PFA molecule is bound directly to the clay (without contacting any amending carnitines or cholines), green bars correspond to assisted-binding instances in which the PFA molecule is bound to CM-carnitine through an amending carnitine, and blue bars correspond to assisted-binding instances in which the PFA molecule is bound to CM-choline through an amending choline. Error bars correspond to the standard error across the quintet MD simulations.

For CM-carnitine and CM-choline, the modifying molecules primarily bound to the clay surface through their positively charged quaternary ammonium groups. Carnitine bound to the surface through its quaternary ammonium group in over 90% of its binding instances, and choline bound to the surface through its quaternary ammonium group in over 80% of its binding instances. For CM-carnitine assisted-binding, PFOA frequently bound to CM-carnitine through hydrogen bond formation between the hydroxyl group of carnitine (Figure 22C & 22D). In this binding mode, PFOA simultaneously bound to clay interlayers through its fluorinated carbon chain (Figure 22C & 22D). PFOA also frequently participated in assisted-binding to CM-carnitine through its fluorinated carbon chain (Figure 22C & 22D, 35% of assisted-binding instances). For the assisted-binding of PFOS, PFOS predominantly bound to CM-carnitine by forming hydrogen bonds to hydroxyl group of carnitine with its sulfonate group (Figure 22C & 22D, 47% of assisted-binding). Additionally, PFOS participated in assisted-binding to CM-carnitine through its fluorinated carbon chain (Figure 22C & 22D, 23% of assisted-binding). For CM-choline assisted-binding, PFOA predominantly bound to CM-choline through hydrogen bond formation between the hydroxyl group of choline and its carboxyl group (Figure 22E & 22F, 48% of assisted-binding). PFOA also participated in assisted-binding to CM-choline through its fluorinated carbon chain (Figure 22E & 22F, 36% of assisted-binding). For the assisted-binding of PFOS, PFOS predominantly bound to CM-choline by forming hydrogen bonds to the hydroxyl group of carnitine with its sulfonate group (Figure 22E & 22F, 61% of assisted-binding). Additionally, PFOS participated in assisted-binding to CM-choline through its fluorinated carbon chain (Figure 22E & 22F, 28% of assisted-binding).

The predominant binding modes of PFOA and PFOS to CM or CM-carnitine uncovered in this study at pH 7 are largely the same to the predominant binding modes uncovered in our recently published study at pH 2, simulating the conditions of the stomach (Wang et al., 2021b). However, because the carboxyl group of carnitine was considered to be protonated at pH 2, the carboxyl or sulfonate groups of PFOA or PFOS, respectively, also formed hydrogen bonds with the protonated carboxyl group of carnitine in addition to the hydroxyl group of carnitine (Wang et al., 2021b). Our previous computational study did not investigate PFOA or PFOS binding to CM-choline.

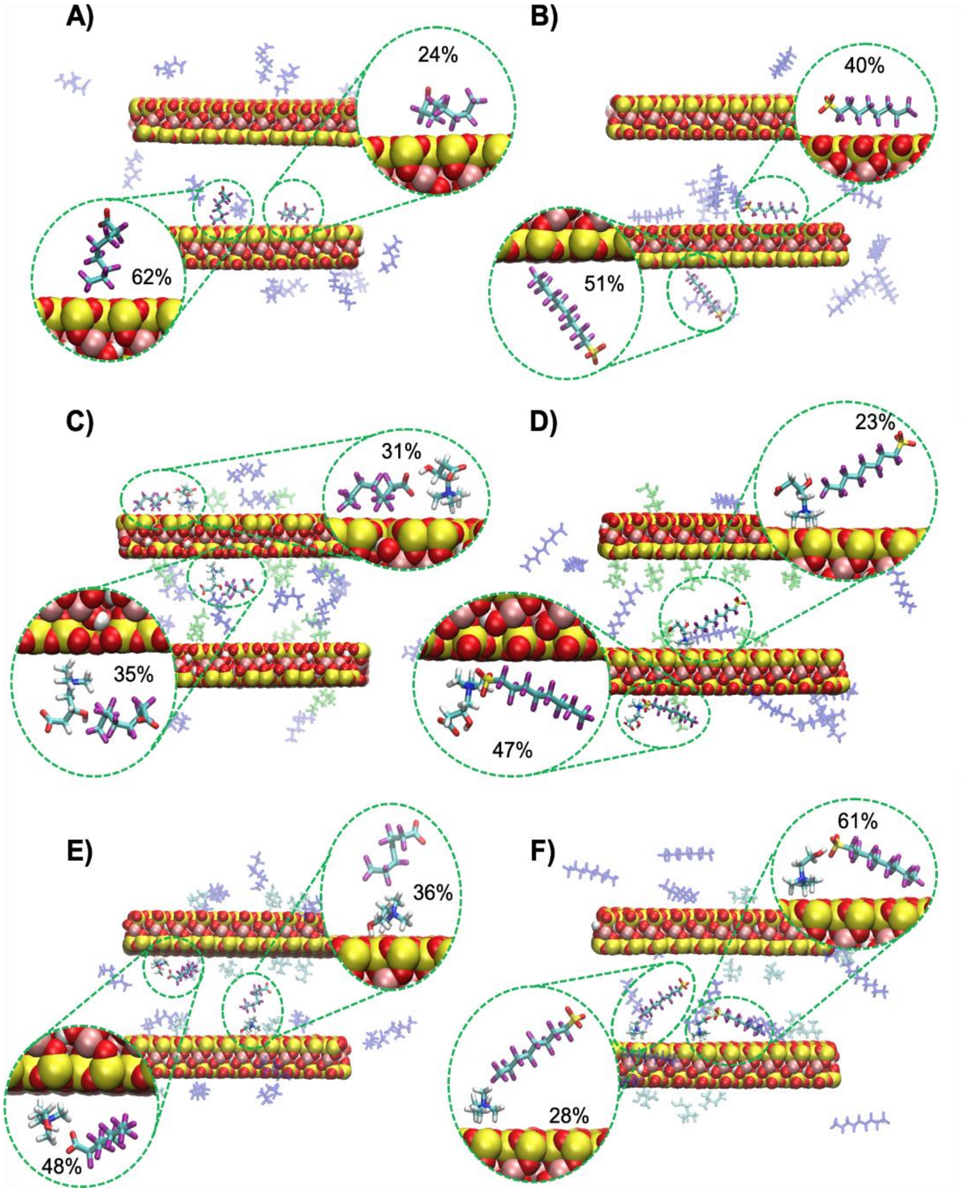


Figure 22. Molecular graphics images of representative prominent binding modes for (A) PFOA and (B) PFOS binding to CM, (C) PFOA and (D) PFOS binding to CM-carnitine, and (E) PFOA and (F) PFOS binding to CM-choline. Zoomed in images of the prominent binding modes are encircled by dotted lines and are reoriented to facilitate the comparison of different binding modes.

