Evaluating Fungus-Inoculated Biochar as a Cost-Effective, Sustainable Media for Stormwater Treatment

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Abstract

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Urban stormwater runoff is a leading contributor to water quality degradation in the U.S., negatively affecting both human and aquatic ecosystems. To mitigate these impacts, many cities have implemented green stormwater infrastructure like bioretention systems. However, the increase in acutely toxic organic contaminants transported by stormwater runoff has prompted the investigation of bioretention system media amendments to improve stormwater quality downstream. Previous studies have shown that biochar, mycoremediation, and phytoremediation serve as sustainable, effective mechanisms to remediate contaminated systems. Therefore, this study investigated the use of Mountain Crest Gardens (MCG) biochar, derived from pyrolyzed conifer softwood, as a contaminant sorbent and growth substrate for fungal degrader *Trichoderma harzianum* via fungal inoculation, to enhance the removal of common stormwater contaminants (sulfamethoxazole, benzotriazole, and acetaminophen). Additionally, this study evaluated the influence of *T. harzianum*-inoculated biochar on the plant health and phytoremediation capacity of the native perennial *Juncus patens*. Laboratory batch isotherm studies and an 18-week greenhouse mesocosm experiment revealed that *T. harzianum*-inoculated MCG biochar improved removal efficiency and sorption capacity across stormwater contaminants, in comparison to *T. harzianum* and MCG alone. The mesocosm study further demonstrated that *Juncus patens* enhanced the removal of all three contaminants in soils amended with TH@MCG biochar in comparison to mesocosms amended with *T. harzianum* or MCG only. These findings suggest that TH@MCG biochar, particularly when combined with *Juncus patens*, offers a promising approach for bioretention systems to sustainably treat stormwater runoff. Utilizing fungus-inoculated biochar alongside plants with phytoremediation potential presents a cost-effective, sustainable solution for remediating contaminated stormwater.

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I am eternally grateful for my advisor and committee chair, Dr. Jessica Ray. Without her compassion, support, and encouragement as I navigated graduate school while grieving a parent, this accomplishment would not have been possible. I would also like to thank my committee member, Dr. Mari Winkler for supporting this project and providing expert feedback on my research. In addition, I would like to express my gratitude for Dr. Korena Mafune, who provided constant support throughout the completion of this study, as well as encouragement as a fellow first-generation college graduate. Further, I extend my appreciate to the entirety of the AIMS Lab for providing an encouraging environment to learn, grow, and make mistakes. A special thank you to Dijia Bao, Kiana Gholamy, Dr. Fanny Okaikue-Woodi, and Reagan Beers for the time they invested into completing this project. It truly takes a village, and I have been lucky enough to work with a team of caring, intelligent, and inspiring scientists. Thank you to the UW Future Rivers Program supporting this project and providing valuable opportunities for me to grow as a scientist.

Finally, I dedicate this thesis and this milestone to my parents, who taught me what it means to protect, love, and care for the environment. My father was a school bus driver for 30 years, and my mother has been a nurse assistant for 23 years. They came to the U.S. from El Salvador with only the clothes on their backs, but they gave my brother and me the world. My parents worked day and night to provide me opportunities to succeed, and their love will always be reflected in my accomplishments. Thank you to my brother, sister-in-law, niece, and nephew for reminding me to smile and laugh in even the hardest times. An additionally thank you to all my friends I have made in Seattle. You have all taught me what it means to share a community, which is a gift not many people get to experience. I am eternally grateful for my friends, family, and community. Thank you for believing in me, even when I struggled to believe in myself.

Quisieron enterrarnos, pero no sabían que éramos semillas.

¡Sí Se Pudo:!

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1. INTRODUCTION

1.1. The Rise of Urban Stormwater Runoff

In terrestrial environments, there are many opportunities for stormwater to infiltrate soils, nourish vegetation, transport nutrients, regulate runoff, and recharge aquifers within ecological systems.[6,7] However, as urbanization increases, there are fewer opportunities for urban stormwater runoff to perform these crucial environmental services.[6,7] For example, in Seattle, WA, the urban tree canopy has declined approximately 2% in the past seven years, which has been primarily replaced by impervious structures (**Figure 1.1**).[8,9] The loss of urban trees, which play an important role in reducing stormwater runoff through interception, evapotranspiration, and infiltration, has significantly impacted stormwater management in urbanized spaces. [8,9] This, combined with the increase in built infrastructure, has promoted the mobilization of harmful pollutants by stormwater runoff to the environment and to downstream water sources.[8,9] Urban stormwater runoff most commonly conveys complex mixtures of contaminants including pesticides, heavy metals, and hydrocarbons from vehicles and homes, as well as suspended solids such as pavement, bacteria, vehicle parts, and construction debris.[1-5]

The rising pollutant loads of dissolved contaminants and suspended solids transported by urban stormwater runoff into watersheds detrimentally impacts both human and aquatic ecosystem health.[9] In the U.S., urban stormwater runoff is recognized as the fastest-growing contributor to surface water degradation.[5] The lack of proper treatment, flow management, and environmental filtration of stormwater runoff often results in increased mortality of sensitive aquatic species within receiving water bodies (i.e. coastal ecosystems).[10] In humans, chronic exposure to mismanaged stormwater runoff and runoff-induced flooding increases the risk of health complications and long-term health consequences including waterborne diseases, respiratory issues, and cancer.[11]

The loss of green spaces, the rise in high intensity storm events, and the degradation of aquatic ecosystems prove to be of even greater concern as the effects of climate change continue to intensify.[12] Both existing data and climatic models that focus on rainfall patterns predict that stormwater runoff volume, peak flow level, and storm event frequency will all vary to extremes of either dry conditions or overly wet conditions on a temporal or regional level. This is expected to increase as a repercussion of anthropogenically induced climate change.[13] Much of the existing urban stormwater infrastructure within the U.S. is not equipped to face these growing changes in stormwater runoff management and volume. Flooding and high intensity storm events only exacerbate the potential human and ecological health risks urban areas face due to contaminated runoff.[10,11] Higher intensity and frequency storms are prone to overwhelm existing drainage systems leading to flooding and (re)mobilization of pollutants. Additionally, these changes in storm events can increase contaminants loads carried into surface and groundwater stores, both of which are used as drinking water supplies and these treatment systems.[11–13] Drought conditions driven by climate change can worsen these issues by concentrating pollutants and reducing the natural dilution of contaminants in waterbodies during dry periods, placing additional stress on water supplies.[14]

According to the Environmental Protection Agency (EPA), many urban areas across the U.S. are already experiencing the combined challenges of urban heat islands—where dense infrastructure

absorbs and retains heat, raising temperatures compared to surrounding vegetated, rural areas—alongside increased contaminated water supplies and exposure to polluted floodwaters. These impacts disproportionately affect low-income communities and communities of color, intensifying environmental and health risks.[2,4,14] Many urban areas have incorporated green stormwater infrastructure (GSI) to manage stormwater runoff using natural processes to mitigate the effects of urban stormwater runoff.[12,15] The incorporation of these systems have also been found to reduce urban heat island effects by providing cooling via vegetation and minimizing heat trapping pollutants in the air.[7-10] Through incorporation of vegetation, these systems are effective at providing urban ecological habitats, sequestering carbon, improving water quality, and reducing flooding.[13] Nonetheless, the majority of existing systems were designed using outdated precipitation frequency analyses.[12,13] Climate change models and observed shifts in precipitation patterns suggest that existing systems are inadequate to handle the anticipated changes in stormwater quality and volume driven by climate change.[12,13]

Inequitable access to safe, healthy environments is a growing disparity that needs to be addressed, and its effects are reflected in urban ecosystem and human health.[1-8] Modifying green stormwater infrastructure (GSI) systems can be a cost-effective, accessible bridge to addressing these intertwined urban environmental issues.[16,17] Therefore, by exploring both the complexities urban stormwater runoff present, as well as the potential amendments that can be made to address the vulnerabilities of these systems, we can effectively mitigate a range of climate-induced environmental issues.

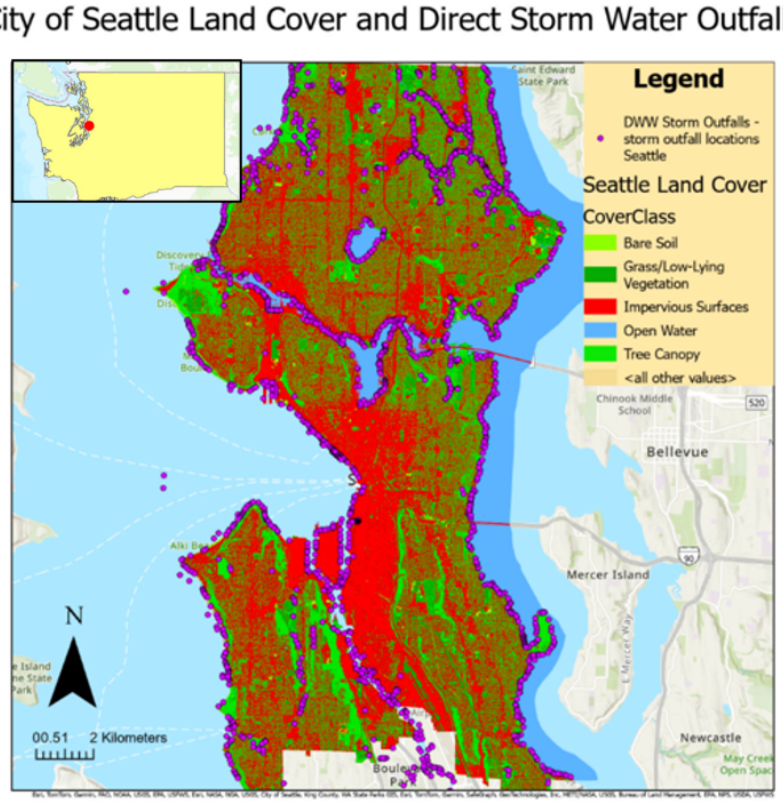


Figure 1.1 ARCGIS map produced using City of Seattle, King County GIS database and USGS maps to portray Seattle land cover. This map depicts the land cover classes within the city of Seattle, emphasizing the difference in vegetated versus impervious land. Further, this map also includes point in Seattle where stormwater runoff is directly released into a waterbody, meaning it receives

no treatment prior to entering the ocean. Together, the map shows how much potential there is for stormwater runoff to flow through impervious structures directly into Lake Washington and the Puget Sound.

1.2. Sources and Impacts of Stormwater Pollution

Designing effective GSI for urban stormwater runoff is challenging due to the variability and geographically-dependent composition of its contaminants.[6,18,19] In areas with combined sewer systems, where stormwater and wastewater share the same infrastructure, heavy storm events can overwhelm these systems, leading to mixtures of untreated stormwater and raw sewage overflowing into nearby water bodies. [1,8] This overflow introduces wastewater pollutants like pharmaceuticals, personal care products, and harmful bacteria the environment, contributing to water quality degradation, oxygen depletion, and excess nutrient levels. [1,11,12] Although most urban areas rely on separate sewer systems, which transport and discharge stormwater runoff separately from sewage, these systems amplify aquatic pollution risks by discharging high volumes of untreated surface runoff into receiving waters. [1,2,8] The absence of environmental buffers and increased flood events have turned urban stormwater runoff into non-point source pollution, carrying contaminants from roads, sidewalks, buildings, and greenways like lawns (**Table 1.1**).[1,2] Pollutants such as suspended solids, fertilizers, pesticides, soaps/detergents, oil/petroleum, heavy metals, and microplastics are transported into water bodies, harming aquatic systems.[1,9,10]

Table 1.1: Adapted from a study by Okaikue-Woodi et al., the table presents a compiled list of common stormwater contaminants from the National Stormwater Database, including common concentration ranges. Data was collected 2001-2018 from over 5000 storm events, and samples were collected from varying location types within urban areas.[6]

Constituent Category	Contaminant	Average Concentration	Unit
Physicochemical Parameter	pH	7.3 ± 0.8	
	Total Suspended Solids (TSS)	133 ± 260 mg/L	
	Chemical Oxygen Demands (COD)	77 ± 91	
	Oil/grease	10 ± 1	mg/l
	Total Kjeldahl Nitrogen (TKN)	2.0 ± 3.5	
	NO_2^-+NO_3^-	0.9 ± 1.3	
	Total P	2.0 ± 3.5	
Metals	Zn	160 ± 356 µg/L	
	Cu	26.5 ± 54.6	
	Pb	24.4 ± 60.6	
	Ni	7.2 ± 14.7	µg/L
	Cr	7.1 ± 13.5	
	Cd	1.5 ± 5.5	
Organic/Biological	Fecal Coliforms	55,151 ± 282,910	#/100 mL
Trace Organic Compounds	Total petroleum hydrocarbons	3.9 ± 4.4	mg/L
	benzene	84.7 ± 79.3	µg/L
	2-chloroethylvinylether	3.4 ± 2.6	
	chloroform	74.8 ± 159.5	
	dichlorobromoethane	0.8 ± 0.5	
	1,1-dichloroethane	0.6 ± 0.1	
	1,2-dichloroethane	1.5 ± 3.6	
	methylchloride	5.2 ± 4.1	
	methylenechloride	12.2 ± 9.4	
	tetrachloroethylene	1.5 ± 1.0	
	toluene	1.5 ± 2.1	
	1,1,1-trichloroethane	2.4 ± 2.0	