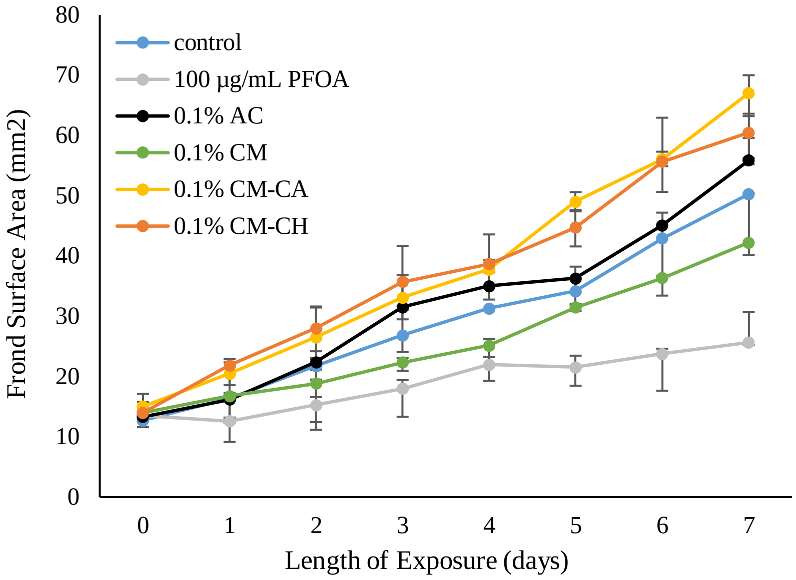
4.3.3 Lemna minor assay

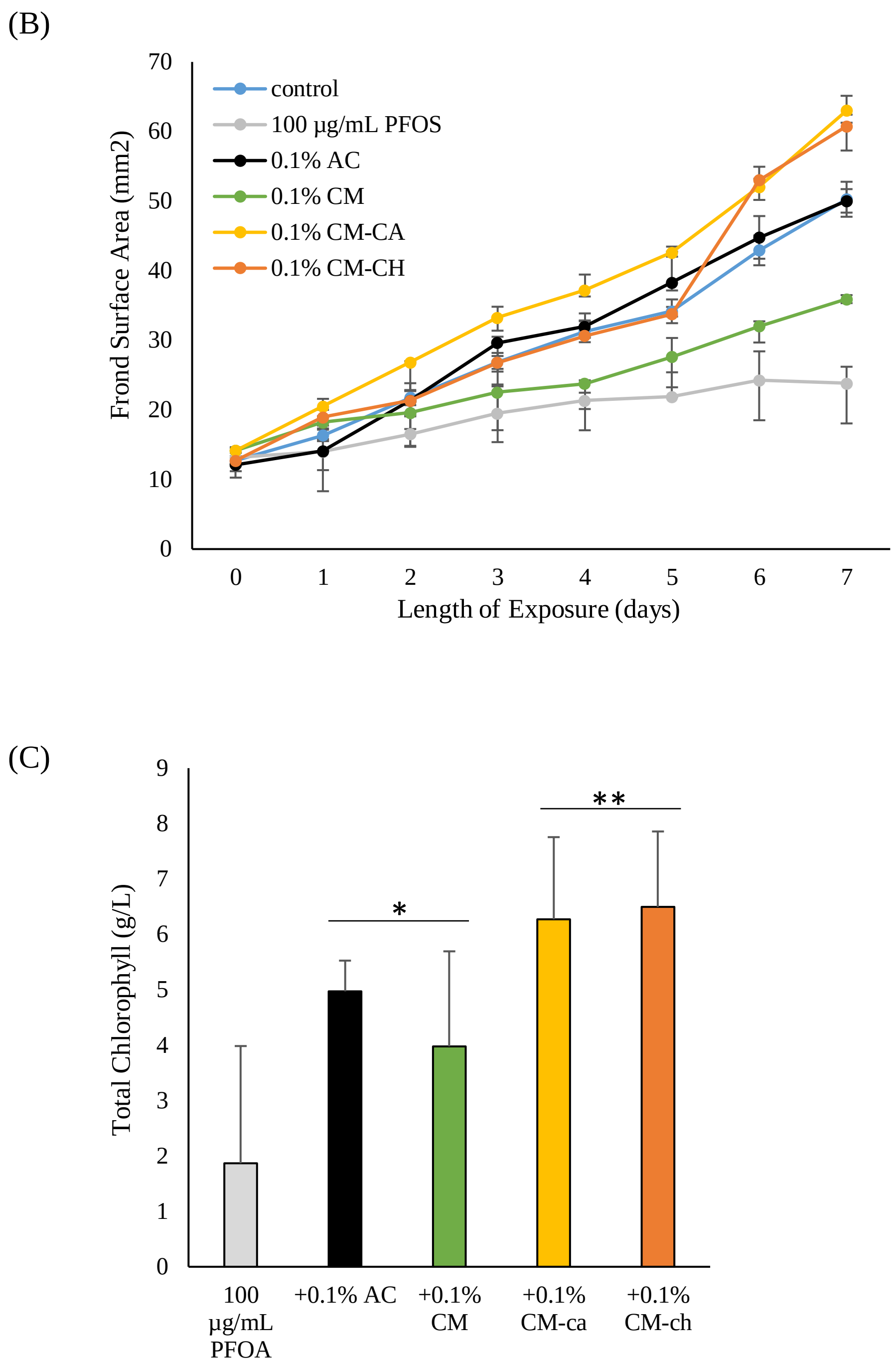
The *Lemna minor* assay is widely accepted and has been extensively studied as a relevant method to identify and characterize potential hazards of chemicals and toxicity on vegetative growth. In this study, we expanded on this traditional application to include sorbent safety and efficacy testing. These studies were important in evaluating sorbent safety to a plant organism and the sorbent's ability to protect the plants from chemical toxicity.

Dose-response experiments (0 to 100 µg mL^{-1}) were conducted and 100 µg mL^{-1} PFOA and 100 µg mL^{-1} PFOS were identified as the concentrations that significantly reduced lemna growth over the 7-day exposure period (53% and 57% reduction in frond surface area with 100 µg mL^{-1} PFOA and 100 µg mL^{-1} PFOS, respectively). These exposure levels also decreased chlorophyll content by 42.1% and 37.0% for PFOA and PFOS, respectively. The phytotoxicity of PFOA and PFOS (at similar and higher concentrations to those used in this study) has been seen with various plant species (Zhao et al. 2013). These exposure concentrations were used in subsequent sorbent studies, in which sorbents were tested at various inclusion levels (0.05 to 1.0%) to protect against chemical toxicity. Optimal protection against PFAS toxicity was achieved with 0.1% sorbent inclusion (Table 3). Figure 23A and 23B show increased *L. minor* surface areas with 0.1% sorbent inclusion compared to PFOA and PFOS controls. With CM-carnitine and CM-choline inclusion, *L. minor* surface area increased by 134% and 129% compared to PFOA control, respectively, and by 152% and 164% compared to PFOS controls, respectively. Additionally, chlorophyll content (chlorophyll a and b) significantly increased in the sorbent treatment groups compared to the PFOA (Figure 23C) and PFOS (Figure 23D) controls. Chlorophyll content increased from 1.0 g L^{-1} in the PFOA exposure group to 6.27 g L^{-}1 and 6.5 g L^{-1} in the CM-carnitine and

CM-choline treatment groups, respectively. Chlorophyll content increased from 1.62 g L^{-1} in the PFOS exposure group to 6.04 g L^{-1} and 4.02 g L^{-1} in the CM-carnitine and CM-choline treatment groups respectively. These studies indicated that the modified clays were consistently more effective than the parent CM clay, similar to what was seen in the isothermal analyses. Importantly, these screening toxicity and sorbent efficacy studies were important in validating the isothermal results *in vivo* before conducting more extensive hydroponic plant studies.

(A)




(B)
Frond Surface Area (mm2)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Length of Exposure (days)
control
100 µg/mL PFOS
0.1% AC
0.1% CM
0.1% CM-CA
0.1% CM-CH
(C)
Total Chlorophyll (g/L)
9
8
7
6
5
4
3
2
1
0
*
**
100 µg/mL PFOA
+0.1% AC
+0.1% CM
+0.1% CM-ca
+0.1% CM-ch
Exposure Group

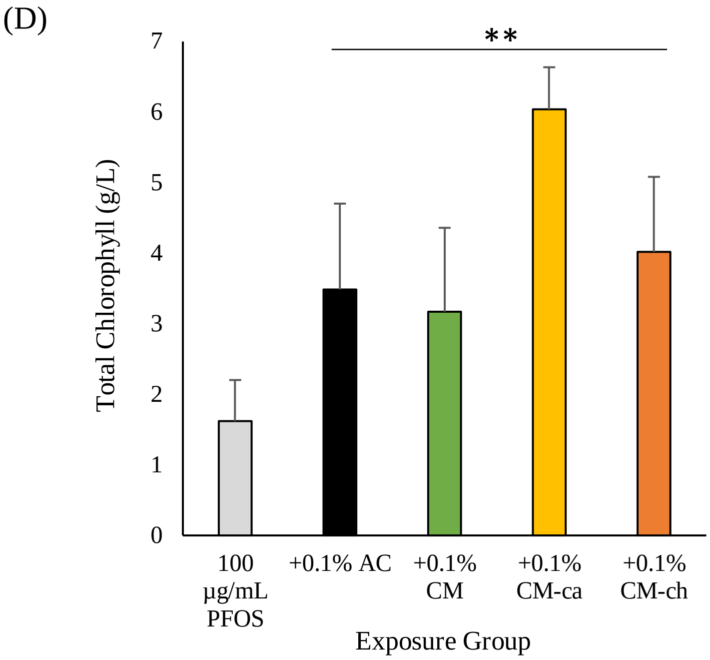


Figure 23. Frond surface area of *Lemna minor* over the 7-day exposure period exposed to 100 ppm PFOA (A) and 100 ppm PFOS (B) and protection of lemna with 0.1% sorbent inclusion. Chlorophyll content of lemna exposed to PFOA (C) and PFOS (D) measured at the end of the exposure period (*$p < 0.05$; **$p < 0.01$).

	Exposure	Chlorophyll Concentration (g/L)	Frond Surface Area (mm^2)	Growth Rate (μ)	Growth Inhibition
Control	Media	2.00	50.27	0.20	-
PFOA	100 ppm	1.90	25.64	0.09	-53%
	100 ppm + 0.1% AC	5.00	55.84	0.20	+124%
	100 ppm + 0.1% CM	3.98	42.23	0.16	+73%
	100 ppm + 0.1% CM-carnitine	6.27	67.02	0.21	+134%
	100 ppm + 0.1% CM-choline	6.50	60.47	0.21	+129%
PFOS	100 ppm	1.62	23.83	0.08	-57%
	100 ppm + 0.1% AC	3.49	50.04	0.20	+139%
	100 ppm + 0.1% CM	3.17	35.94	0.13	+57%
	100 ppm + 0.1% CM-carnitine	6.04	63.01	0.21	+152%
	100 ppm + 0.1% CM-choline	4.02	60.76	0.22	+164%

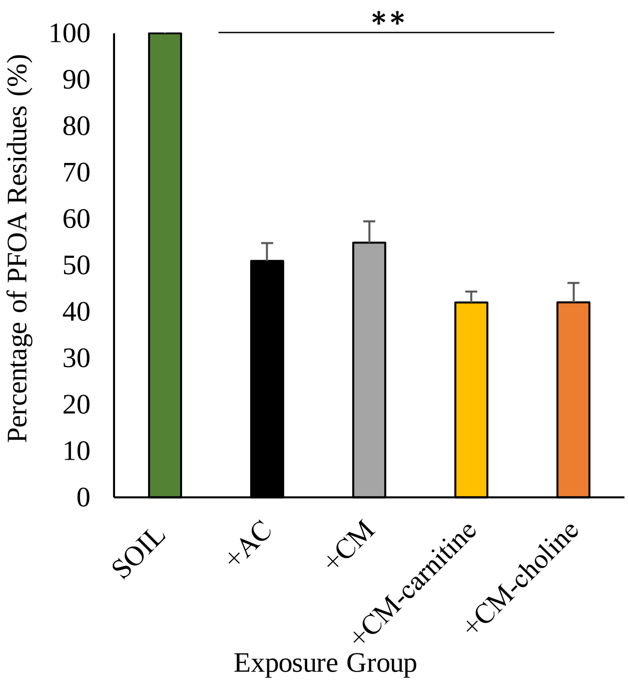
Table 3. Chlorophyll concentrations and frond surface areas measured for all exposure groups for both PFOA and PFOS. Growth rates were calculated based on the frond surface areas, and growth inhibitions were calculated from the growth rates.