Further, road, industrial, and agricultural runoff can transport complex organic contaminants like polycyclic aromatic hydrocarbons (PAHs), tire-derived chemicals and pesticides into receiving

water bodies, and even into groundwater stores.[20–22] Residential areas also contribute to this issue as pet waste, fertilizers, cleaning chemicals, and even heavy metals from building structures are conveyed into watersheds after storm events. [1,5,11] Many of these stormwater runoff pollutants have been classified as contaminants of emerging concern (CEC) by the Environmental Protection Agency, or "chemicals and toxics that have been found in waterbodies that may cause ecological or human health impacts and are not currently regulated."[23] CECs like pharmaceuticals and personal care products have been found to be acutely toxic in aquatic systems, leading to reproductive complications, behavioral issues, and higher mortality rates within aquatic organisms as well as changes to microbial communities.[24] Many endocrine disrupting chemicals have been identified as CECs and are commonly detected within stormwater runoff.[25,26,27] Chronic exposure to endocrine disrupting chemicals including surfactants, pesticides, pharmaceuticals, brominated flame retardants, PAHs, polychlorinated biphenyls **(**PCBs), and phthalates has been linked to several human health issues such as declining male fertility, birth defects, and breast and testicular cancer.[25]

The dangers presented by contaminated stormwater runoff are intensified by the vast amount of persistent organic pollutants, from pharmaceuticals to Per- and polyfluoroalkyl substances (PFAS), that disrupt aquatic ecosystems and cause detrimental harm to coastal communities.[6] This is especially apparent in coastal habitats that serve as essential coastal buffer zones such as wetlands.[10] Increasing amounts of trace metals, pesticides, and other contaminants have made it difficult for systems like wetlands to perform their essential ecosystem functions (e.g. nutrient cycling, flood mitigation, water purification).[18,28] Further, high volume storms often lead to flooding that exacerbate these issues by mobilizing contaminants, causing algal blooms (via excess nutrients) and coastal erosion.[10,18,28,29]

In Washington, stormwater runoff has been identified as the leading source of urban water pollution, with approximately one-third of state waters being too polluted to meet water quality standards.[30] Stormwater runoff disproportionately impacts low-income and marginalized coastal communities, who often face the highest risks of water-related health risks and the lowest recovery rates from flooding events.[31] As an example, the Duwamish River in Seattle, WA, an EPA Superfund site polluted with PCBs, is largely comprised of refugees and immigrants who have historically relied on fishing due to socio-economic barriers.[32] This led to their exposure to toxic substances like PCBs, dioxins, arsenic, lead, mercury, and pesticides through bioaccumulation in the fish.[32] Many of these compounds carried by stormwater from anthropogenic sources can be acutely toxic, causing generational harm on keystone species within the Pacific Northwest.[33] Therefore stormwater runoff contamination is both a public health and ecological conservation issue.[20-22] When designing green stormwater infrastructure, it is essential to consider the complexities of urban runoff and aim to minimize its potential effects on the quality of receiving waters.

1.3. Existing Stormwater Management GSI and Flow Management Systems

The rapid degradation of aquatic systems from anthropogenic pollution coupled with the impending effects of climate change has led many cities globally to explore the implementation of stormwater management systems.[6,34] The introduction of stormwater best management practices (BMPs), or devices and methods to mitigate the volume and quality of polluted stormwater runoff, are a practical solution to preventing flooding following heavy storm events and remediating

contaminated stormwater runoff, such as permeable pavement and infiltration trenches.[6,306,34,35] When accounting for the need for green spaces in urban areas, the implementation of structural BMPs are found to be beneficial, nature-based solutions. Bioretention systems, including constructed wetlands, rain gardens, bioswales, and retention basins are multifunctional and provide opportunities to reduce contaminated stormwater runoff flooding events while also providing environmental benefits.[6,34,35] Bioretention systems are commonly comprised of sand, gravel, soil, mulch and vegetation layers that reduce flooding by slowing or trapping stormwater runoff and, depending on the design, filter contaminants within runoff (**Fig 1.2**).[6,34,35]

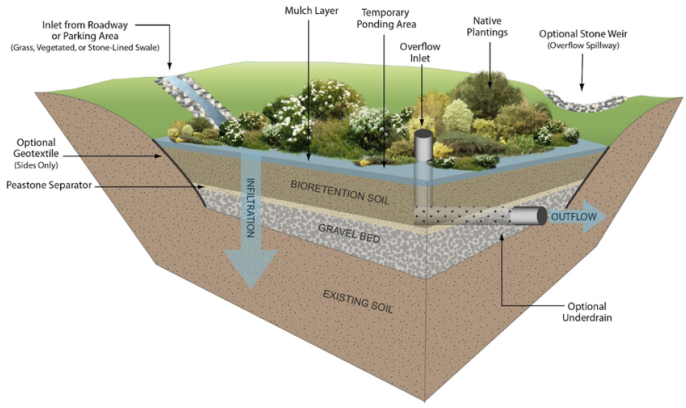


Figure 1.2: Example design by Geosyntec Consultants of a bioretention system, which include aspects like gravel layers and overflow inlets to provide more opportunities for natural stormwater filtration before it is released to an outflow drain . [1]

These systems often leverage naturally occurring processes to support runoff remediation like soil filtration and adsorption of contaminants as well as biological processes that manage the watershed nutrient supply.[6,34,36] While bioretention systems help control runoff flow rates and reduce suspended solids mobilized by urban runoff, they may be ineffective at reducing concentrations of dissolved contaminants like heavy metals, trace organic compounds, and nutrients (**Fig. 1.3**).[6,37] Additionally, the retention of contaminated stormwater in stormwater wetland basins for example, poses long-term threats as the accumulation of these pollutants without treatment can increase health risks for local biota supported by stormwater BMPs.[38,15] Thus, there is a need to modify bioretention systems to immobilize and degrade organic contaminants within stormwater runoff. Improving stormwater runoff quality not only reduces aquatic pollution, but also provides opportunities for groundwater recharge post-filtration.[6,19,37]

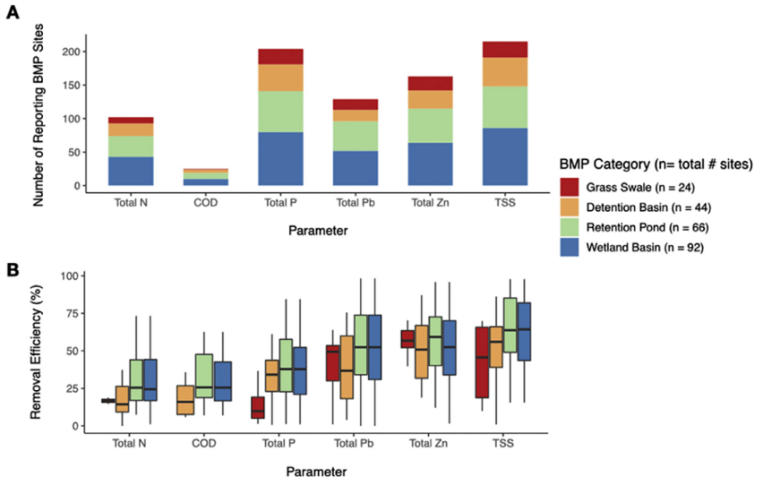


Figure 1.3: Data from the International Stormwater Best Management Practices (BMP) Database to illustrate (Figure A) the number of sites reporting data and the data limitations across different green stormwater infrastructure systems, emphasizing the limited contaminants recorded for each system. The lower graph (Figure B) depicts contaminant removal efficiency at each BMP site that reported influent and effluent concentration measurements across multiple storm events. COD = Chemical Oxygen Demand; TSS = Total Suspended Solids [6]

1.4. Media Amendments within Bioretention Systems

The increase in anthropogenic contaminants transported by stormwater runoff to water bodies has led to the investigation of bioretention system media amendments to improve stormwater quality downstream.[6,15,37,38] Conventional media amendments including sand, compost, and wood chips have been incorporated into these bioretention system soils to encourage natural filtration of stormwater runoff.[1,27] Research has shown that conventional media, both organic (e.g., biochar, compost, woodchips) and inorganic (e.g., sand, zeolite, clays), are effective at removing suspended solids from synthetic stormwater in lab and field-scale experiments.[6,39] Organic media amendments can facilitate removal of select organic contaminants, while inorganic media are more efficient at filtering out solids, pathogens, nutrients, metals, and hydrocarbons.[6,37,39] In fact, organic media have been highly studied due to their ability to support contaminant remediation and foster microbial growth, encouraging biotransformation of organic pollutants.[1]

However, both organic and inorganic conventional media lacks remediation efficiency across a range of contaminants, as well as the necessary physical and chemical properties to effectively adsorb or degrade complex stormwater contaminants.[6,37,39–42] Engineered media amendments have been proposed to enhance existing remediation capabilities through physical and chemical augmentation.[1,19] Recent studies suggest that incorporating engineered media along with conventional media in bioretention systems offers a sustainable, cost-effective solution to further reduce stormwater pollution urban areas with limited environmental filtration opportunities.[1, 27-39] Previous studies have explored using polymer clay composites, coated sands, and chemically modified zeolite to filter contaminants via adsorption or degradation.[6,37,43] For example, researchers have leveraged surface modifications and functional group interactions with contaminants to improve the remediation potential of sand (e.g. manganese oxide-coated sands for trace metal complexation)[6]. Other studies study chemically activated biochar using potassium hydroxide to improve its surface potential for organic contaminant sorption (e.g. KOH-activated biochar to improve PFOS sorption)[44]. Often, engineered media are produced to more efficiently

target and remove specific contaminants for stormwater remediation. However, many of the tested modification processes can often be costly, time-consuming, prone to degradation over time, or limited in their remediation efficiency across a range of common and emerging stormwater contaminants. [6,37,39] These issues restrain their applications within bioretention systems.[45]

1.5. Biochar Contaminant Removal Efficiency within Stormwater Infrastructure

Among organic stormwater treatment media, biochar has been the most studied and commonly used in application.[6,37] Biochar is a carbonaceous sorbent material that is produced at relatively low costs by pyrolyzing organic materials like plant biomass at high temperatures (300-800°C) in an oxygen-free environment.[6,37,42,46] The high porosity and surface area, as well as favorable functional groups of biochar allow it to have high remediation capabilities across a broad range of stormwater contaminants.[6,39,42–44,46,47] Biowaste including tree cuttings, distiller grains, rice husks, crop residues, poultry litter, and sewage sludge have been effectively repurposed feedstock to produce biochar.[48] The remediation potential of biochar is highly driven by the biomass feedstock used and the pyrolysis temperature set point.[48] Feedstock with higher lignin, mineral content, and carbon content outputs are likely to be preferred for biochar production.[49] Conducting pyrolysis at higher temperature increases micropore formation and surface area, but greatly impacts nutrient availability (e.g. N, H, and O)[48,49] Faster pyrolysis rates at higher temperatures results in less biochar and more bio-oil biproducts.[4,50] Most biochar production methods involve conducting slow rate, high temperature pyrolysis to produce biochar with greater surface area, accessible nutrients, and aromatic carbon.[50] Notably, because higher temperatures result in greater micropore formation and surface area, there are greater opportunities for contaminant sorption due to the increased storage capacity the pores provide.[37-40]

Biochar has been found to be capable of not only adsorbing organic pollutants, excess nutrients, and trace metals, but also effective at supporting soil structure and fertility.[6,37,41,47,51,52] The nutrient availability of biochar can be leveraged to support plant and microbial growth. Additionally, the porous structure of biochar provides habitats within soils for microorganisms to live and reproduce in.[39,40] Many studies have found that woody, crop, and herbaceous based biochar prepared at higher temperatures tend to have greater cation exchange capacity, in addition to greater surface areas and nutrient availability. These benefits allow for improved soil nutrient retention and pH, which can potentially enhance soil microbial communities and plant health within bioretention systems.[49,53] A review of 22 studies on herbaceous and woody based biochar-enriched soils revealed that biochar reduced soil compaction, increasing water retention, stabilized soil structure, and enhanced the soil microbial community.[54] The hydraulic benefits and improved water retention capacity of biochar-enriched soils can be extremely useful when considering high volume storm events and flood mitigation via biochar-enriched bioretention systems.[54] Additionally, reduced soil compaction and healthy soil microbial communities greatly supports for vegetation growth within these systems.[54,55]

Many studies have found that soils containing biochar are "redox-active"— or the ability to donate, accept, or transfer electrons—facilitating both microbial and abiotic degradation of pollutants (e.g. heavy metals, nutrients, and organic compounds).[56–59] Thus, the benefits of biochar have been leveraged in many bioretention system studies as an amendment to remove chemical and microbial pollutants from urban stormwater runoff. [42] An example of a commonly used commercially available biochar is Mountain Crest Biochar, which is made through the commercial gasification

of conifer softwood at 1100–1400 °C (Mountain Crest Gardens, MCG, GrowPro Inc., California, USA). Ray et al. and Urlich et al. explored the use of MCG biochar for the removal of multiple complex organic contaminants within a synthetic stormwater mixture.[40,51] In a different study, Ray et al. conducted a column experiment using 3 wt% biochar enriched sand to test the removal capacity of seven common organic contaminants at 10 ug/L concentration, within a synthetic stormwater mixture containing organic matter (DOC, 5 mg-C/L).[40] This study observed complete removal of 2,4-dichlorophenoxyacetic acid, tris(2-chloroethyl) phosphate, diuron, fipronil, perfluorooctanoic acid and perfluorooctanesulfonic acid, and 80–100% atrazine removal.[40] Similarly, using 0.2 wt% biochar enriched sand columns, Ulrich et al. observed 70–100% removal of prometon, benzotriazole, atrazine, diuron and tris(3–chloro-2- propyl) phosphate with initial concentrations of 20 ug/L per contaminant within a synthetic stormwater mixture containing organic matter (10 mg-C/L DOC) over a longer study duration.[60] Biochar maintains high removal efficiency across mixed contaminant stormwater systems at a lab scale; however, individual contaminant removal rates are variable and are often influenced by the other contaminants within stormwater runoff.[6,37,40,47,52,60]