4.3.4 Soil studies

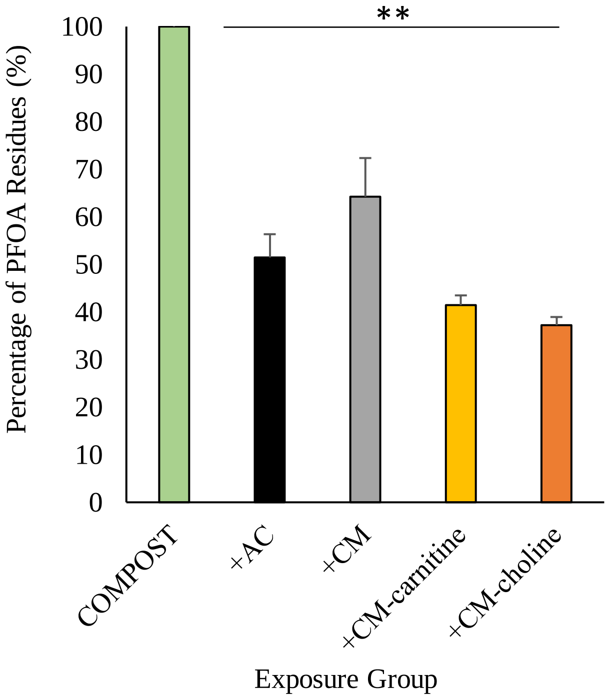
Soil studies were conducted to determine the efficacy of sorbents in reducing PFAS bioavailability from soil. Sorbent inclusion was higher in this study than the isotherms and *L. minor* assay due to the organic matter (OM) content of soil and compost. Doses of up to 30% have been used in media with high OM content (Bjerkli, 2019; Bräunig et al., 2021). Preliminary studies in our laboratory that evaluated the efficacy of various sorbent inclusion levels (0.1 – 5%) showed 2% inclusion of AC, CM, CM-carnitine, and CM-choline was optimal. Soil and compost samples (1 g) were spiked with 1 μg g^{-1} PFAS. This was chosen as the exposure level because it is higher than typical environmental levels (Rankin et al., 2016; Strynar et al., 2012;), lower than levels detected at contaminated sites (Brusseau et al., 2020), and comparable to concentrations used in studies focused on translocation of PFAS from soil to plants (Garcia-Valcarcel et al., 2014; Lal et al., 2020; Zhao et al., 2013). Figure 24 shows reduced bioavailability of PFOA and PFOS from spiked soil with inclusion of AC, CM, CM-carnitine, and CM-choline compared to bioavailability from spiked soil without sorbents. Results showed the modified CM-carnitine and CM-choline sorbents reduced PFOA bioavailability from soil by 58.0% and 57.9%, respectively, and from compost by 58.5% and 62.8%, respectively. CM-carnitine and CM-choline reduced PFOS bioavailability from soil by 77.5% and 79.4%, respectively, and from compost by 60.8% and 60.5%, respectively. The modified clays were consistently more effective than the parent CM clay. We expect that at lower and more environmentally-relevant doses of PFAS than used in this study, CM and modified-CM sorbent efficiencies would be higher.

Few studies have evaluated clay sorbent inclusion to reduce PFAS bioavailability from soil. One study used up to 30% inclusion of a commercially available sorbent (RemBind®) and found that between 10 and 30% inclusion reduced PFOA and PFOS leaching from AFFF-contaminated soil (< 2.6 ng mL^{-1} PFOA and < 547 ng mL^{-1} PFOS) up to 99% (Bräunig et al., 2021). MatCARE™, another commercially-available sorbent (palygorskite-based material modified with oleylamine) retained PFOS in soil with negligible release (0.5 – 0.6%) at 10% inclusion, with the best retention in soil with lower OM, lower pH, and higher clay content (Das et al., 2013). A study focused on a firefighting training facility found that PFOS leaching was reduced 35% with 3% montmorillonite clay (Hale et al., 2017). Thus, the CM-carnitine and CM-choline amended clays at low inclusion levels (2%) could present promising strategies to significantly reduce PFAS bioavailability from soil.

(A)



(B)



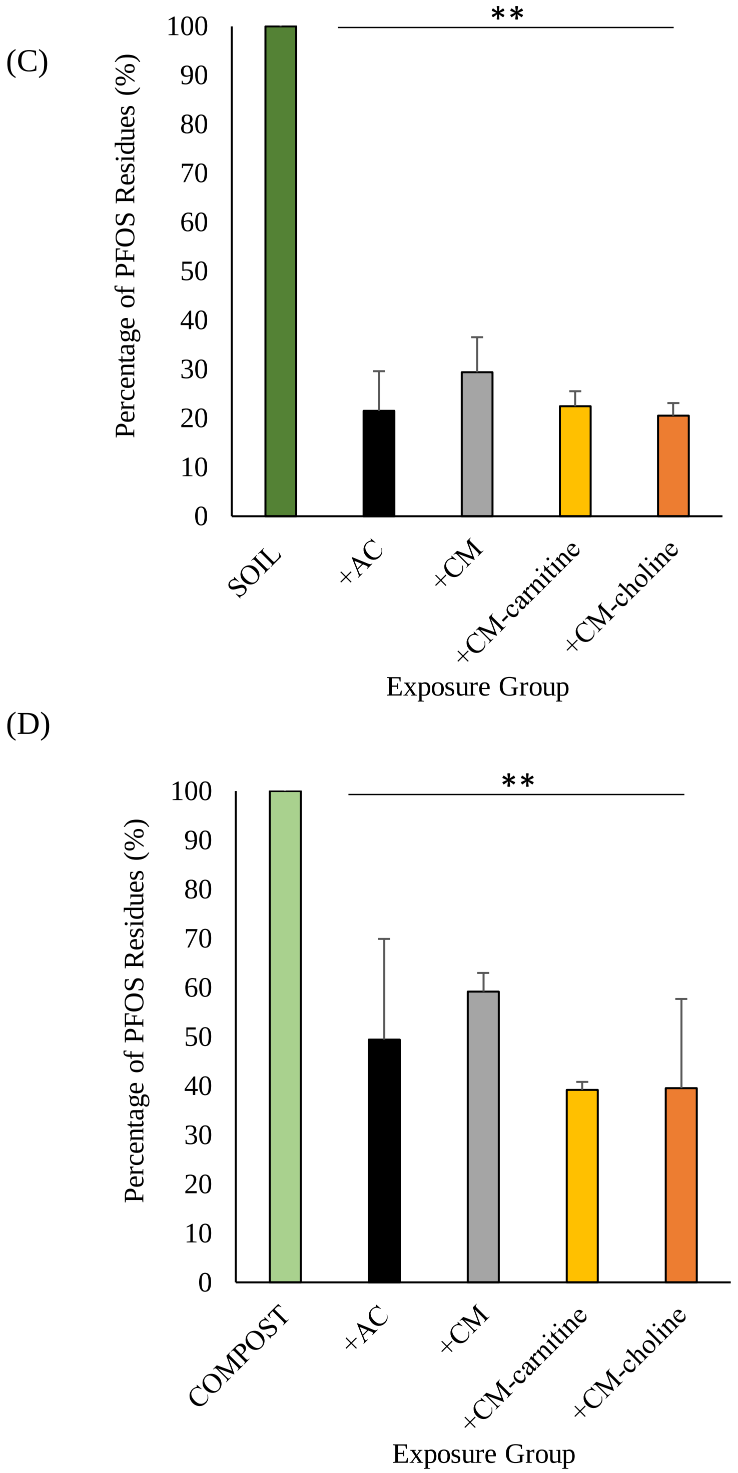
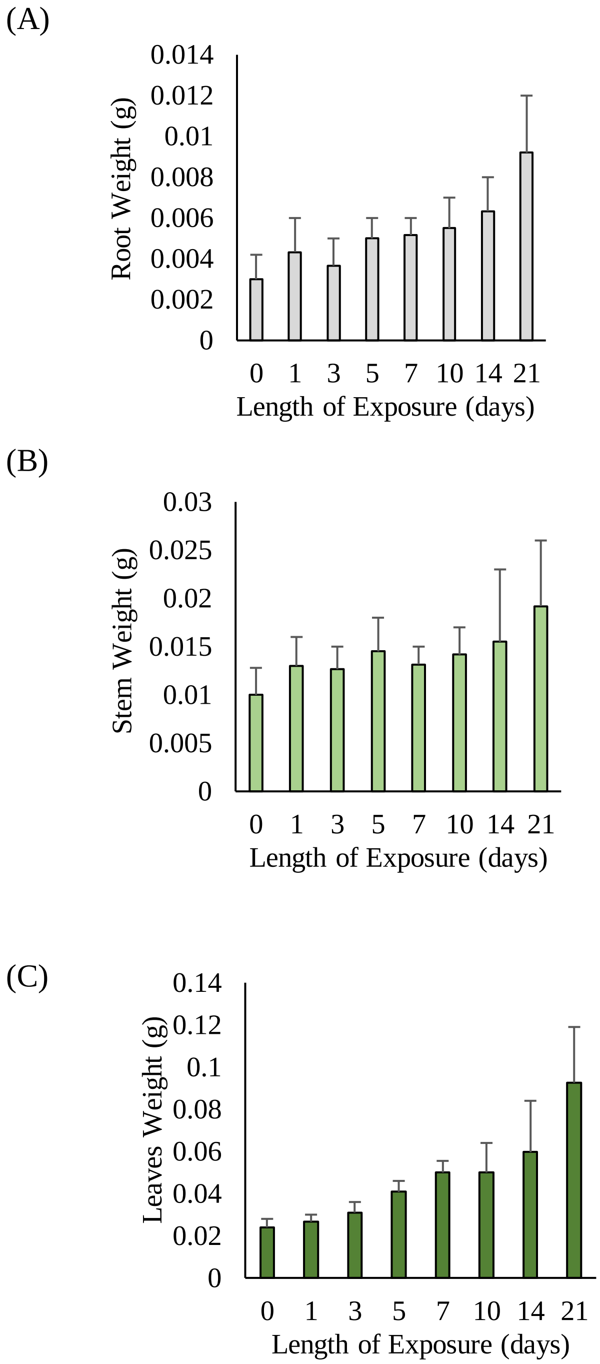
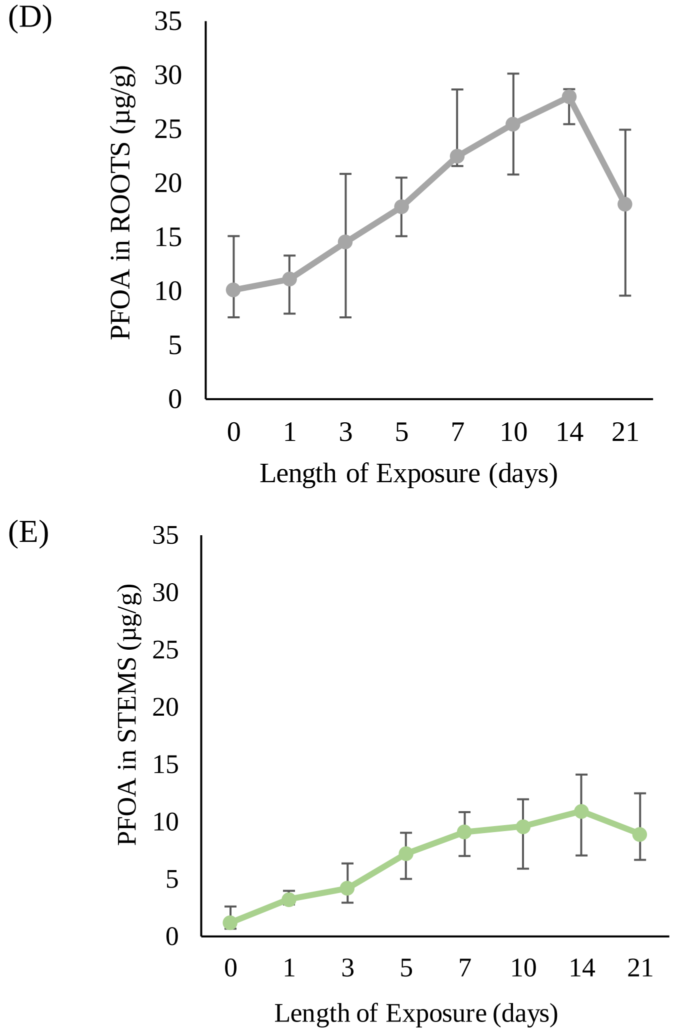


Figure 24. Soil studies showing significant reduction of PFOA (A, B) and PFOS (C, D) bioavailability from soil and compost with 2% inclusion of AC, CM, CM-carnitine and CM-choline (38 to 74%). Amended clays were consistently more effective than the parent clay. ($**p < 0.01$).

4.3.5 Plant uptake studies

Plant uptake studies were important in evaluating translocation of PFAS to cucumber sprouts and sorbent efficacy in reducing PFAS translocation. Cucumbers were chosen as the model plant in this study because PFAS compounds, especially PFOA and PFOS, tend to accumulate in fruit vegetables and have been shown to have high transfer rates from water and soil (Lechner & Knapp, 2011; Liu et al., 2019). Time course studies were conducted to evaluate PFOA and PFOS translocation to cucumber roots, stems, and leaves over 21-day exposure periods. Cucumber sprouts were exposed to PFOA or PFOS in both the soil and the nutrient solutions of hydroponic systems. Dry weights for cucumber sprouts exposed to PFOA measured for roots (Figure 25A), stems (Figure 25B) and leaves (Figure 25C) were not significantly different than controls. Cucumber components were extracted and PFOA was measured in roots (Figure 25D), stems (Figure 25E), and leaves (Figure 25F). Over the 21-day exposure period, PFOA concentrations increased from 10.1 to 18.0 µg/g in roots, from 1.2 to 8.9 µg/g in stems, and from 1.7 to 22.5 µg/g in leaves. As indicated by the proportions of total PFOA in the cucumber components, PFOA began to accumulate in the leaves by the end of the exposure period (Figure 25G). Dry weights for cucumber sprouts exposed to PFOS measured for roots (Figure 26A), stems (Figure 26B) and leaves (Figure 26C) were also not significantly different than controls. Over the 21-day exposure period, PFOS concentrations increased from 12.1 to 27.0 µg/g in roots (Figure 26D), decreased from 15.2 to 13.7 µg/g in stems (Figure 26E), and increased from 1.4 to 21.3 µg/g in leaves (Figure 26F). Similar to PFOA, PFOS began to accumulate in the leaves at the end of the 21-day exposure period (Figure 26G). We expect that this accumulation would increase if the plants continued to grow past the 21-day exposure. Because PFOA and PFOS exist in ionized forms at pH 7, potential mechanisms behind the translocation of PFOA and PFOS to cucumber sprouts could be facilitated diffusion and active transfer (Zhao et al., 2013).




(D)
PFOA in ROOTS (µg/g)
0
5
10
15
20
25
30
35
0
1
3
5
7
10
14
21
Length of Exposure (days)
(E)
PFOA in STEMS (µg/g)
0
5
10
15
20
25
30
35
0
1
3
5
7
10
14
21
Length of Exposure (days)