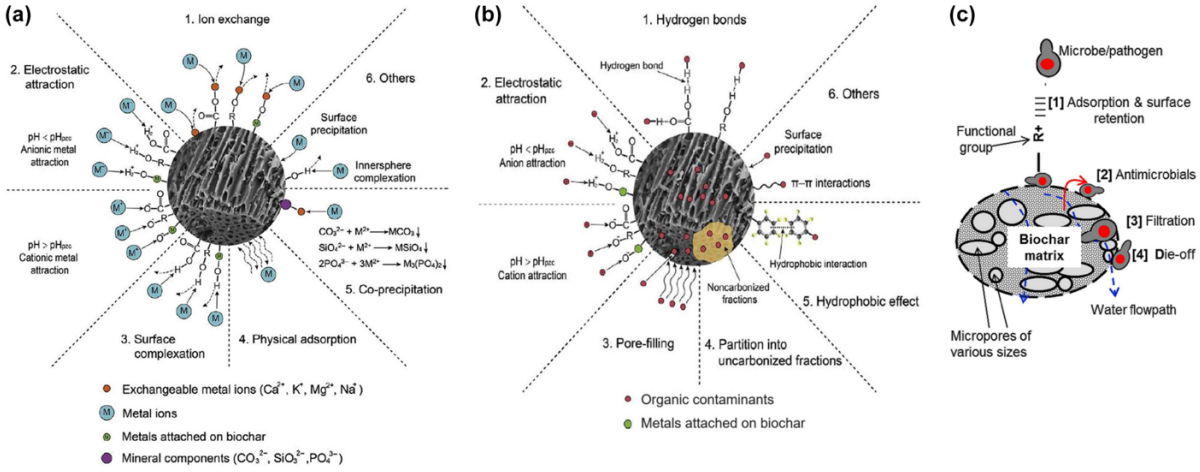


Figure 1.4: Biochar-pollutant interaction mechanisms for the removal of contaminants; (a)biochar-heavy metal interactions (b) organic contaminant-biochar interactions (c)microbe-biochar interactions for the remediation of contaminants within aqueous solutions.[2]

1.5.1. Biochar within Bioretention Systems

It is important to consider varying removal rates among different contaminants and how the stormwater runoff composition (e.g. contaminant particle size, interacting contaminants) can impact removal. For instance, a large concern amongst these studies is how organic matter particulates might hinder the sorption capacity of biochar by blocking sorption sites and reducing biochar surface area. Understanding how biochar influences environmental soils, microbial communities, and stormwater runoff quality can be understood in greater detail through studies more representative of real-world bioretention systems. Therefore, field-based and mesocosm studies of biochar-amended bioretention systems using simulated stormwater mixtures provide detailed insights into the removal efficiency microbial impacts of biochar under environmentally relevant conditions.

A study by Akpinar et al. examined the effects of adding wood-based biochar to bioretention soil media, which typically contain soil, compost, and sand, to improve plant growth, stormwater infiltration, and excess nutrient removal within bioretention systems.[55,61] The study used one-gallon pots to mimic bioretention soil media, layered with high and moderate sand proportions, wood-based biochar (4% w/v), and a sawdust-fines or compost-mulch mix (depending on variable tested).[55,61] All pots were planted with a native perennial often used for bioretention systems, *Pancium virgatum* (switchgrass). They were then kept in a growth chamber simulating day/night cycle and subjected to storms for 20 weeks then a 10-week drought period.[55,61] Bioretention systems have been known to struggle with maintaining plant growth and survival due to the volume of sand incorporated into these systems. This study found that incorporating biochar (4% w/v) into bioretention soil media with high sand content improves plant height and plant drought resilience (e.g., delays wilting under drought conditions).[55] The incorporation of biochar also significantly reduces excess nutrient leaching, which is a common issue for compost/mulch-based bioretention soil media.[55,61] However, this study found that biochar did not improve plant performance (e.g. total plant shoots, total biomass) significantly over compost-mulch bioretention media.[55,61] Further research on how biochar-amended soils can be improved to both remediate contaminants and support vegetation are needed.

Ulrich et al. conducted a mesocosm experiment to compare the removal efficiency of MCG biochar and granular activated carbon (GAC) for trace organic contaminants in stormwater biofilters.[52] Vegetated columns (50 cm long, 15.24 cm diameter) were configured with a planted layer (sand or sand-compost), a sorbent layer (biochar or GAC), and a drainage layer (sand, small gravel, and pea gravel).[52] The filters were watered with synthetic stormwater containing trace organic contaminants over six months, and sand-only filters served as controls.[52] The findings of this study report that the use of biochar is not only more cost effective, but also more efficient at removing trace organic contaminants, likely due to its particle size and density.[52] The biochar amendment (6.7 wt%) maintained greater than 99% trace organic contaminants removal throughout five months of dosing (equivalent to approximately 1.3 years of runoff volume), relative to less than 50% removal of most trace organic contaminants by unamended biofilter columns.[52] Additionally, the study found that biochar amended biofilter columns resulted in improved removal of total organic carbon, total nitrogen, nitrate, and total dissolved phosphorus by greater than 60%.[52] The study emphasizes the need for future studies to explore the fate of retained trace organic contaminants by biochar and pathways for degradation within these systems. [52] The study noted that although leached dissolved organic matter from bioretention media, in addition to nutrients and heavy metals, may foul biochar by reducing its sorption capacity for trace organic contaminants, their interaction with biochar surfaces could potentially enhance electron transfer processes. [52] The immobilization of dissolved organic matter, heavy metals, and nutrients may also support microbial and fungal amendments within bioretention soils, providing an accessible nutrients and energy source.[62] Overall, many studies indicate MCG biochar as a superior choice for future environmentally-relevant studies exploring biochar performance within bioretention systems.[40–42,51,60]

As depicted in **Figure 1.4**, biochar can remove inorganic and organic contaminants via adsorption, pore-filling, precipitation, ion exchange, hydrophobic interactions, electrostatic interactions, surface complexation, and inter-molecular hydrogen bonding.[6,37,39,59,63] These mechanisms are facilitated by the physical and chemical characteristics of the biochar, which are heavily dependent

on the feedstock type, pyrolysis temperature, and chemical modifications of the material during production.[6,63] These properties contribute to immobilization and capture of contaminants, but captured contaminants can remain chemically active within its pore structures and at its surface. Therefore, while biochar can mediate biotic and abiotic degradation of contaminants via electron exchange and conductivity, biochar generally does not degrade or transform contaminants independently without the presence of microbial activity or a reducing agent for example.[43,63,64] Many studies have explored engineered modifications of woody biochar (e.g., chemical modification using oxidants, physical modifications via steam activation during production, surface coating using bacteria)[65] to enhance abiotic and biotic degradation of contaminants.[43,63,65] However, many of these modifications have not been tested over extended periods, pose threats to soil physiochemical properties and biota, and often fail to succeed within environmental applications.[63,65,66] Therefore, there is a need to modify woody, biowaste-based biochar to effectively immobilize and degrade a range of contaminants at mixed concentrations, while also maintaining its physiochemical strengths and environmental benefits.

1.6. Mycoremediation and Fungal Inoculation of Media

To avoid the potential complexities of introducing more contamination via leaching of chemically modified biochar, researchers have explored the potential of modifying biochar using microorganisms.[67] Many commonly occurring, environmental microorganisms have found to be capable of biotransformation and biodegradation of inorganic and organic contaminants into carbon, nutrients, and energy sources. [36,67,68] Select fungi and bacteria have even found to serve as biopesticides against pests and pathogens within soils, reducing pesticide contamination from agricultural runoff and providing nonhazardous alternatives to agricultural methods.[69] Bioremediation via bioaugmentation, or the practice of introducing living organisms to remove pollutants from natural systems, has been highly studied and implemented to sustainably support environmental cleanup efforts.[67,68,70] For example, the introduction of prokaryotes with the capability to perform biotransformation or degradation of contaminants has been found to be a much more cost effective and a less environmentally damaging option in comparison to other common remediation techniques such as soil washing.[70–72] The inoculation, or the introduction of a microorganism into a medium to grow on or within biochar is a new topic of research exploration. This process provides the organisms "microhabitats" for proliferation, increasing opportunities for bioremediation of contaminants. [70–72] Bacteria use the porous structure of biochar as a habitat for growth and biofilm formation, where they attach to biochar surfaces and form a microbial community structure that coats the particulate. Biochar also serves as a nutrient source for these microbial communities, providing carbon and nitrogen for their use.[73] This synergy facilitates transformation of chemical pollutants captured by biochar into energy sources by the microbial community inhabiting the biochar. Bacteria immobilized, or isolated, onto biochar have been found to improve the removal of heavy metals, dyes, benzenes, polyaromatic hydrocarbons, petroleum hydrocarbons, and antibiotics in water and soils.[73]

A common obstacle with bacteria-inoculated biochar amendments is that individual bacterial strains have low mortality rates when applied to large-scale applications with a pre-existing microbial community.[74] Many bacteria are limited to single-contaminant degradation and lose efficiency in mixed-contaminant systems.[73] For instance, in soils contaminated with heavy metals and PAHs, microbial enzymatic activity decreases, which is the mechanism responsible for their capacity to perform degradation.[75] Mixed contaminant systems often lead to reduced microbial

activity and diversity due to selective pressure.[75] Some studies suggest inoculating a bacterial consortium, or a symbiotic bacterial group, for mixed-contaminant remediation; however, successful remediation using bacteria is highly dependent on biochar nutrient content, contaminant concentrations, and environmental conditions (e.g. aeration, temperature, moisture content).[64,73,76] Additionally, many studies on bacteria-inoculated biochar have found that the combination of persistent free radicals in biochar interacting with biodegradable hydrophilic contaminants (e.g. phenols, PAHs, and organic acids) can trigger "microbial cytotoxic effects," which inhibit microorganisms bonding to or trapping in biochar.[64,73,76,77] Bacterial bioremediation is more successful in mixed contaminant, environmental systems when combined with phytoremediation, or the use of plants to remove, immobilize, or reduce the toxicity of environmental contaminants.[73,74,76,78]

Due to the complications presented by bacteria-based remediation, myco-remediation poses as a much more effective solution to stormwater remediation. Myco-remediation, or the removal of environmental pollutants using fungi, leverages the robustness of fungi, their tolerance to harsh environmental conditions, and their ability to produce various enzymes to break down a range of pollutants.[79] Fungi are chemoheterotrophic organisms, meaning they can produce a range of both intracellular and extracellular enzymes to perform decomposition.[79] This metabolic function allows them to break down both organic and inorganic pollutants such as PAHs, pesticides, chlorophenols, lead, cadmium, and copper.[80–82] Fungi are also unique in their low energy source specificity, which allows them to convert a wide range of compounds into carbon and energy sources because their catabolic enzymes are not highly selective when performing degradation.[80,81] Within soils, many fungal species form mutualistic relationships with plants, using their extensive mycelium, or far-reaching network of fungal filaments, to access and deliver nutrients to plant roots. In exchange, plant roots provide sucrose and a surface for fungal colonization.[83] The presence of diverse fungal communities within soils are also attributed to promoting carbon and nutrient cycling, carbon sequestration, and plant health,.[84]

Existing studies have explored amending soils with plant growth-promoting fungi (such as *Aspergillus, Penicillium, and Trichoderma*) and biochar due to their ability to promote plant resilience and degradation of pollutants such as dyes, heavy metals, and organic pollutants (e.g. acid fuschin and synthetic dyes, lead and cadmium[85], organochlorine and organophosphorus pesticides[86]).[87] Like bacteria, plant growth-promoting fungi have been found to successfully colonize and form habitats within biochar, while also using its surface as a nutrient source.[88,89] The fungal species *Trichoderma* has proved to be a favorable species for myco-remediation efforts because it not only serves as a remediation agent, but also as a biopesticide for plant pathogens, and as a biofertilizer.[90] Multiple studies highlight *Trichoderma sp.* as an effective fungi for myco-remediation of highly complex contaminants in water, soil, and marine environments using their ability to adapt to a range of environmental conditions and habitats, while contributing to plant and soil health.[85,91 91,92] Unlike many bacteria, *Trichoderma sp.* has also been found to have a high tolerance of contaminated and nutrient-limited soils.[93] Specifically, the environmentally ubiquitous plant growth-promoting fungi *Trichoderma harzianum* has been found to degrade heavy metals, pharmaceuticals, diesel fuel, and various polycyclic aromatic hydrocarbons (PAHs).[91,92] In agricultural applications crop seedlings inoculated with *Trichoderma harzianum* have led to improved resiliency against salinity, drought, temperature-related stress in addition to pathogen and parasite resistance. [93]

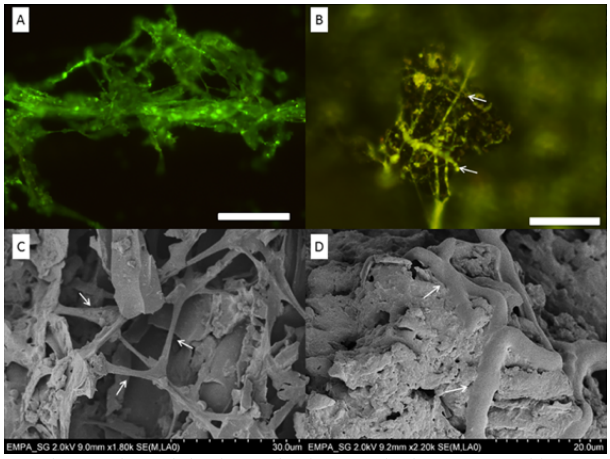


Figure 1.5: Colonization of biochar substrate by Trichoderma harizanum is demonstrated in A & B after genetic tagging with green fluorescent proteins (to study fungal degradation methods against contaminants (100 µm scale bar). Images (C)& (D) Show non-tagged *Trichoderma harizanum* colonization of biochar using a (C) 30 µm and (D) 20 µm scale bar. Arrows point towards hyphae of *TH*.[3]