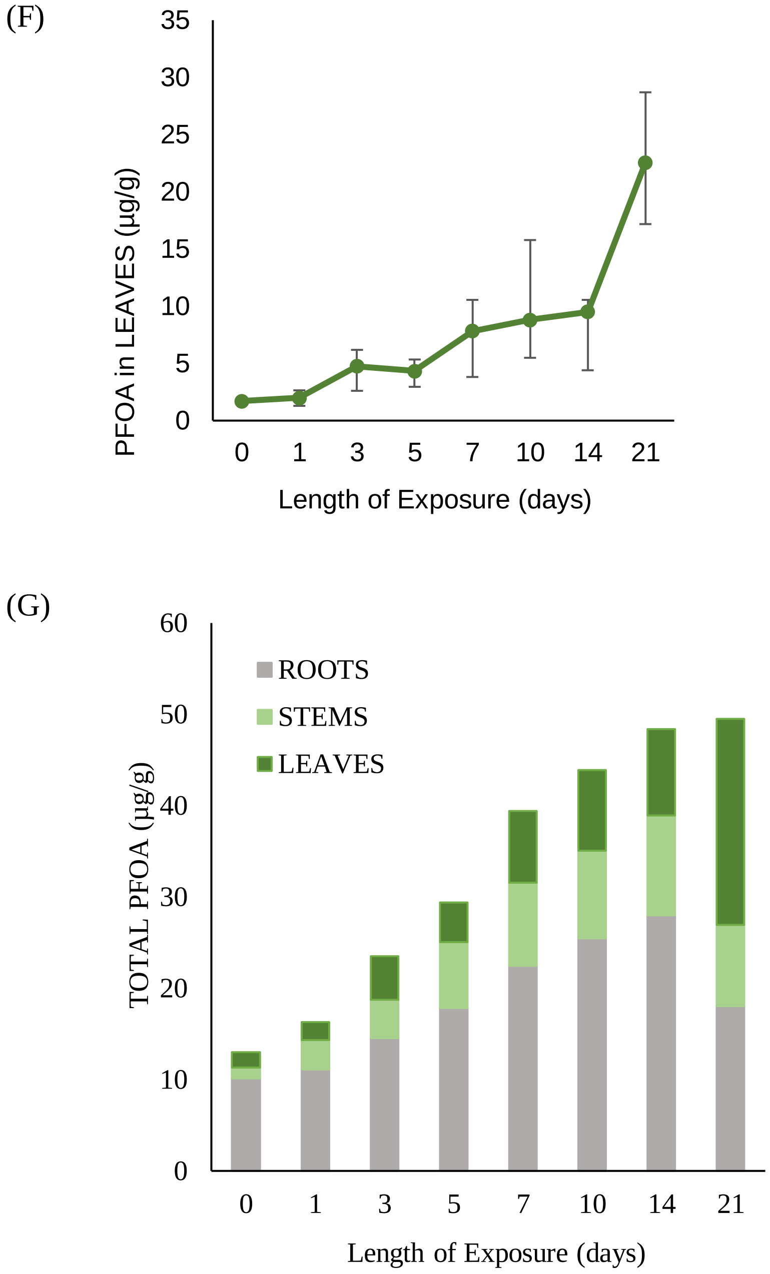
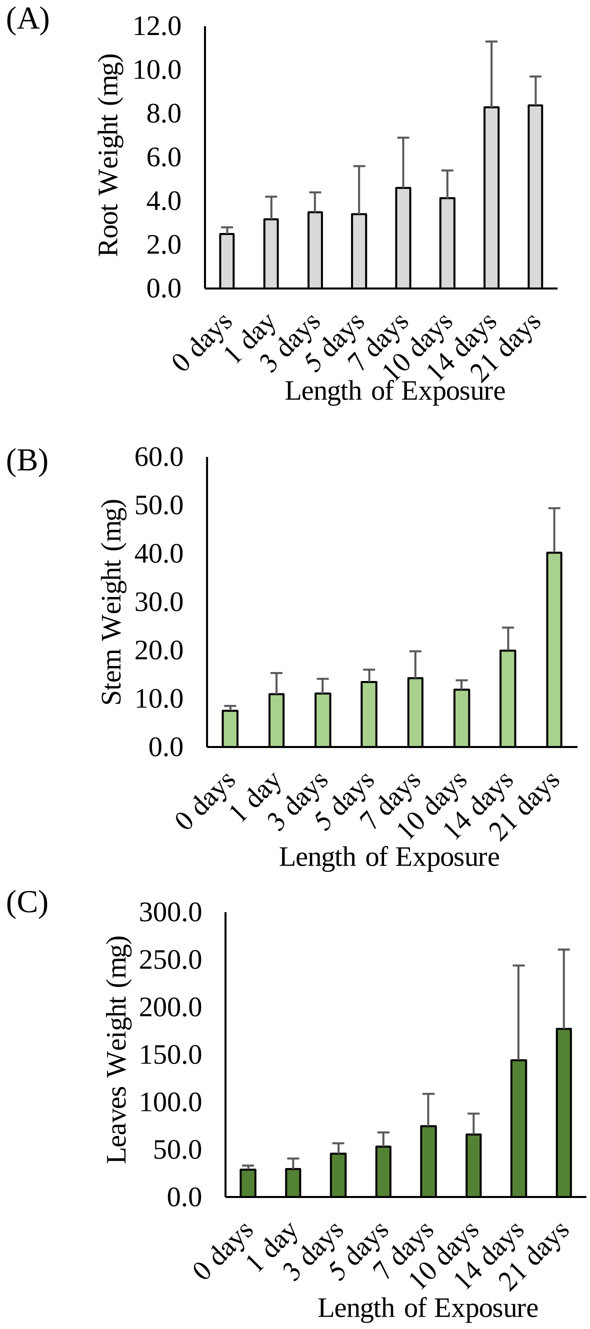
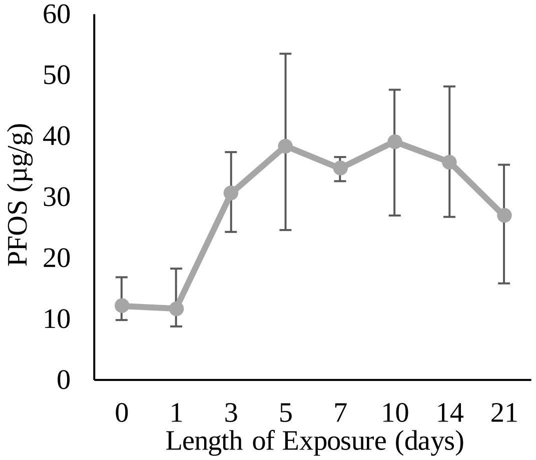


Figure 25. Time course plant study showing uptake of PFOA by cucumber sprouts. Root dry weight (A), sprout dry weight (B) and total sprout length (C) were measured over the duration of the 21-day exposure period. PFOA concentrations were measured in roots (D), stems (E), and leaves (F). Proportions of PFOA in the sprouts compartments suggest accumulation of PFOA in the leaves by the end of the exposure period (G).

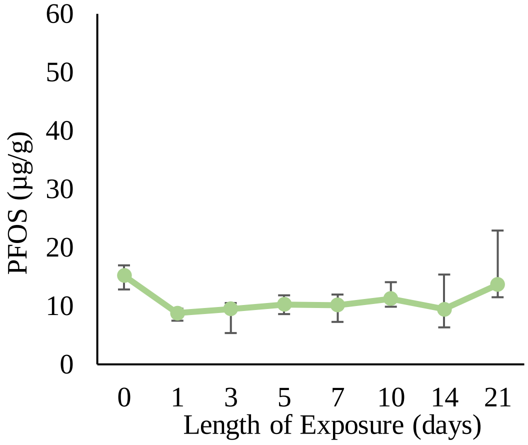

(A)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Root Weight (mg)
0 days
1 day
3 days
5 days
7 days
10 days
14 days
21 days
Length of Exposure
(B)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Stem Weight (mg)
0 days
1 day
3 days
5 days
7 days
10 days
14 days
21 days
Length of Exposure
(C)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Leaves Weight (mg)
0 days
1 day
3 days
5 days
7 days
10 days
14 days
21 days
Length of Exposure

(D)



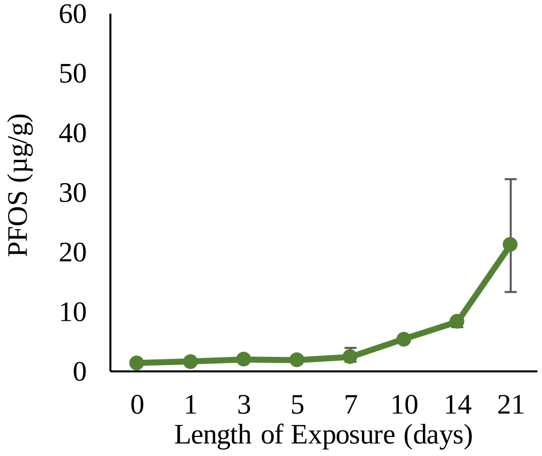
60
50
40
30
20
10
0
PFOS (µg/g)
0
1
3
5
7
10
14
21
Length of Exposure (days)

(E)



60
50
40
30
20
10
0
PFOS (µg/g)
0
1
3
5
7
10
14
21
Length of Exposure (days)

(F)



60
50
40
30
20
10
0
PFOS (µg/g)
0
1
3
5
7
10
14
21
Length of Exposure (days)

(G)

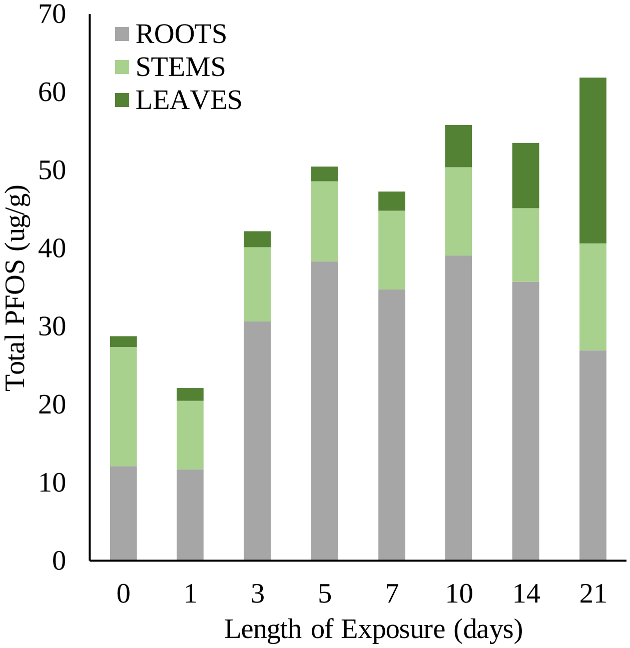
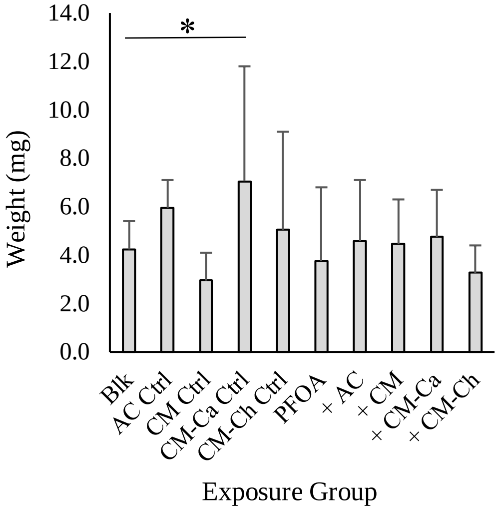


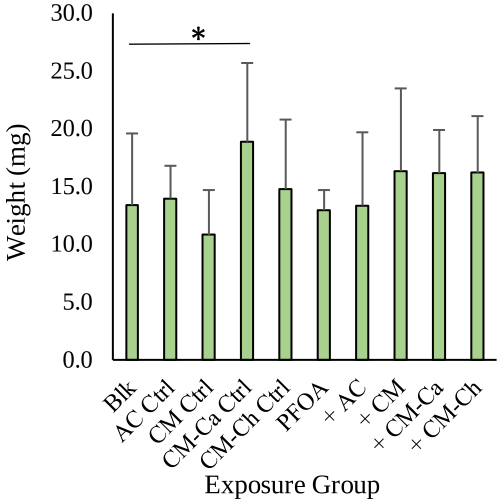
Figure 26. Time course plant study showing uptake of PFOS by cucumber sprouts. Root dry weight (A), sprout dry weight (B) and total sprout length (C) were measured over the duration of the 21-day exposure period. PFOS concentrations were measured in roots (D), stems (E), and leaves (F). Proportions of PFOS in the sprouts compartments suggest accumulation of PFOS in the leaves by the end of the exposure period (G).

Sorbent inclusion studies were conducted to determine sorbent ability to reduce PFAS translocation to cucumber sprouts. Root, stem, and leaves dry weights were measured for all groups at the end of the exposure period. In the roots (Figure 27A), stems (Figure 27B), and leaves (Figure 27C) of cucumbers in the PFOA study, the CM-carnitine control group had significantly higher growth compared to the blank group ($p < 0.05$). L-carnitine has been shown to be important in fatty acid metabolism and cell homeostasis in plants as well as promoting cell division and plant growth (Jacques et al., 2018; Oney-Birol, 2019). These positive effects on plant growth could explain the increased growth seen in the CM-carnitine control group. At 2% sorbent inclusion, all tested sorbents were able to significantly reduce PFOA translocation to cucumber roots, stems, and leaves (Figure 27D, E, F). CM averaged 51.2% reduction for all plant compartments, while CM-carnitine and CM-choline averaged 63.4% and 64.7%, respectively. For PFOS, there were no significant differences between exposures groups for root (Figure 28A), stem (Figure 28B), and leaves (Figure 28C) dry weights. Clay-based sorbents were highly effective, reducing PFOS residues by an average of 50.1%, 69.9%, and 67.2% for CM, CM-carnitine, and CM-choline, respectively (Figure 28D, E, F). The modified CM clays were more effective in reducing PFOA and PFOS translocation to cucumber sprouts than the parent CM clay. This increased efficacy along with the beneficial growth effects of L-carnitine suggest that modified CM clays could present a valuable soil amendment strategy for PFAS remediation and protection of plants.

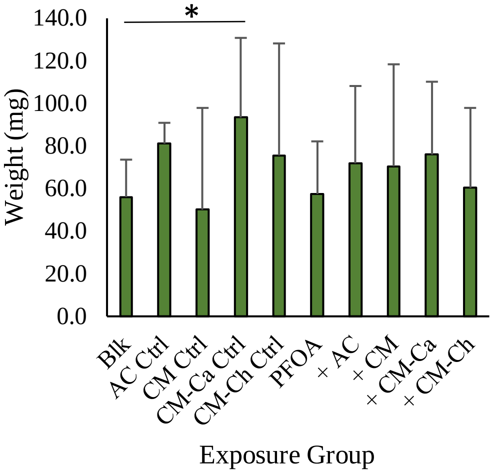
(A)



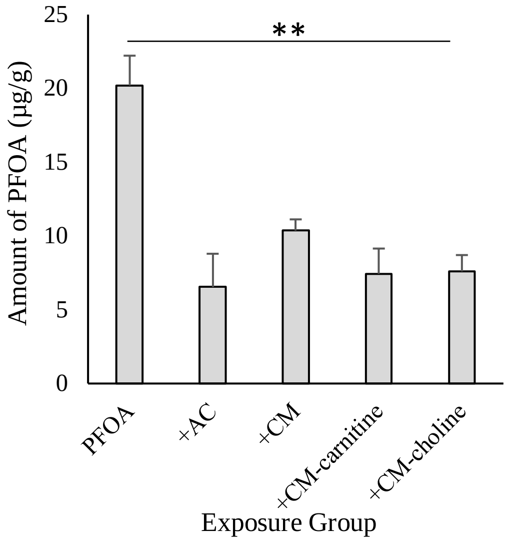
(B)


30.0
25.0
20.0
15.0
10.0
5.0
0.0
*
Weight (mg)
Blk
AC Ctrl
CM Ctrl
CM-Ca Ctrl
CM-Ch Ctrl
PFOA
+ AC
+ CM
+ CM-Ca
+ CM-Ch
Exposure Group

(C)

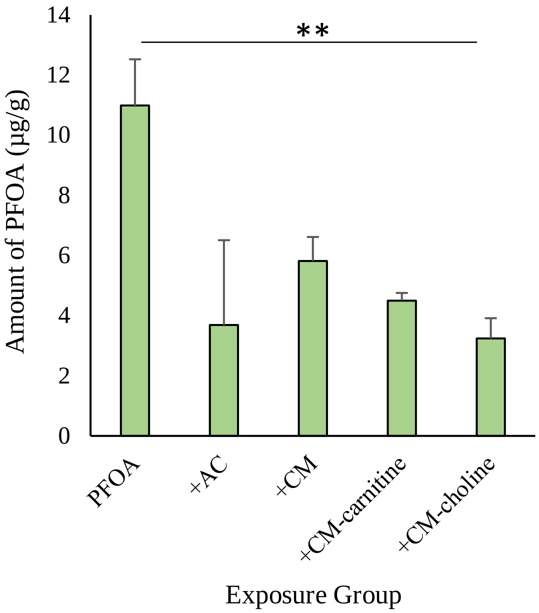

140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
*
Weight (mg)
Blk
AC Ctrl
CM Ctrl
CM-Ca Ctrl
CM-Ch Ctrl
PFOA
+ AC
+ CM
+ CM-Ca
+ CM-Ch
Exposure Group

(D)



25
20
15
10
5
0
Amount of PFOA (µg/g)
**
PFOA
+AC
+CM
+CM-carnitine
+CM-choline
Exposure Group

(E)



14
12
10
8
6
4
2
0
Amount of PFOA (µg/g)
**
PFOA
+AC
+CM
+CM-carnitine
+CM-choline
Exposure Group

(F)

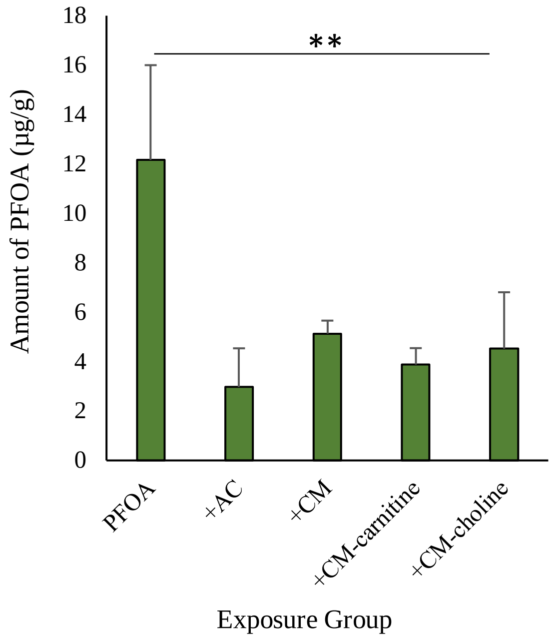
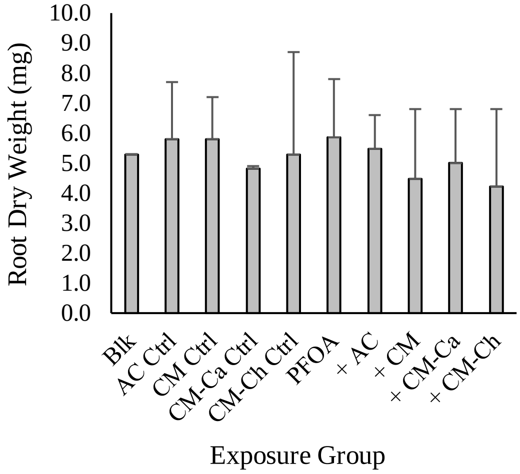
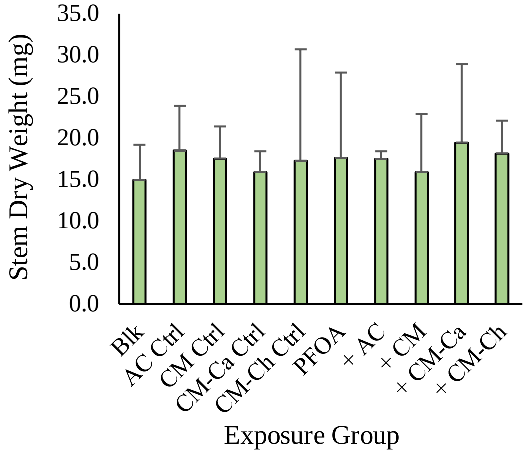


Figure 27. Plant study showing translocation of PFOA to cucumber sprouts and reduction of PFOA translocation with sorbents. Root (A), stem (B), and leaf (C) dry weights were measured for all exposure groups. Sorbents significantly reduced PFOA residues in cucumber roots (D), stems (E), and leaves (F) (*p < 0.05; **p < 0.01).