Limited studies have been published on the remediation efficiency of biochar (BC) inoculated with *Trichoderma harzianum (TH)* (so-called "*TH@BC*"); however, studies exploring the incorporation of fungi and biochar for soil and wastewater remediation thus far are promising. [85,87,94,95] Existing studies have found the combined effects of biochar and *T. harzianum* have been effective in remediating heavy metals within soils.[85,87,94,95] One study found that the incorporation of biochar reduced the lead within soils and decreased lead uptake by plants, while *T. harzianum* improved lead removal, plant growth, and reduced absorption in plant tissues.[94] Another study found that biochar was capable of immobilizing zinc (Zn) and lead (Pb), while increasing nutrient availability.[96,97] In this study, *T. harzianum* enhanced plant growth and reduced heavy metal toxicity. [96,97] It was found that the incorporation of *T. harzianum* along with biochar within contaminated soils encouraged Zn retention within plant roots but reduced the translocation of Zn to its roots.[96,97] Furthermore, the combined amendments were found to improve plant performance within polluted soils, alleviate oxidative stress, and reduce heavy metal uptake, making the combination effective for bioremediation and crop production in contaminated environments. [96,97]

The influence of biochar and the fungal strains *Trichoderma harizanum,* and *Bacillus subtilis* on soybean growth in soils contaminated with cadmium (Cd) was evaluated through a pot study experiment.[98] The combined application of *Trichoderma harizanum* and *Bacillus subtilis* along with biochar was more effective than the fungi-only systems and the biochar-only systems. [98] Together, the collective amendments reduced Cd bioavailability, improved photosynthetic activity and growth of soybeans, and improved soil properties (e.g. pH, aeration, water retention, improved microbial productivity and population composition). [98] These studies explored the incorporation the microorganisms into soils, as opposed to inoculation onto biochar, which leaves much room for the exploration of biochar-fungal interactions as well as fungal proliferation within soils when established on biochar. For instance, a study in which biochar was inoculated with arbuscular mycorrhizal (AM) fungi can grow on and within biochar, accessing pore spaces within biochar that are too small for plant roots to access.[88] AM fungi found biochar to be an attractive substrate, or a substance that fungi can grow within as well as decompose into a nutrient source, especially in nutrient-limited soils. [88] Additionally, this study found that loading biochar with phosphorus

encouraged fungal colonization and allowed for AM fungi to uptake phosphorus and translocate it to plant roots it formed symbiosis with within the experiment.[88] The translocation of phosphorus to plant roots from the fungi improved six-fold in comparison to systems where fungal hyphal contact with the biochar was prevented using mesh.[88] Plant root access to immobilized contaminants sorbed within biochar micropores can potentially play an important role in phytoremediation (remediation via plants). Exploring microbial and plant degradation of stormwater contaminants may hold opportunities for regeneration of biochar, as immobilized contaminants may be used as a nutrient source for select fungi and plants.

Previous studies have highlighted the many benefits of incorporating plant-growth promoting fungi into soils due to symbiotic interactions with biochar, increased contaminant removal, improved soil health, and enhanced plant growth.[83,88,93,95] Understanding the remediation potential of biochar inoculated with a commonly occurring, plant-growth promoting fungi like *Trichoderma harzianum* at a larger scale under environmentally-relevant conditions could improve the future of stormwater runoff remediation.[90] Furthermore, the combined amendment of *TH@BC* can likely support plants capable of remediating pollutants for further removal of stormwater contaminants.

1.7. Enhanced Phytoremediation via Fungi-Inoculated Biochar Soil Amendments

Fungi-inoculated biochar has been identified as a method to support plant growth and plant resilience amid environmental stressors, though this research is limited. [88,89,99] The strengths of these amendments within soils together can enhance plant biomass yield and resilience, signaling an opportunity to improve remediation using plants. [85,87,94–99] Phytoremediation is a commonly used approach for environmental remediation, in which plants are used to degrade, immobilize, or uptake pollutants such as heavy metals, trace organics and excess from the environment.[100] Many existing bioretention systems use plants that perform phytoremediation to support the filtration of stormwater runoff, reduction evapotranspiration of contaminants from runoff, and mitigation of potential flooding events.[101] The collection and slow release of stormwater runoff within these systems provides plants the opportunity to uptake or degrade contaminants overtime. Additionally, many plants roots carry rhizomicrobiomes (or root microbial communities) and root exudates which support microbial biodegradation of contaminants.[100,101]

The mechanism by which a plant conducts phytoremediation and its efficiency to remove a select pollutant is highly dependent on the plant species.[101,102] Plants have been used for the remediation of an array of pollutants including heavy metals, nutrients, surfactants, and organic compounds like PCBs, PAHs, and chlorinated solvents (**Figure 1.6**).[100,103] The five general phytoremediation techniques plants can include: (1) absorption of contaminants from water by plant roots (i.e., rhizofiltration); (2) uptake of contaminants from the soil by plant roots (i.e., phytoextraction); (3) degradation of contaminants in soil or water by plants' metabolism (i.e., phytotransformation); (4) plant stimulation of microbial contaminant degradation of contaminants in the root zone within soil or water (i.e., phyto-stimulation); and, (5) reduction of contaminant mobility in soils by plant roots (i.e., phytostabilization).[100] The complications of relying on phytoremediation are three-fold: i) slow plant growth processes limit runoff remediation efficiency; ii) often, plant roots are too shallow to access the stormwater runoff contaminants; and iii) the relocation of contaminants into plant biomass as opposed to degradation is unfavorable due to future ecological health threats and potential for re-release of absorbed contaminants.[103]

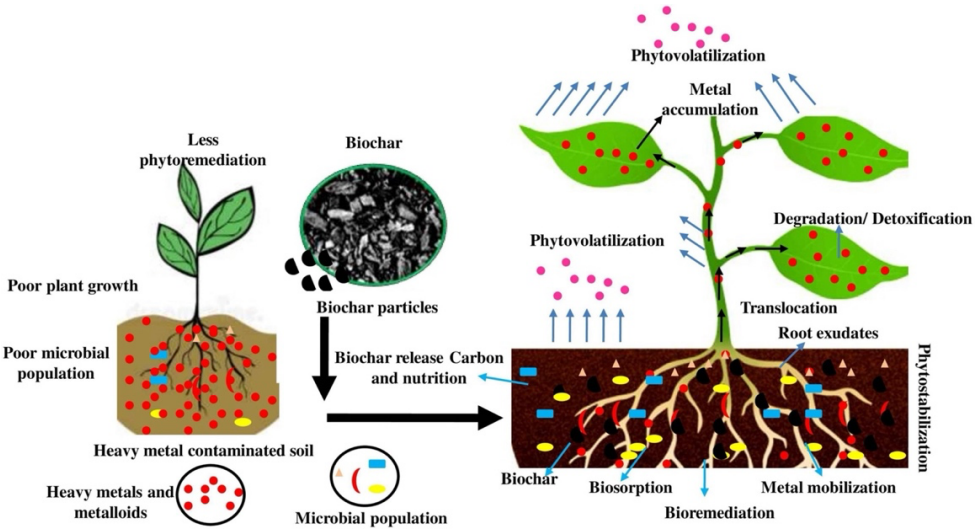


Figure 1.6: Graphical depiction of the benefits biochar has on phytoremediation and soil microbial communities. Improved soil conditions lead to enhanced bioremediation and phytoremediation of heavy metal contaminated soils.[4]

Fungus-inoculated biochar holds great potential to address these complications and support sustainable, cost-effective remediation of stormwater runoff within bioretention system. Yao et al. investigated how *Trichoderma harzianum* and biochar influence the phytoremediation ability of *Brassica juncea* (mustard plant). Under this combined treatment, biochar was found to support the proliferation and stability of beneficial soil microbes, aid the transformation of contaminants into bioavailable forms for phytoremediation, and improve soil quality (e.g. cation exchange capacity, organic matter content, available nutrients) resulting in enhanced contaminant removal.[87] Biochar was found to boost biomass production of *Brassica juncea*, while *T. harzianum* aided the activity of antioxidant enzymes and facilitated contaminant transport within the plant.[87] In a subsequent study (Yao et al. 2024), investigated soil amendments to enhance phytoremediation of infiltration systems contaminated by arsenic and cadmium using *Miscanthus sinensis* (perennial grass). Researchers found that the incorporation of the endophytic fungi *T. harzianum* and *Paecilomyces lilacinus* (applied to soil) along with biochar enhances phytoremediation in *Miscanthus sinensis.* This combined treatment also enriched the soil microbial community, and it shifted the reliance of phytoremediation on fungal diversity and soil nutrients to bacterial diversity and available contaminants. *T. harzianum* was found to stimulate *Miscanthus sinensis* production of organic acids that allow its root system to transform and adsorb contaminants within the soil. [87,104] Futhermore, *T. harzianum* was found to support remediation at greater soil depths, as their extensive hyphal network has been found to reach up to a meter below the soil surface. [103,105] Fungal inoculum can transport contaminants to plant root systems, allowing plants to access depths they otherwise couldn't.[104] These studies confirmed that the dual amendment supported the remediation of toxic elements and improved soil quality at deeper soil levels (below root growth region). [87,104]

Another study exploring the remediation of metals (Cu, Mn, and Zn) within soils deployed a consortium of fungal isolates (*Beauveria bassiana, Metarhizium anisopliae, Pochonia chlamydosporia, Purpureocillium lilacinum,* and *Trichoderma asperella*) applied to plant roots and biochar to improve the phytoremediation potential of plant species *Jacaranda mimosifolia.*[106] The study concluded that the fungal consortium paired with biochar significantly supported the translocation (from plant roots to plant shoots) and accumulation of heavy metals within the plant

across contaminants and increased plant root and shoot mass. [106] Additionally, the study determined that biochar significantly reduced leaching of the contaminants which indicates that the fungal consortium and biochar treatment could be used to decrease the risks of groundwater contamination.[106]

The incorporation of biochar has been found to positively affect fungal hyphae length, plant root length, plant available water, and hydraulic conductivity within conventional bioretention soil media.[85,87,94–99] Despite fungal growth improvements observed by biochar amendments within soils, their co-application has been limited to agricultural contexts and heavy metal pollution, as opposed to the stormwater runoff remediation. [85,87,94–99] There is very limited research on how fungus and biochar impact phytoremediation of aqueous contaminants, and the plants examined within existing studies are very limited to herbaceous and agricultural plant species.[100] Depending on the plant species tested, their ability to perform phytoremediation may also be deterred by their tolerance to organic and inorganic contaminants.[107] Because wetlands are buffer systems known to naturally process and remove contaminants, their plants have been identified as phytoremediation contenders for bioretention systems.[108] For instance, many species within the halophyte *Juncus* genus have been highly identified as capable of performing phytoremediation of organic pollutants and heavy metals in soils, wetlands, and wastewater runoff via phytoextraction and phytostabilization.[109] The *Juncus* genus is also known for its fast growth rate, water flow rate management abilities, and their tolerance under environmental stressors (contaminated soils, drought). *Juncus effuses,* for example, has been found to not only tolerate, but also remove common stormwater runoff contaminants including trimethoprim, metoprolol, benzotriazole, carbamazepine, caffeine, propranolol, sulfamethoxazole, furosemide, mecoprop, and diclofenac, and irbesartan.[110] The species *Juncus patens*, which is native to the western coast of the United States, has not been assessed within these contexts. Its versatility and presence across environmental systems shows promising potential for tolerance of contaminated systems and may serve as an effective remediation approach within stormwater BMPs. Furthermore, studies on the efficiency of constructed wetlands find better treatment performance when native plants are tested for phytoremediation due to their pre-existing tolerance to the existing climate and soil microbial community.[107]

1.8. Maximizing remediation within Bioretention Systems

Previous studies have shown that biochar, myco-remediation, and phytoremediation serve as sustainable, effective mechanisms to remediate contaminated systems. Moreover, as emerging contaminants becoming increasingly present within stormwater runoff, it is important to explore runoff remediation solutions that are cost-effective, reproducible, and time efficient. There is very little research on the synergy between these three amendments beyond the remediation of heavy metal-contaminated soils.[87] Therefore, there is a need for research that explores the remediation of contaminated runoff using phytoremediation supported by fungus-inoculated biochar. We seek to leverage biochar as a reaction center to simultaneously facilitate myco-remediation using MCG biochar inoculated by *Trichoderma harzianum*, and promote transport of sorbed pollutants to plants capable of phytoremediation via fungal hyphae networks.[88] As known degraders of wood and other cellulose-based materials, the fungal inoculum holds the potential to decompose contaminants sorbed onto biochar as well as use the media as an energy source.[80] Studies surrounding this remediation relationship have not used biochar as a growth substrate for fungal

inoculum for the remediation of mixed contaminant runoff. Additionally, exploring the potential contaminant remediation benefits of a common rush native to the West Coast of the Unites States, *Juncus patens*, provides opportunities for regionally applicable amendments to bioretention systems.[111] Thus, we will explore how fungus-inoculated biochar influences phytoremediation as well as contaminant removal within environmentally relevant conditions to accurately assess their remediation capacity of stormwater runoff within bioretention systems.

2. Objectives and Hypotheses

The **overall objective** of this thesis is to assess how bioaugmented biochar can impact contaminant removal in stormwater runoff through laboratory and greenhouse scale studies. The **first** objective of this thesis is to assess differences in stormwater runoff contaminant removal of Mountain Crest Gardens (MCG) biochar and *Trichoderma harzianum* inoculated MCG (*TH@MCG*) through batch sorption isotherm studies. This first objective seeks to evaluate the removal capacity of both media within a synthetic stormwater matrix containing multiple trace organic stormwater runoff pollutants. We hypothesize that *T. harzianum*-inoculated biochar will improve removal efficiency of MCG biochar across contaminants within a synthetic stormwater matrix.