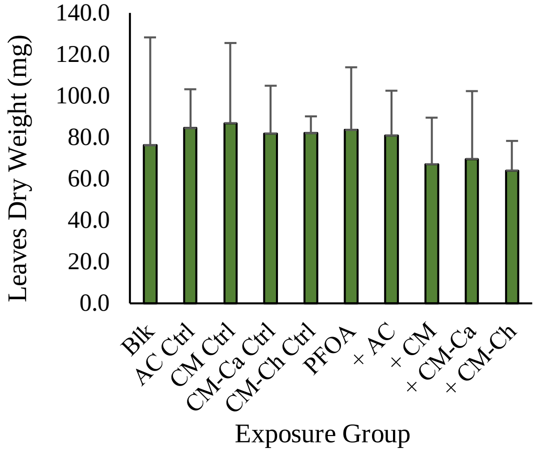
(A)



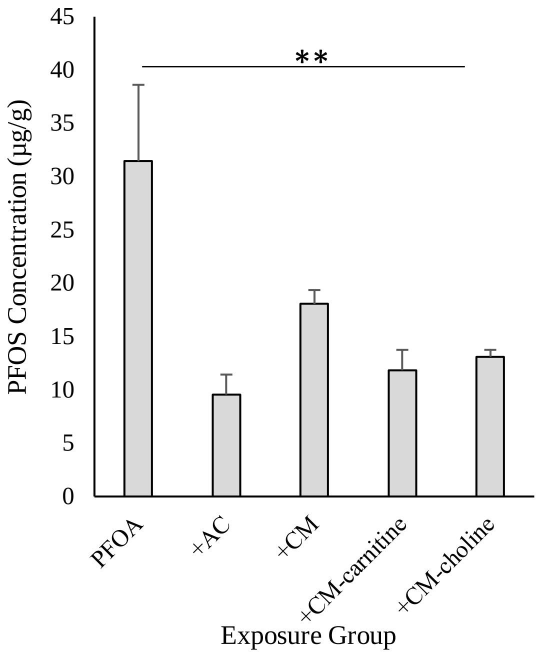
(B)


Stem Dry Weight (mg)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Blk
AC Ctrl
CM Ctrl
CM-Ca Ctrl
CM-Ch Ctrl
PFOA
+ AC
+ CM
+ CM-Ca
+ CM-Ch
Exposure Group

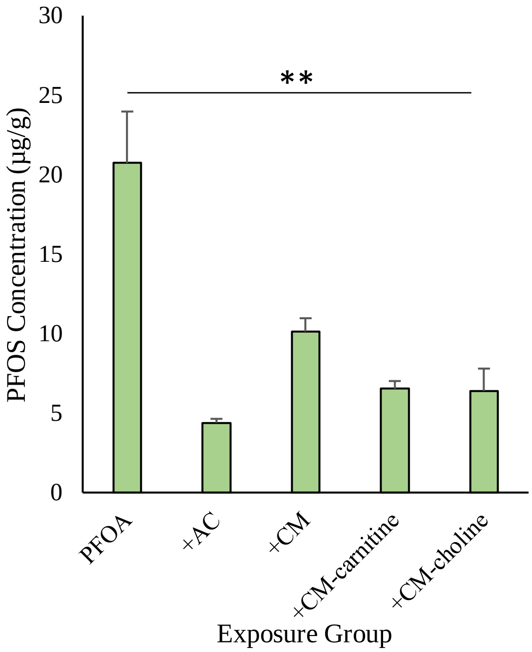
(C)


Leaves Dry Weight (mg)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Blk
AC Ctrl
CM Ctrl
CM-Ca Ctrl
CM-Ch Ctrl
PFOA
+ AC
+ CM
+ CM-Ca
+ CM-Ch
Exposure Group

(D)


45
40
35
30
25
20
15
10
5
0
PFOS Concentration (μg/g)
**
PFOA
+AC
+CM
+CM-carnitine
+CM-choline
Exposure Group

(E)


30
25
20
15
10
5
0
PFOS Concentration (μg/g)
**
PFOA
+AC
+CM
+CM-carnitine
+CM-choline
Exposure Group

(F)

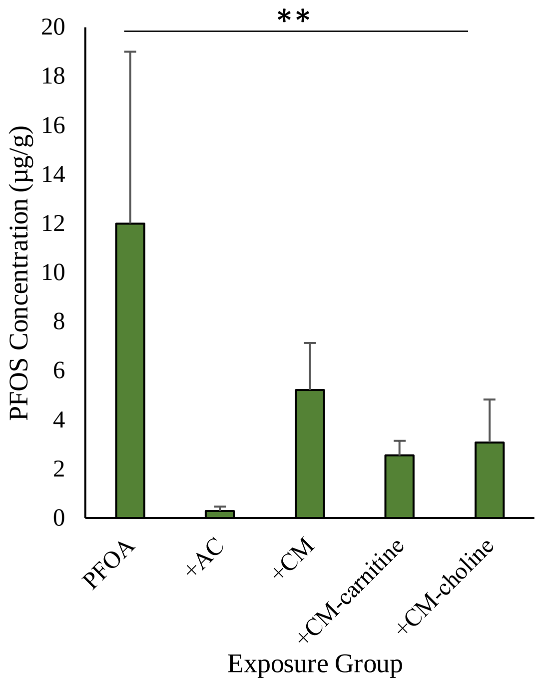


Figure 28. Plant study showing translocation of PFOS to cucumber sprouts and reduction of PFOS translocation with sorbents. Root (A), Stem (B), and leaf (C) dry weights were measured for all exposure groups. Sorbents significantly reduced PFOS residues in cucumber roots (D), stems (E), and leaves (F) (**$p < 0.01$).

AC was also able to significantly reduce PFOA and PFOS residues in cucumber sprouts (averaged 69.9% and 74.2% for PFOA and PFOS, respectively). AC and other carbon-based sorbents have been widely used and shown to be effective for contaminant immobilization in soil. However, it is important to note that some studies have suggested that AC can negatively impact soil and plant health. AC has been shown to reduce microbial denitrification and nutrient availability (Bonaglia et al., 2019). AC can also reduce macrofauna living in sediment and decreased survival and growth rates of benthic invertebrates (Janssen & Beckingham, 2013). To reduce these effects and to potentially enhance plant health, modified clays alone or in combination with AC could enhance PFAS remediation from soil and benefit plant growth (Samuelsson et al., 2015). Studies have shown that combinations of AC and clay-based sorbents can enhance binding efficacy of contaminants such as metals and can increase nitrogen availability (Han et al., 2019; Zhu et al., 2009). Nutrient-modified CM clays alone or in combination with AC could present a promising strategy to reduce chemical bioavailability from soil.

4.4 Conclusions

Adsorption with modified CM clays presents a promising strategy for PFAS remediation of soil and reducing PFAS translocation to produce. The efficacy of this technique is supported by *in vitro*, *in vivo*, and *in silico* results that show sorbents can tightly bind PFOA and PFOS and can reduce bioavailability from soil and translocation to cucumber sprouts. CM-carnitine and CM-choline were consistently more effective in binding PFOA and PFOS than the parent CM clay due to increased hydrophobicity of clay surfaces. Importantly, these sorbents are environmentally-friendly, energy-efficient, and practical. Additional studies are needed to investigate the field efficacy and long-term potential of these sorbents in reducing PFAS bioavailability. Future studies including AC, CM, modified CM clays, and mixtures of these sorbents will investigate the effects of sorbents on nutrient utilization in plants and the mitigation of other environmentally relevant chemicals following natural disasters, chemical spills, and emergencies.

5. SUMMARY AND CONCLUSIONS

5.1 Summary

The overall goal of this study was to develop and characterize novel, broad-acting sorbents that can reduce the bioavailability of environmental chemicals in soil and reduce their translocation to plants, thus reducing the risk of exposure. Carbonaceous and clay-based sorbents were tested for ability to bind lipophilic, hydrophilic, and amphiphilic chemicals using *in vitro*, *in vivo*, and *in silico* methods. Activated carbon (AC), calcium montmorillonite clay (CM), calcium montmorillonite clay modified with L-carnitine and choline (CM-carnitine and CM-choline), and acid processed calcium montmorillonite clay (APM) were excellent sorbents for a variety of environmental test chemicals.

CM and APMs were effective binders of dieldrin, which is a hazardous legacy organochlorine pesticide that is still detected in water and soil even years after discontinuation. Both APMs had enhanced capacities for dieldrin, compared to the parent CM clay. Isothermal results for collapsed-CM and collapsed APM clays showed that clay interlayer surfaces were important binding sites for dieldrin, but not the only sites available for binding. Enthalpy values (> -20 kJ mol^{-1}) that were derived from thermodynamic studies suggested that dieldrin binding to CM and APM involved exothermic interactions, indicating tight binding of dieldrin, or chemisorption. Results from the hydra assay showed that CM and APM significantly protected the hydra from dieldrin toxicity (60% and 70%, respectively), and both sorbents at a 1% inclusion level significantly reduced dieldrin bioavailability from soil (up to 65%) over a range of chemical concentrations. This study indicated that APM had significantly enhanced binding potential for dieldrin compared to AC and CM.

Based on isothermal analyses, AC and CM were shown to be effective sorbents of aminomethylphosphonic acid (AMPA) which is the main metabolite of the widely used herbicide glyphosate. Isothermal results from collapsed CM suggested that active interlayer surfaces are important in binding AMPA, but not the only sites involved in the sorption of AMPA. Thermodynamic studies suggested that CM bound AMPA with a high heat of sorption, indicating chemisorption interactions. The hydra assay showed that both AC and CM provided 90% protection of the hydra from AMPA toxicity, and that AMPA was more toxic to the hydra than glyphosate (the parent) at the same dose. In soil studies, 1% w/w inclusion of AC and CM reduced AMPA bioavailability by 53% and 43%, respectively. Plant uptake studies showed translocation of PMG and AMPA to cucumber sprouts and metabolism of PMG to AMPA. Inclusion of 1% w/w AC and CM reduced AMPA residues in cucumber sprouts grown in both soil and compost by up to 70% and 57%, respectively. In this study, CM had comparable binding efficacy to AC. Importantly, soil studies suggested that combinations of AC and CM could enhance the binding of AMPA.