The **second objective** of this thesis is to determine whether the native wetland grass *Juncus patens* is capable of phytoremediation, and if so, whether *T. harzianum*-inoculated biochar enhances the phytoremediation capacity of the *Juncus patens*. To evaluate trace organic removal during infiltration performed by *T. harzianum*-inoculated biochar and how *Juncus patens* impacts remediation, we performed a mesocosm greenhouse pot study that mimics stormwater biofiltration systems. We hypothesize that *Juncus patens* will improve removal efficiency of contaminants and *T. harzianum*-inoculated biochar will improve plant health and growth.

Past studies have found that *Trichoderma harzianum* can degrade organic pollutants such as pharmaceuticals and personal care products within wastewater systems, as well as PAHs, heavy metals, and pesticides within contaminated soils.[79,87,112] Therefore, we hypothesize that inoculating biochar with this fungus will not only promote contaminant adsorption onto the biochar, but also support its hyphal network expansion and consequently, contaminant removal via mycoremediation. In a previous study, researchers amended heavy metal contaminated soils with biochar and plant seeds capable of phytoremediation, and the soils were then sprayed with *Trichoderma harzianum* as a microbial treatment.[52] Their study concluded that *Trichoderma harzianum* and biochar enhanced phytoremediation in addition to improving the microbial community and soil quality.[87] Thus, we hypothesize that *T. harzianum*-inoculated biochar will support phytoremediation in reaction systems containing *Juncus patens*. By incorporating these three amendments into greenhouse biofiltration systems, we plan to systematically investigate the individual and synergistic stormwater treatment capabilities to ultimately identify a solution to mitigate the negative environmental impacts of polluted stormwater runoff.

3. Methods and Materials

To evaluate the efficiency of fungus-inoculated biochar for contaminant removal in stormwater runoff, this study is divided into two main components: (1) laboratory batch isotherm studies and (2) a greenhouse mesocosm pot experiment. The batch isotherm studies were conducted to compare the removal efficiency of Mountain Crest Gardens (MCG) biochar (Mountain Crest Gardens, GrowPro Inc., California, USA, 351 m^2/g surface area) with and without *Trichoderma harzianum* inoculation. These tests were performed using a synthetic stormwater matrix with three representative trace organic contaminants at concentrations ranging from 25 µg/L to 5000 µg/L. The second component involved a greenhouse mesocosm experiment designed to simulate bioretention systems, where we assessed the contaminant removal efficiency of MCG biochar, *Trichoderma harzianum* inoculated MCG (*TH@*MCG), and *Trichoderma harzianum* alone. Each pot in the mesocosm study was layered with sand, gravel, and soil to replicate existing bioretention systems and was irrigated weekly with synthetic stormwater containing environmentally relevant contaminant concentrations to mimic storm events.

Material Preparation and Laboratory Experimentation

3.1. Fungal Strain Selection *Trichoderma harzianum (TH)* and Growth

The fungal strain *Trichoderma harzianum Rifai* (ATCC 60850) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), and packaged in a cryovial containing lyophilized spores. The fungus was then cultivated and maintained on Potato Dextrose Agar (PDA) plates (1.5% w/v agar) at 20 C. Prior to the subsequent tests, a fresh transfer of *T. harzianum* to PDA took place, and the fungus was grown for a a total of five days prior to use in batch and greenhouse studies. The biochar tubes were then inoculated with T. harzianum mycelia and spores under aseptic conditions. The tubes were incubated for five days at 20 C and biochar was observed for successful fungal establishment under a stereomicroscope (Fig.4.2).

3.2. Preparation and Characterization of Biochar

3.2.1. Material Uniformity and Cleaning

Mountain Crest Gardens (MCG) biochar is commercially produced from the waste byproduct of mixed softwood (high Ponderosa pine content) using a combustion energy generation at 1400 °C in a downdraft (Mountain Crest Gardens, GrowPro Inc., California, USA, 351 m^2/g surface area).[40] Upon receipt, the MCG biochar was ground using a coffee grinder and sieved to 595 µm (NO. 30) to 297 µm (NO. 50) to achieve uniform particle size. It was then rinsed with deionized water, dried at 90°C overnight in a VWR 1500E incubator (VWR International, Radnor, PA), and stored in a sealed bottle to prevent contamination.

3.2.2. MCG Biochar Characterization

Proximate carbon analysis, zeta potential analysis, and BET surface area were previously reported in another study assessing MCG biochar treatment for PFAS removal.[44] Images of the MCG biochar were captured using scanning electron microscopy (SEM, ThermoFisher Scientific, Waltham, MA Apreo VP).

3.2.3. Fungal Inoculation of Biochar (for Batch Isotherm Experiments)

20 mg of MCG was measured into 50 mL centrifuge tubes to prepare for the batch sorption isotherm tests. These tubes then received two spores and three gentle scrapes of mycelia from the plates were added onto MCG biochar using sterile loops. In addition, X amount of PDA was added to enhance establishment of *TH* onto the biochar. After five days, X amount of PDA was added into the centrifuge tubes to support further fungal establishment. In total, the *TH* was given seven days to establish onto the MCG prior to experimentation.

3.3. Stormwater Mixture

All chemicals used were certified ACS reagent grade or equivalent unless otherwise noted. Synthetic stormwater (SSW) was produced to mimic the composition of real stormwater. This mixture was used in batch tests as well as the greenhouse pot experiments. Stormwater constituents and concentrations were referenced from Okaikue-Woodi et al. 2020. (Table 3.1).

Table 3.1 Synthetic stormwater composition used to mimic the cations and anions present in real stormwater.[6] Suwanee River natural organic matter (SRNOM) was added (10 mg-C/L concentration) in addition to the synthetic stormwater matrix in select experiments.

Constituent	Concentration (mM)
Ca^{2+}	0.75
Mg^{2+}	0.075
Na^{+}	1.932
SO_4^{2-}	0.33
HCO_3^{-}	1.00
Cl^{-}	1.65
NO_3^{-}	0.256
$H_2PO_4^{-}$	0.016
SRNOM	10 mg-C/L concentration

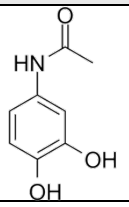
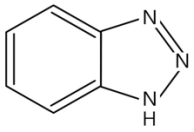
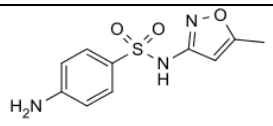
The cation stock solution was prepared with calcium chloride ($CaCl_2$, Sigma Aldrich, MO) and magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, Thermo Scientific, MA) in ultrapure water at concentrations 100x higher than in the final mixture. The anion stock solution was prepared separately with sodium sulfate (Na_2SO_4, Thermo Scientific, MA), sodium bicarbonate ($NaHCO_3$, Fisher Chemical, MA), sodium nitrate ($NaNO_3$, Thermo Scientific, MA), and sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$, Fisher Chemical, MA) in ultrapure water at concentrations 100x as well. While stormwater runoff commonly includes ammonium, NH_4^+ [6,40,113], it was excluded from our synthetic stormwater mixture to the limit accumulation of an additional variable that may influence the microbial community within experiments. For example, high concentrations of ammonium have been found to change microbial community structure [114–116], inhibit fungal growth[117,118], and decrease microbial enzymatic activity in soils[119]. Suwannee River natural organic matter (SRNOM, 1R10N, International Humic Substances Society, St. Paul, MN) was

added to the SSW mixture for sorption isotherm studies to represent naturally organic matter within stormwater at the concentration of 10 mg-C/L.[6,40,113]

3.3.1. Organic Compounds

All batch sorption and greenhouse pot studies received Acetaminophen (ACT, Spectrum Chemical MFG Corp, NJ), benzotriazole (BZT, TCI America, OR), and sulfamethoxazole (SMX, TCI America, OR), and all organic compounds used were certified ACS reagent grade. These pharmaceutical compounds were selected as representative trace organics as they are commonly detected in stormwater. Their chemical properties and characteristics are reported in Table 3.2. Concentrations of the three compounds of interest in Table 3.2 within batch test and pot effluent samples as well as the presence of transformation products were assessed using High Performance Liquid Chromatography (HPLC) equipped with a UV diode array detector (Instrument details in Section 3.8).

Table 3.2: Characteristics and Properties of Study Contaminants

Trace Organic Compound	Structure	MW (g/mol)	Log K_{ow}	Sources	Typical Environmental Concentrations
Acetaminophen, ACT		151.16 g/mol	0.46	Analgesic drug	7.9–85.0 ng/L[120]
Benzotriazole, BZT		119.13 g/mol	1.44	Anti-corrosion agent, UV inhibitor	<0.2–8529.8 $ng \cdot dm^{-3}$ (surface water)[121]
Sulfamethoxazole, SMX		253.27 g/mol	0.89	Anti-microbial drug	440 $\mu g \cdot dm^{-3}$ (drinking water) [121]

3.4. Batch Sorption Isotherm Experiment

3.4.1. MCG Batch Sorption Isotherm Tests

Two 120-hr batch sorption isotherm tests were performed using only MCG biochar and *TH@*MCG biochar to determine the maximum sorption capacity of each contaminant onto the media. The tests were performed in the SSW mixture (see Table 3.1). To assess the adsorption capacity of the MCG biochar for the select trace organics, batch sorption isotherm experiments were conducted using 100 mg/L of sieved, washed, and dried MCG biochar in 50 mL centrifuge tubes at the initial concentrations specified in Table 3.4. Each concentration was prepared in triplicate and equilibrated over five days using a Fisherbrand Multi-Purpose Tube Rotator (Fisher Scientific, Waltham, MA) at 40 rpm. Control experiments were also performed in duplicate in the absence of

MCG biochar to account for losses of trace organics during the experiment. The isotherm test was repeated using the same synthetic stormwater matrix in the absence of SRNOM to understand the effect of dissolved organic matter on the removal of trace organics in the reaction system and to assess contaminant partitioning to the dissolved SRNOM. After five days, samples from the tests are filtered using 0.22 µm cellulose acetate syringe filters (Thermo Scientific,Waltham, MA) and analyzed on the HPLC-UV.

3.4.2. TH@MCG Batch Sorption Isotherm Tests

The batch isotherm test was also conducted with MCG inoculated with *Trichoderma harzianum* (i.e., *TH*@MCG) with an adjusted experimental set up to maintain biological activity. MCG was coated as described and allowed to establish for seven days prior to beginning the batch test. 500 mL Erlenmeyer flasks were used for each batch sorption number, and the flasks were sealed with parafilm and stirred on a shaker table during the five-day equilibration period. Samples were taken in triplicate from each flask, filtered using 0.22 µm cellulose acetate syringe filters as well.

Table 3.4: Batch Isotherm sorption test parameters, using MCG and *TH*@MCG (*TH* inoculated MCG) within the synthetic stormwater matrix

Test Number	Treatment System	SRNOM (10 mg-C/L)	Trace Organics	Initial Concentration (µg/L)
1*	MCG	Yes	BZT, ACT, SMX (mixed) BZT, ACT, SMX (individual)	25
				50
				250
				500
				2000
				5000
2*	MCG	No	BZT, ACT, SMX (mixed) BZT, ACT, SMX (individual)	25
				50
				250
				500
				2000
				5000
3	TH@MCG	Yes	BZT, ACT, SMX (mixed)	25
				50
				250
				500
				2000
				5000
4	TH@MCG	No	BZT, ACT, SMX (mixed)	25
				50
				250
				500
				2000
				5000

*****= control experiment was repeated under the same experimental conditions in the absence of the SRNOM to evaluate the sorption capacity of the organic matter included in our experiments.
"**Mixed**" indicates the experiment was conducted with all three contaminants at the respective concentration.
"**Individual**" indicates the experiment was repeated for each contaminant individually.

Greenhouse Mesocosm Study Design and Pot Preparation

Greenhouse mesocosm studies were conducted to observe removal efficiency of the three select trace organic contaminants with and without a plant host present. Conducting a greenhouse mesocosm study allows for greater statistical significance as a result of replicated experiments while maintaining defining biological complexities and characteristics of natural systems that lab-scale experiments often exclude. [122,123] Many studies have found that plants and bacteria aid the removal and degradation of organic contaminants in bioretention cells; however, there is a large knowledge gap around the role fungi play and their community structure within in bioretention systems.[124] Very few studies have investigated the remediation capability of fungi and biochar together as bioretention amendments at larger scales; therefore, this study aimed to understand the capacity of *TH@*MCG in a more environmentally-relevant system as well as how it influences plants with phytoremediation potential.[125]

Greenhouse pot studies were conducted within the University of Washington Life Sciences Building Biology Greenhouse, which is temperature, humidity, and lighting controlled to mimic suitable environmental conditions. The study spanned 19 weeks to assess the remediation abilities of MCG and/or *Trichoderma harzianum*-innoculated MCG and its potential to support phytoremediation. Assembled pots were randomly arranged on a single bench within the greenhouse prior to beginning the study to avoid watering and lighting proximity biases (Figure 3.1). The bench was lightly watered daily by the overhead misting system within the greenhouse, and pots were watered with synthetic stormwater once a week using deionized water sourced from the greenhouse.

3.5. Pot Assembly

One-gallon pots were lined with cheese cloth mesh (Cotton Farm from Mediterranean, Acacia Trading LLC, Atlanta, GA) to prevent loss of sorbents and media from drainage holes. Subsequently, the bioretention layers, as pictured in Figure 3.1, were added as follows (from bottom to top): 383 grams of coarse gravel (Sky Nursery, Shoreline, WA), 530 grams coarse gravel (Sky Nursery, Shoreline, WA), 402 grams sand (Sky Nursery, Shoreline, WA), 1,858 grams of soil (natural soil collected from the Center of Urban Horticulture at the University of Washington, Seattle, WA), and an inch of headspace was allotted for water. Mass values were determined by dividing the pot height into sections based on typical bioretention system layering (vegetation layer, soil, coarse sand, and gravel). [1,126] The pot configurations were prepared in triplicate (**Fig 3.1**). For pots receiving MCG or *TH@MCG*, approximately 9.3 grams (or 0.5% wt) of the media was mixed into the soil and then layered into the pot. To collect and image samples of MCG within the soils after the study concluded, 500 mg of the allotted 9.3 grams of media per pot were added in 2.36 x 2.75-inch nylon tea baggies (Insiswiner, distributed through Amazon) and mixed within the soil during pot assembly.

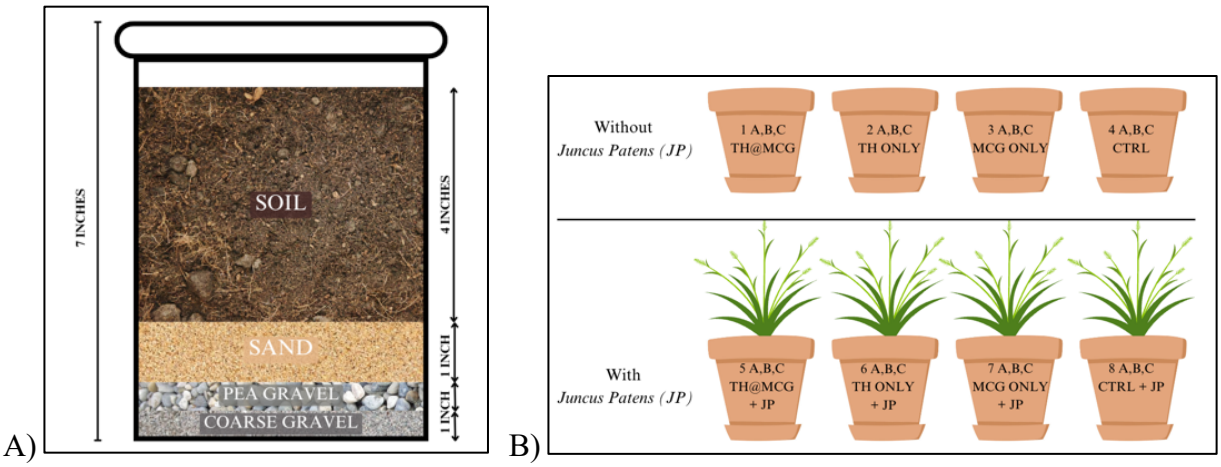


Figure 3.1: A) Diagram depicts the physical composition of each pot B) One gallon pot configuration completed in triplicates to explore each variable's impact and mimic a bioretention system composition (24 pots total). C) Pots randomly arranged on single greenhouse bench to avoid bias when watering.

3.5.1. Plant Introduction and Characterization

As shown in Figure 3.1, each variable was tested within two sets of triplicates, one with plants and one without plants. Plant pots received single 2" plant plug of *Juncus patens* (hereafter, *JP*; Go Natives! Nursery, Shoreline, WA) that were kept in a shaded area and bottom-watered prior to purchasing. Plants were watered on alternate days with deionized water only two weeks to account for potential failure to establish within the soil after being transplanted into pots. Average initial heights of the plants were collected, and heights were recorded after 10 and 19 weeks. Plant yellowing and observational changes in plant health were also recorded at weeks 10 and 19 of the greenhouse study.

3.5.2. Fungal Inoculation of MCG and T.H. Pot Soils

To prepare the soil amendments, 9.3 grams of MCG were measured out and divided into two Petri dishes with 232.5 µl of potato dextrose broth. For the THBC treatment, PDA agar plugs (0.4 mm in diameter) of T. harzianum spores and mycelium were place into each of the Petri dishes containing biochar. The plates were parafilmed and incubated at 20 C for five days prior to incorporation into experimental soils. The five day period was selected to keep consistent with isotherm batch incubations, and in both experimental cases, was observed to be a sufficient amount of time for the fungus to proliferate on the biochars. Pots amended with *Trichoderma harizanum* only received 15 mL of liquid culture of the fungus, and the liquid culture was uniformly distributed onto the soil surface as it was layered into the pots.

Approximately 9.3 grams of MCG were measured out and divided into two petri dishes. After five days of for fungal cultivation, two spores and three gentle scrapes of mycelia from the agar plates were added onto MCG biochar using sterile loops. An approximate 0.5-inch segment of agar from the fungal plates were also added into MCG dishes. The MCG was gently mixed, and X amount of PDA was added onto the biochar to support fungal establishment. The plates were sealed and refrigerated for another five days prior to incorporation into experimental soils. Pots amended with *Trichoderma harizanum* only received 15 mL of liquid culture of the fungus, and the liquid culture was uniformly distributed onto the soil surface as it was layered into the pots.

3.6. Pot Sampling

3.6.1. Watering, Sample Collection, and Analysis Preparation (Weeks 1-10)

Once *J. patens* plugs were transplanted into the test pots, they were allowed a two-week acclimation period post transplanting. During this period, they were watered every two days. After this period, all pots were watered once a week with the synthetic stormwater spiked with 100 µg/L of each contaminant. A new 14.5-liter stock of synthetic water was prepared prior to watering each week, and each pot received 600 mL of the stormwater mixture each week during watering. Leachate samples were collected from the plastic plant saucers beneath each pot within 30 minutes of watering. A 15-ml leachate sample from each pot was filtered using a 0.2 µm cellulose acetate filters (Thermo Fischer Scientific, Waltham, MA) to remove debris prior to analysis.

To measure leachate samples for trace organic analysis, all 1-mL HPLC vials were prepared by incorporating 675 µL of effluent sample and 25 µL of HPLC-grade methanol (Avantor, Inc., Radnor, Pennsylvania). Additionally, 250 ug/L spike (100uL) of each of the three organic contaminants was also incorporated into each sample vial because sample concentrations were consistently below the detection limit of the instrument. Total organic carbon (TOC) concentrations of the effluent samples were measured bi-monthly using the Shimadzu (Kyoto, Japan) TOC-L analyzer, in which TOC vials received 1mL of filtered sample and 8 mL of ultrapure water for each pot for analysis. pH measurements were also collected from filtered effluent samples bi-monthly using Thermo Scientific™ Orion™ Star A211 pH Benchtop Meter (Thermo Fischer Scientific, Waltham, MA).

3.6.2. Watering, Sample Collection, and Analysis Preparation (Weeks 10 - end)

Due to the increasing amount of organic matter within effluent samples following week 9, 15-mL effluent samples were collected without filtration, and were immediately centrifuged for one hour at 4150 rpm using Thermo Scientific Megafuge 16R Centrifuge (Thermo Fischer Scientific, Waltham, MA). At week 10, we increased the initial concentration of each contaminant to 10 mg/L to accelerate reaching the breakthrough point of the study. After centrifugation, samples were filtered with 0.22 µm cellulose acetate syringe filters and stored in the refrigerator for later analysis. Following the concentration increase, HPLC samples were diluted (1:10 sample:ultra-purewater) to maintain sample concentrations within the instrument limit of detection. All HPLC vials of pot effluent samples after week 9 received 97.5 µL of pot effluent sample, 25 µL of HPLC-grade methanol, and 877.5 µL of ultrapure water.

3.7. Pot Destruction and Sample Analysis

After 19 weeks of watering the pots with SSW, the experiment was destructively harvested to take soil samples, measure plant and root biomass, and note general observations on plant health. J. patens above-ground biomass was cut to soil level, rinsed, labelled, and dried at 60°C for 72 hours to determine dry plant weight. The soil was then removed from the pot and collected from within the compacted soil mass for analysis. Plant roots were separated from the compacted soils and washed with deionized water, then dried and stored for future analysis as well. The submerged mesh baggies were extracted and frozen at 0°C in plastic bags for scanning electron microscope

(SEM) imaging. Prior to analysis, samples with the same experimental system were combined (triplicates were condensed into a single sample), and dried for imaging post-study.

3.7.1. Plant Root and Shoot Extraction

Following pot deconstruction, plant roots were rinsed carefully with water, cleared of soil before air drying at room temperature for 48 hours, then oven dried for 72 hours at 60 °C. Plant shoots were dried for 48 hours at 60 °C in the same oven, shaken to remove remaining dirt, and weighed. After plant roots were dried, a root extraction was performed to recover any trace organics adsorbed into plant roots during the greenhouse study. The root extracts were collected by adding 500 mg of ground plant roots into 40 mL of 80% HPLC-grade methanol. The root solution was then sonicated for 30 minutes, filtered using a Whatman Grade GF/B Glass Microfiber Filter under vacuum (Cytiva, Marlborough, MA), centrifuged for an hour, and then filtered using a 0.2 μm cellulose acetate filters (Thermo Fischer Scientific, Waltham, MA).

3.7.2. TOC Analysis

In addition to collecting HPLC measurements, total organic carbon (TOC) concentrations in pot study effluent samples were measured using a Shimadzu TOC-L Analyzer with an ASI-L autosampler. Samples were acidified with 1 M HCl to remove inorganic carbon by the instrument, employing the instrument's high-temperature (680 °C) combustion catalytic oxidation method. The TOC calibration curve was generated using potassium hydrogen phthalate (KHP, Fisher Chemical, MA) standards at concentrations ranging from 1 mg/L to 35 mg/L. Prior to analysis, samples were filtered through 0.2 μm cellulose acetate filters (Thermo Fischer Scientific, Waltham, MA) to eliminate particulates and prevent blockages in the injection system.

3.8. HPLC-TOC-UV Analysis of Synthetic Stormwater

The organic contaminant concentrations of batch test and pot study samples were analyzed using a Dionex 68 Ultimate 3000 High Performance Liquid Chromatography (HPLC) with a diode array detector and an Ascentis® C18 column (2.1 69 mm x 15 cm, 3 μm). An isocratic elution method was used to separate the trace organics following batch sorption studies: 18% methanol mobile phase and 82% 0.1% formic acid (v/v) stationary phase. For greenhouse study pot samples, BZT and SMX were analyzed using the same method described above. However, ACT concentrations were measured using an 8% methanol and 92% 0.1% formic acid (Agilent Technologies, CA) (v/v) elution method to minimize interference with background peaks associated with the organic matter within samples. All pot samples were spiked with 25 uL of HPLC grade methanol (Avantor, Radnor, Pennsylvania) to assist in the separation of the contaminants from the effluent samples. Analytical standards of SMX, BZT, and ACT were used to create the calibration curve, which ranged from 25 ug/L to 800 ug/L of each contaminant.

Table 3.3. HPLC Analysis Characteristics for Synthetic Stormwater Compounds [127]

Compounds	Wavelength (nm)	Retention Time (min)
Acetaminophen, ACT	244	6.19±0.03; 14.8±0.16 (8% MeOH method)
Benzotriazole, BZT	260	18.0±0.09
Sulfamethoxazole, SMX	266	22.0±0.13

4. Results and Discussion

This section encompasses data summarizing MCG biochar characterization, isotherm batch studies using MCG or *TH@*MCG, and an 18-week greenhouse pot experiment.

4.1. Biochar Characterization and Elemental Composition

Physiochemical characteristics and elemental compositions of both MCG biochar is reported based on previous studies (**Figure 4.1**). [44,128] Bioretention systems are commonly amended with green waste compost derived from organic materials such as yard waste, wood waste, food waste, and manure.[129] MCG biochar proves to have a higher surface area and small pore sizes in comparison, which is associated with high adsorption capacity.[44] The high porosity of biochar has also been found to support plant-water availability and nutrient retention capacity.[130,131] The high surface area and porosity of MCG, paired with findings of increased microbial communities in studies exploring biochar-enriched soils, supports the findings that micropores within MCG provide microhabitats that protect microbes against microbial grazers. [76,132]

Table 4.1: Physiochemical properties of MCG Biochar[44]

Media Type	BET SA (m^2/g)	Pore size (nm)	Elemental Analysis				Proximate Carbon Analysis		
			C%	H%	N%	O%	VC%	FC%	Ash%
MCG Biochar[44]	801	2.21	81.33 ± 0.31	1.21 ± 0.05	ND	12.82	11.5 ± 0.2	83.5 ± 0.3	5.0 ± 0.5

Furthermore, soil microbial communities have been found to use biochar as a carbon and nutrient source, enhancing microbial growth and resilience.[56,98,133] Additionally, the abundance of active hydroxyl, carboxyl, sulfonic acid group, amino, imino, and acylamino hydroxyl and carboxyl functional groups support microbial cell adhesion and proliferation. [53,97,133] Further, MCG is characterized by its negative surface charge and limited identifiable Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) surface functional groups, signaling high aromatic carbon content capacity to interact with hydrophobic compounds.[44] This physical property has also been found to support wood-based biochar resistance against microbial degradation and chemical reactions within soils, allowing it to maintain its stability within the environment.[48,134,135] The negative surface charge of the MCG biochar can also facilitate high adsorption affinities for positively charged ions and help to improve soil health by binding to plant nutrients and hydrogen ions.[136] Therefore, MCG biochar can provide greater opportunities for contaminant sorption in while also providing many soil benefits.[133-137]

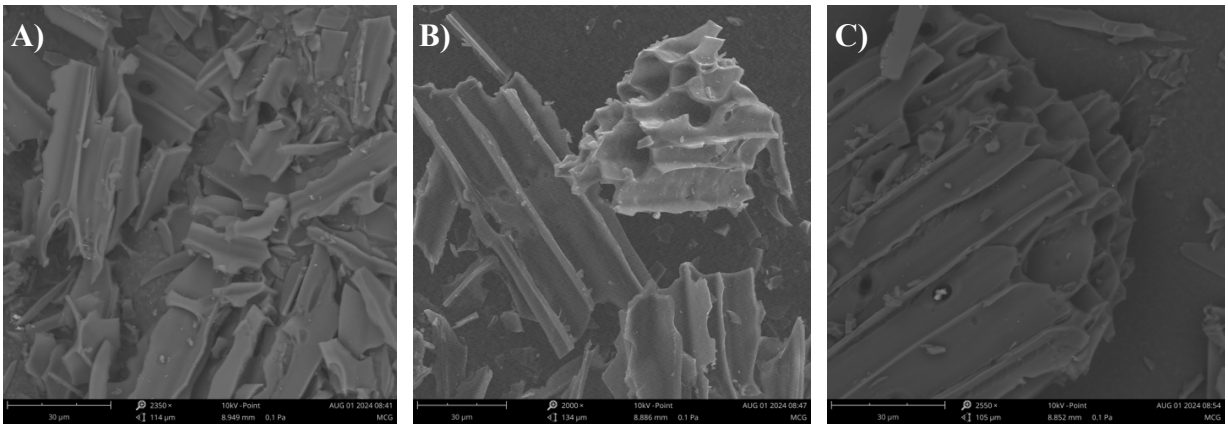


Figure 4.1: SEM images of unmodified MCG biochar after sieving and washing (described in Methods section 3.2.1) at using a 30-micrometer scale. Images (B) and (C) exemplify the macropores and micropores MCG has that allow for contaminant adsorption and microbial growth.