AC, CM, CM-carnitine, and CM-choline were all effective sorbents of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). All 4 tested sorbents had high capacities and affinities for both fluorosurfactants (PFOA and PFOS). CM-carnitine and CM-choline had significantly enhanced capacities for PFOA and PFOS compared to the parent clay. Molecular dynamics (MD) simulations confirmed that the L-carnitine and choline modifications improved the binding of PFOA and PFOS to sorbents. Thermodynamic studies showed that all clay-based sorbents had high enthalpies, suggesting tight binding of both PFOA and PFOS. In *Lemna minor* studies, Inclusion of 0.1% CM-carnitine and CM-choline significantly increased frond surface area and total chlorophyll content compared to the PFOA and PFOS exposure groups. Importantly, these modified clays enhanced lemna growth compared to the media control group. In soil studies, 2% inclusion of AC, CM, CM-carnitine and CM-choline significantly reduced PFOA and PFOS bioavailability (38 to 74%). The same inclusion level applied to the plant studies resulted in up to 70% reduction in PFOA and up to 74% reduction in PFOS residues in cucumber roots, stems, and leaves. The modified clays were consistently more effective than the parent clay. Importantly, cucumber sprout growth was favored in the CM-carnitine control groups, suggesting that this modification may be nutritive to plants. The L-carnitine- and choline-modified clays were consistently more effective in binding PFOA and PFOS than the parent CM clay.

These results collectively indicated that carbonaceous and clay-based sorbents can effectively bind a wide range of chemicals. Processed and modified CM clays could present a novel and effective strategy to reduce chemical bioavailability from soil and translocation to plants. Because these novel sorbents are environmentally-friendly and can provide enhanced binding for various chemicals compared to AC and the parent CM, inclusion of these sorbents could improve soil remediation efforts based on immobilization of chemicals. Sorbent mixtures could be developed and optimized to bind chemicals and chemical mixtures of concern at a particular location.

5.2 Cost Considerations

Clay-based sorbents utilized as soil amendments present an environmentally-friendly and economically-feasible strategy to reduce chemical bioavailability. Adsorption of chemicals in environmental media is favored over other remediation methods (e.g., filtration, electrochemical treatment, extraction) due to its lower cost, higher efficiency, and applicability to a range of environmental chemicals (Akpomie & Dawodu, 2016). Carbonaceous sorbents have been widely used in the remediation of environmental media due to their abilities to sorb a range of chemicals. Although ACs are highly effective sorbents of diverse environmental chemicals, the production of ACs is expensive and can result in secondary contamination. Clays such as montmorillonites and various modified montmorillonites are considered to be promising low-cost strategies for soil remediation.

Carbonaceous sorbents such as granular and powder ACs are widely used in a variety of applications because of their sorption capabilities, however it is important to consider the costs of these products for large-scale applications. The production of AC is an expensive process. Depending on the quality, ACs can cost $0.80 to $1.10 per kg and can range up to an estimated $9 USD per kg (Kausar et al., 2018; Kurniawan et al., 2006;). Additionally, issues with disposal and regeneration can contribute to operational costs. Powdered AC sorbents are popular due to their low initial cost; however, they are difficult to regenerate and can be expensive in terms of operational costs for continuous usage (Kennedy et al., 2018). Also, the fine particles of powdered AC, the dust created through their use, and their flammability can make them difficult to apply and handle. Granular AC is more expensive up front and is more often used in water filtration than in soil applications. Biochars present cheaper alternatives to activated carbons, but these materials are not as well-characterized in terms of adsorption mechanisms and efficacy. Biochar derived from coconut shell costs $0.25 per kg, and biochar derived from peanut hulls can cost an average of $2.00 per kg (Kurniawan et al., 2006). Additionally, these materials often require extra processing costs.

Many adsorption studies have investigated the use of clay minerals as soil amendments due to their lower costs, lack of toxicity, and active surfaces for diverse chemicals. Natural clays have been evaluated as alternative sorbents to AC for environmental applications because they are naturally-occurring and do not present any risks to environmental health (Boufatit & Khouider, 2004; Iannicelli-Zubiani et al., 2015). Montmorillonite clays are ideal sorbents due to their structures and abilities to bind various chemicals within their interlayers, at edge sites, or on basal surfaces. The abundance of montmorillonites in nature contributes to their low cost (an estimated $0.04 – 0.12 per kg) (Srinivasan, 2011). The parent CM clay used in this study is commercially-available and used worldwide as a food and feed additive for the mitigation of mycotoxin exposures and as an anti-caking agent (Phillips et al., 2019). The ability of montmorillonite clay to bind diverse environmental contaminants, its cost-efficiency, and its availability make it ideal for large-scale soil remediation efforts.

Studies have shown that organoclays can also be more cost-effective and environmentally-friendly than ACs and biochars (Nourmoradi et al., 2016; Shattar et al., 2019). Organoclays alone or in combination with AC have been shown to greatly enhance water filtration and can be significantly more cost-effective (Alther, 2002). Including organoclays in groundwater filtration can enhance remediation efforts as these clays can be 7 times more effective in removing oil than AC, and can cut costs by 50% as they do not have to be replaced as often as AC (Alther, 2010). Including organoclays in sediment capping and soil stabilization has been shown to be a cost-effective alternative to AC alone (Sarkar et al., 2012). Importantly, studies have shown that these modified clays can be regenerated after applications in wastewater treatment as well as soil and groundwater remediation (Zhu et al., 2009). Based on the reported efficacy and cost-efficiency of these sorbents, organo-modified clays could be an economically-feasible strategy to reduce chemical bioavailability from soil.

Acid-processed clays have been reported to be effective sorbents of chemicals in the environment and have been used as low-cost alternatives to AC (Akpomie & Dawodu, 2016; Amari et al., 2018). Acid activation of clays is widely used in various applications due to the low costs associated with the acid activation process (Toor et al. 2015; Zhou, 2011). Acid-activated clays are applicable to a variety of industrial activities and commercial products and have more recently been evaluated for efficacy in environmental applications. These clays are often used for bleaching or decolorization purposes. In industrial applications, sulfuric acid (used in this study) is preferred to other commonly used acids such as hydrochloric because it is less expensive and not as harsh (Komadel, 2016). An example of a commercial use of this type of clay is Poultry Guard®, an acid-activated bentonite clay that is currently mass produced and sold as a poultry litter. The APM clays used in this study are expected to be more cost-effective and economically-feasible. APM clay technology has been described in a non-provisional patent application (PCT/US2019/047356), and a worldwide exclusive license through the Office of Technology Commercialization at Texas A&M University.

5.3 Field Applications

Carbonaceous and clay-based sorbents have been applied to soils to reduce chemical bioavailability and translocation to plants. Immobilization of chemicals in soil is a widely used remediation technique because this process is cost-effective compared to other techniques and presents minimal risks to the environment. Carbonaceous sorbents such as activated carbon and biochar have been used to reduce the bioavailability of chemicals. A field study in Norway using 2% powdered or granular activated carbon significantly reduced PAH leaching from soil for up to 28 months after application (Hale et al., 2012). Sugarcane bagasse biochar amendments significantly reduced bioavailability of heavy metals (cadmium, copper, lead) from soil and protected bok choy plants from exposure (Nie et al., 2018). Smectite clays were used as soil amendments to reduce the bioavailability of metals and translocation to plants (Ou et al., 2018). Application of 0.5% clay resulted in 35.4% reduction in cadmium bioavailability and up to 62% reduction of metal uptake into edible parts of plants. Organoclays have also been used on a large scale to reduce pesticide bioavailability. For example, montmorillonite modified with spermine applied at 1%, 2%, and 5% significantly reduced fluometuron bioavailability and leaching from soil (Gamiz et al., 2010). Organoclays were also effective in immobilizing the pesticide bentazone (Carrizosa et al., 2000). Natural and acid-activated clays were tested for efficacy in reducing molybdenum bioavailability from soil and translocation to alfalfa plants (Alrashidi et al., 2020). Results showed that acid-activated clays significantly reduce molybdenum bioavailability and were more effective than the natural clay. Importantly, both clays had a nutritive effect on plant growth.

The results of this study suggest that novel processed and modified CM clays may be more effective that commercially available sorbents at lower inclusion levels. Additionally, the characterization of sorbent-chemicals relationships and the potential to optimize sorbent combinations for specific chemicals of concern presents an added value to applying these sorbents as soil amendments.

5.4 Conclusions

The main novelty of this study is the development and characterization of broad-acting sorbents that can bind diverse environmental chemicals in soil and decrease their uptake by plants. It is important to evaluate the binding capacities, affinities, and mechanisms as well as validate the safety and efficacy of sorbents in various applications. This information can result in the development of optimized sorbents for specific chemicals and chemical mixtures. Collectively, *in vitro*, *in vivo*, and *in silico* methods successfully characterized sorbent-chemical relationships as well as sorbent safety for living organisms and efficacy in soil applications. The results of this study suggest that calcium montmorillonite (CM) clay along with acid-processed montmorillonite (APM) and montmorillonite modified with L-carnitine and choline (CM-carnitine and CM-choline) could be highly effective in binding pesticides and per- and polyfluoroalkyl substances (PFAS). This is the first report of APMs and nutrient amended clays, i.e., L-carnitine and choline modified CM clays, that were able to reduce chemical bioavailability from soil and translocation to plants. These environmentally-friendly, novel, and broad-acting sorbents could be applied alone or in combination with the more traditionally used activated carbons as soil amendments to bind hazardous chemicals in soil and protect against human and animal exposures. This protection is especially important in cases of natural or anthropogenic disasters that can mobilize contaminants already present in the environment and contribute to this contamination. These sorbents could be applied as mixtures that can be adjusted and optimized based on the chemicals of concern at a specific site. Also, broad-acting sorbents can be added to community lawns and gardens in vulnerable communities to decrease exposures to toxic environmental chemicals. Applying novel soil amendments can reduce chemical bioavailability and translocation to plants, protecting people from exposures through either contact with contaminated soil or consumption of contaminated food products.

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