4.2. *T. harzianum*-inoculated Biochar Establishment and Characterization

Using both stereomicroscope imaging and scanning electron imaging (SEM), fungal establishment was confirmed. *T. harzianum (TH)* was applied to biochar and given sucrose as an additional nutrient source, and images of the material were taken to assess the extent of fungal growth after one week (**Figure 4.2 A**) and two weeks (**Figure 4.2 B, Figure 4.2 C**).

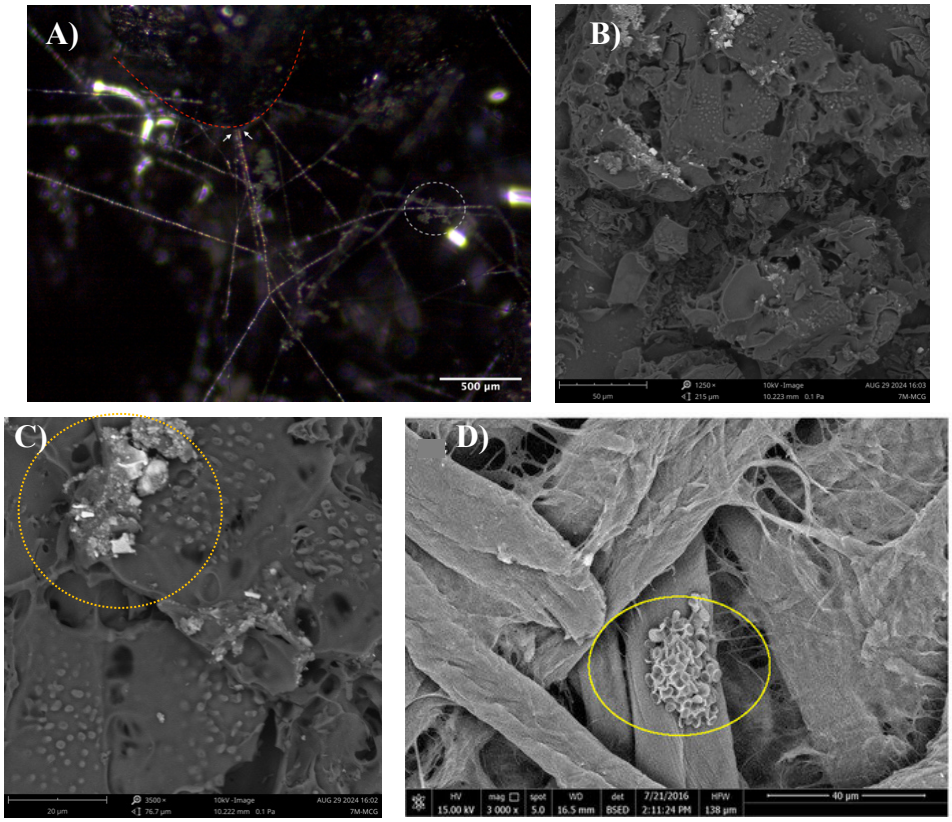


Figure 4.2: (A) Live microscope image of biochar inoculated with *TH* capturing hyphal attachment (white arrows) and reproductive structures (white circle) on BC particles; SEM imaging of *TH*-inoculated biochar at 50 micrometers

(B) and 20 micrometers (C). (D) From Hassan et al., SEM imaging of in intense *T. harzianum* colonization cluster on Whatman paper (40 µm scale), which mirrors the clusters found on *TH@*MCG.[5]

Signs of fungal colonization on MCG and structural changes to the media captured by SEM images may signal that *T. harzianum* can use MCG biochar not only as a growth substrate, but also as an energy source for growth.[88,89,138] Comparing the growth dispersal image in **Figure 4.2 (D)** and **Figure 1.5** from prior studies, it is likely that intense *T. harzianum* colonization has established on and within MCG biochar pores, confirming fungal capabilities to establish hyphal networks within biochar.[139,140] Because fungal establishment appears to be sporadically coating MCG, it is likely that while MCG is serving as a fungal carrier, it can still sorb and capture contaminants within its pores.[88,89]

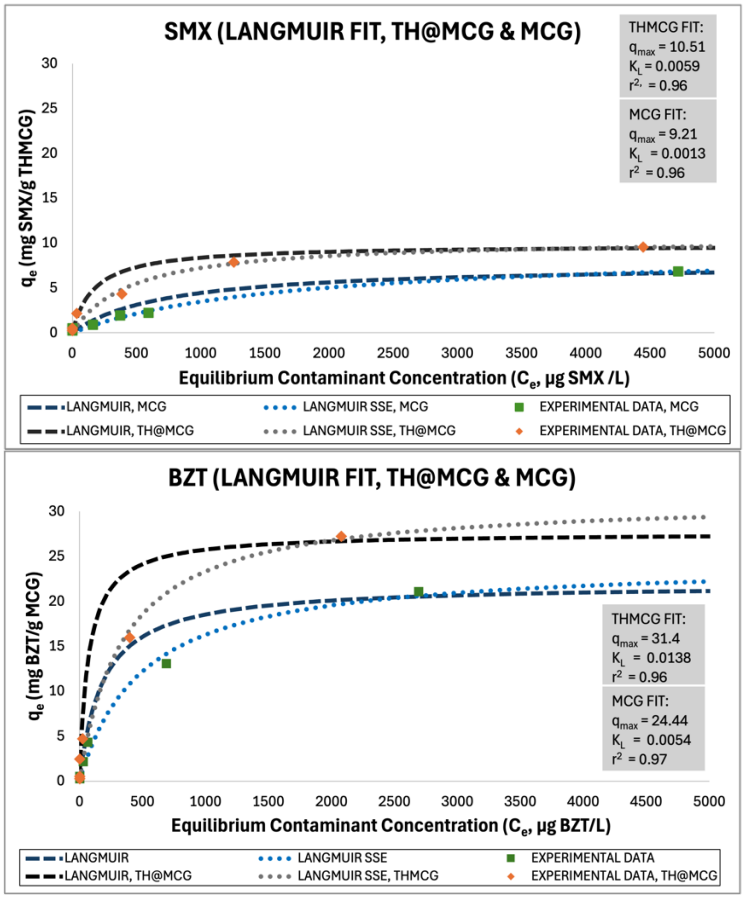
4.3. Batch Isotherm Sorption Studies

Batch isotherm sorption experiments were conducted to access the contaminant removal capacity of MCG biochar and *TH@*MCG within a synthetic stormwater matrix containing BZT, ACT, and SMX varying by concentration and presence of natural organic matter over a five-day period. Batch test results were fitted to the Langmuir and Freundlich isotherm model. However, the Langmuir model resulted in the best fitting of our experimental data, where the amount of contaminant adsorbed per unit weight of biochar at equilibrium (q_e, mg/g) was plotted as a function of the aqueous phase equilibrium concentration of the contaminant in the solution (c_e, µg/L).[141–143] Langmuir SSE fit is determined by minimizing the sum of squared errors between predicted values from Langmuir equation and the experimental data.[141–143]

4.3.1. Mixed Contaminant System (BZT, ACT, and SMX) within SSW Matrix

Figure 4.3 summarizes batch sorption capacity of MCG and *TH@*MCG within a mixed contaminant system of BZT, ACT, and SMX at concentrations between 25 µg/L to 5 mg/L each containing SSW and NOM (10 mg-C/L). Controls were conducted both with and without NOM present to assess sorption of contaminants by NOM and removal by NOM was found to be negligible except at higher concentrations of SMX (**Appendix 1.1**). MCG and *TH@MCG* achieved 100% removal of BZT, ACT, and SMX up to 50 µg/L. BZT was removed up to 87% at 500 µg/L concentration by MCG and up to 95% by *TH@MCG* within a mixed contaminant system. MCG achieved a q_{max} value of 24.4 for BZT, and 9.21 for SMX. These Langmuir fit q_{max} suggest that MCG biochar has the highest adsorption capacity for benzotriazole, followed closely by acetaminophen, while its capacity to adsorb sulfamethoxazole is significantly lower. *TH@MCG* q_{max} values indicate that inoculated MCG has a higher maximum adsorption capacity for BZT ($q_{max,\,BZT}$ = 31.4 mg/g), followed by ACT ($q_{max,\,ACT}$ =26.42 mg/g), and the same decreased capacity for SMX ($q_{max,\,SMX}$ = 10.51 mg/g). This suggests that the inoculation of MCG may have enhanced the adsorption capacity for BZT and ACT compared to non-inoculated MCG. Both media had the lowest affinity for removal of SMX in comparison to ACT and BZT. As previous studies have found, MCG biochar does not maintain the same removal efficiency across contaminants within a mixed system.[6,37,40,60,144] In mixed contaminant systems, compounds compete for binding sites on the biochar surface.[6,37,40,47,52,60,144,145] Consequently, removal efficiency of each contaminant is reduced in comparison to individual contaminant batch test systems (**Figure 4.5**).

Molecular properties of organic compounds including molecular structure, hydrophobicity, aromaticity, and polarity influence their removal by the MCG biochar.[37,40,52,60,144,145] For instance, larger molecules like SMX (in comparison to compact structure of BZT) may have less access to smaller pores within biochar, hindering adsorption potential.[60,63,144–146] It is also likely that the high aromaticity of BZT π-π interactions with MCG, along with its moderate polarity, encouraged higher sorption.[147] SMX maintains moderate polarity (sulfonamide group and heterocyclic ring) and moderate aromaticity (aromatic benzene ring),[148–150] whereas ACT holds higher polarity (from hydroxyl and amide groups) and moderate aromaticity (aromatic benzene ring).[151–153] These compound characteristics would be expected to limit surface interactions and sorption potential with MCG.



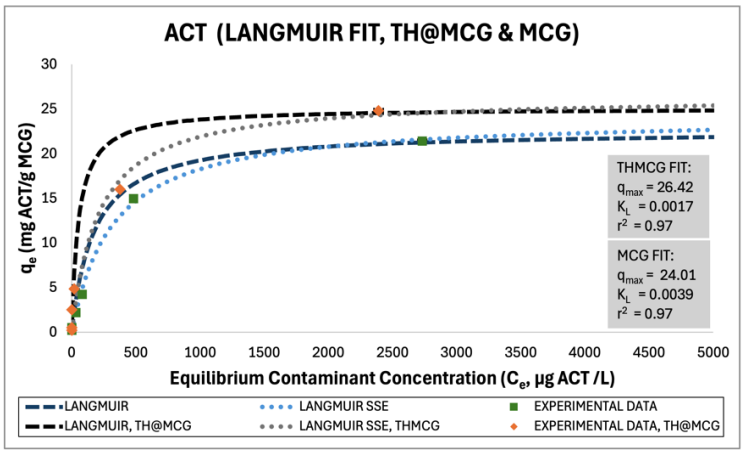


Figure 4.3: Batch isotherm adsorption capacity of MCG and *TH@*MCG (RAW DATA), fit to Langmuir isotherm model (LANGMUIR) and the Langmuir Sum of Square Error fit (LANGMUIR SSE). Fits were determined using experimental batch isotherm test data using 100 mg/L of sorbent at varying concentrations within SSW matrix and 10 mg-C/L NOM (See **Appendix 1.2** for NO NOM control results).

Considering the K_{ow}, or the octanol-water partition coefficient, BZT is the most hydrophobic with the largest K_{ow} ($logK_{ow}$ = 1.44), whereas sulfamethoxazole ($logK_{ow}$ = 0.89) and ACT are more hydrophilic ($logK_{ow}$ = 0.46).[127,154] Hydrophobic interactions often play a large role in competition for adsorption sorption sites by contaminants.[6,37,145] Because BZT has a higher K_{ow} for *TH@MCG* sorption, it is likely its higher q_{max} can be partially attributed to hydrophobic interactions between BZT and *TH@MCG*.[127,154] ACT is less hydrophobic than BZT, but more than SMX, so hydrophobic interactions likely played into ACT removal by MCG and *TH@MCG*.[120,149,154] However, because MCG achieved the largest q_{max} (23.08 mg/g) for ACT removal, other mechanisms like hydrogen bonding are also influencing the remediation capacity of MCG.[6,37,145]

The presence of oxygen-containing functional groups (**Table 4.1**) on MCG may have facilitated hydrogen bonding with ACT (containing hydroxyl and amide groups), resulting in higher sorption than anticipated based on the molecular structure and of $logK_{ow}$ of ACT.[155,156] Further, MCG may have favored ACT due to its smaller size and polar functional groups (carboxyl, hydroxyl groups), likely contributing higher sorption rates to hydrogen bonding and van der Waals forces for this compound.[6,37,40,40,155] According to Shimabuku et al., several studies have found that SMX has a greater affinity for lower biochar produced at lower pyrolysis temperatures that contain more polar functional groups, lower surface areas, and high H:C ratio (more aliphatic).[149] Because MCG is produced high temperatures and has a low H:C ratio that is reportedly around 0.015 (**Figure 4.1**), it may not have been favorable SMX sorption.[44,149] This low H:C ratio also suggests MCG has fewer polar functional groups, making it less ideal for SMX adsorption compared to other media with higher H:C ratios.[44,149] Nonetheless, this study affirms that sorbents with low H:C ratios (indicator for aromaticity) still maintain removal efficient at environmentally relevant concentrations of SMX.[149,150]

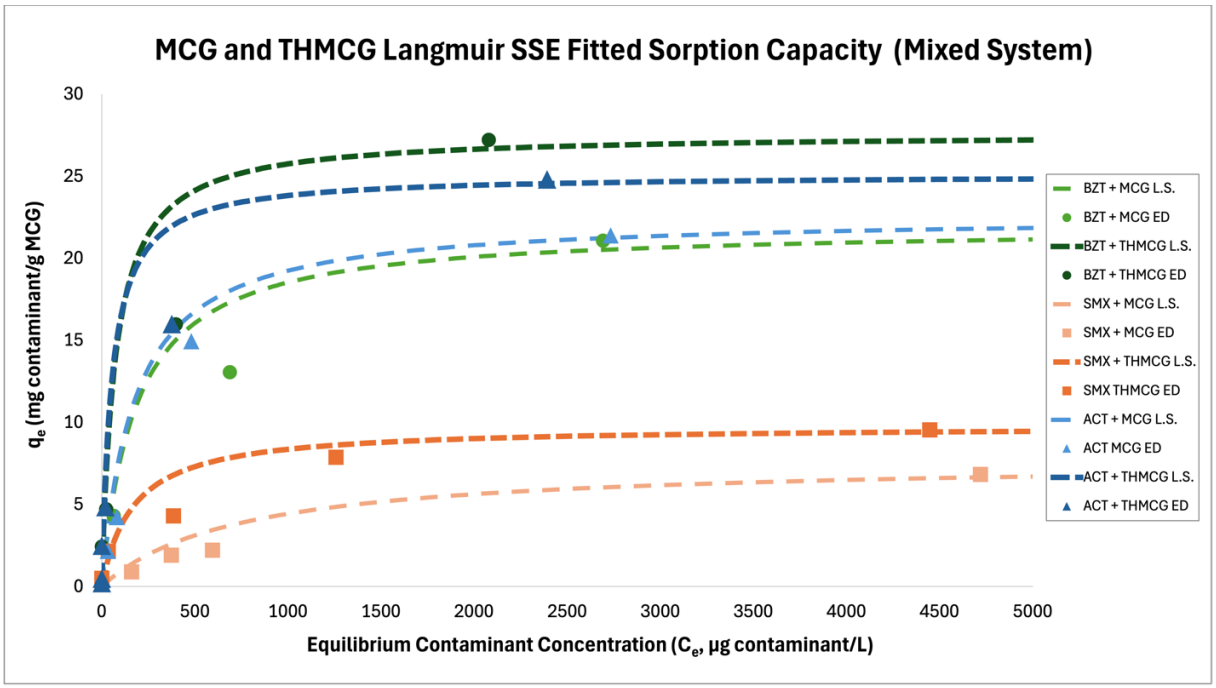


Figure 4.4: Compiled batch sorption experimental data (CONTAMINANT + SORBENT ED) and Langmuir SSE (CONTAMINANT +SORBENT L.S.), portraying competing sorption efficiency within mixed contaminant systems using MCG and *TH*@MCG (same data from **Figure 4.3**). *ED = Experimental Data, LS = Langmuir Fit

Batch isotherm tests conducted using *TH*@MCG resulted in a shift in removal performance, in which BZT had the highest removal rate, followed by ACT, then SMX (**Figure 4.4**). Additionally, the incorporation of *T. harzianum* improved removal efficiency across contaminants. There are many potential reasons why *TH* may have enhanced contaminant removal: (1) Because *TH* is known for producing varying enzymes capable of degrading pollutants,[82] it is possible that contaminant biodegradation occurred during the five-day batch experiment[157]; (2) *Trichoderma sp.,* known for their ability to produce surface-active proteins that form a hydrophobic biofilms on their growth substrates under nutrient-limited conditions, may have increased the surface hydrophobic interactions with BZT through this biological function[157,158]; (3) significant fungal growth may have resulted in greater overall surface area of the media or the production of enzymes that altered the MCG surface chemistry of MCG, resulting in greater immobilization of our contaminants. It is possible that *T. harzianum* had favorable interactions with the MCG surface properties and functional groups which allowed for fungal adhesion to and between char particles to improve sorption.[88,157] As many other microorganisms immobilized onto biochar have exhibited, *TH* also potentially produced organic acids, enzymes, and other metabolites. The production of these substances can alter the chemical properties of the biochar surface and enhance adsorption capacity, degradation capabilities, and environmental adaptability.[124,159] As portrayed in **Figure 4.2** prior, it is also likely that the extensive hyphal network of *TH* growing between particles may have increased surface area for contaminant immobilization.[88,160] Structural changes MCG exhibits after inoculation may also signal modified porosity produced by fungal growth on MCG, which could also improve contaminant capture.[88]

4.3.2. Individual Contaminants (BZT, ACT, and SMX) within SSW Matrix

Additional batch isotherm tests were conducted to assess the sorption capacity of each contaminant individually by MCG at concentrations between 25 µg/L to 5 mg/L over a five-day period within SSW containing NOM (10 mg-C/L). Across contaminants, removal rates were higher in comparison to a mixed contaminant system, likely due to the lack of competition between contaminants. MCG achieved the highest removal of ACT, and the lowest removal of SMX. There was negligible removal of ACT and BZT by NOM when comparing controls with and without NOM, meaning differences between controls with and without NOM were below detection the HPLC limit (20 µg/L) throughout our tested concentration range (25 µg/L to 5 mg/L) (**Appendix 1.2**). However, at higher concentrations of SMX, contaminant concentrations were reduced up to 150 µg/L in systems with NOM. This could indicate that SMX has an affinity to sorb onto dissolved organic matter when it is present at higher concentrations (1-5 mg/L). Previous studies have noted that the presence of NOM negatively impacted SMX sorption by various wood-based biochar types, except those produced at lower pyrolysis temperatures (300-500 °C).[149] Further, this study confirmed that NOM does compete with SMX for sorption sites across biochar types, indicating SMX interactions with MCG are likely influenced by NOM.[149] This finding is also dependent on NOM source, particle size, and concentration, leaving room for further research surround its impact on sorption.[148,149] This study also noted that the hydrogen-to-carbon (H:C) ratio (**Figure 4.1**) was linked to SMX absorption by wood-based biochar, which could also have influenced SMX removal rates**.**[149] This characteristic likely contributed to the relatively lower q_{max} value for SMX observed in experiments, as MCG may not have provided the optimal interaction sites for polar molecules like SMX.[148,149]

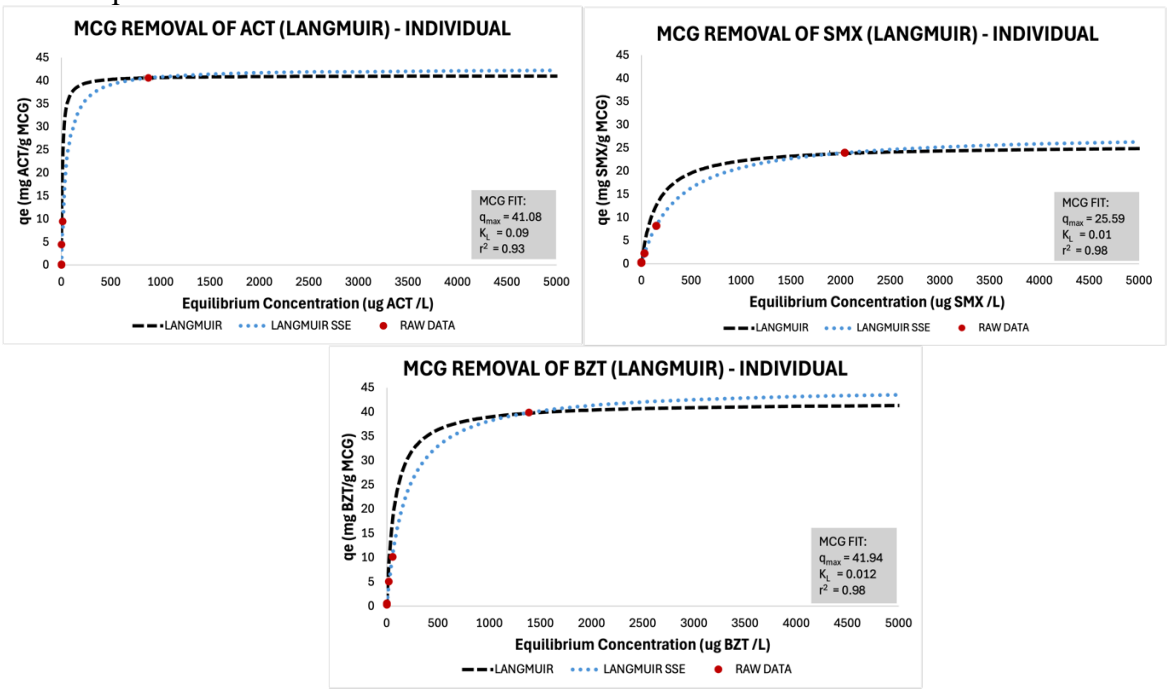


Figure 4.5: Experimental batch isotherm sorption data (RAW DATA) in an individual contaminant system (BZT, ACT, or SMX) fit to Langmuir equation and Langmuir Sum of Error (Langmuir SSE), to produce predicted removal capacity of MCG as concentration of contaminant within system increases. Langmuir SSE improves Langmuir fit by accounting for error terms.

4.4. Greenhouse Mesocosm Study

Greenhouse mesocosm studies were assembled in triplicate to mimic bioretention systems amended with MCG and *TH*@MCG both with and without plant species *Juncus patens*. Pots were watered weekly with synthetic stormwater spiked with 10 µg/L of BZT, ACT, and SMX each (at week 10 concentrations increased to 10 mg/L). Mesocosm effluent samples were collected and analyzed using HPLC-UV to quantify removal the capacity of all three amendments. Results below are pots without plants, separated by individual contaminant (within a mixed system).

Figure 4.6: Greenhouse experimental setup pot with randomized distribution of triplicates (pots with and without plant *juncus patens).*

4.4.1. Mesocosms without plant Juncus patens

SEM IMAGING

Upon completion of the study, mesh baggies containing *TH@MCG* and MCG only within mesocosm soils were extracted and frozen for SEM imaging. Comparing **Figure 4.7** presenting imaging of the media after the study to unmodified MCG (**Figure 4.1**), we observe microbial growth on the surface of both amendments. Additionally, in pots containing *TH@MCG* without plants, it appears the fungal growth we observe in **Figure 4.2** as well as **Figure 4.11** (SEM of pots with plants) is not as apparent. It is likely that the absence of plants may have inhibited sustained *TH* colonization on MCG. Further there was observed microbial growth on MCG only mesocosms, exemplifying that unmodified MCG can serve as a microhabitat for soil microbes.

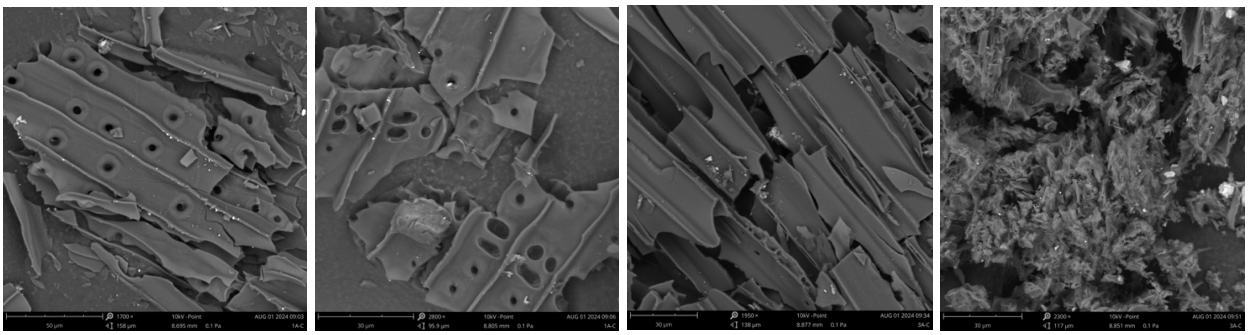


Figure 4.7: (A) and (B) exhibit SEM images of *TH@MCG* biochar taken from mesh baggies buried within no-plant mesocosms at the end of the study at 50- and 30-micron scale; (C) and (D) exhibit SEM images of MCG only pot no-plant mesocosm biochar at the end of the study from the mesh baggies buried within the pots at 30-micrometer scale.

HPLC RESULTS

BZT removal (no plants): All systems exhibited complete removal of BZT until week 8 (prior to contaminant concentration increase within stormwater mixture). Following the concentration increase, pots amended with *TH*@MCG maintained the highest removal rate of benzotriazole in comparison to our other systems. While control pots containing natural soil reduced contaminant breakthrough, it has the lowest BZT removal rate in comparison to MCG, *TH@MCG,* and *TH* amended systems. Pots containing *TH* only, MCG only, and *TH*@MCG maintained relatively similar removal rates within the system (maintaining between 36% - 43% removal rate between weeks 14-18). However, *TH* only had the largest standard deviations after week 12 (followed by MCG only), signaling that their removal efficiency varied more in comparison to *TH*@MCG. Additionally, removal efficiency across amendments appears to begin to reach a plateau around week 12, signaling reduction of sorption capacity due to the elevated concentration of contaminants added (**Figure 4.8)**.

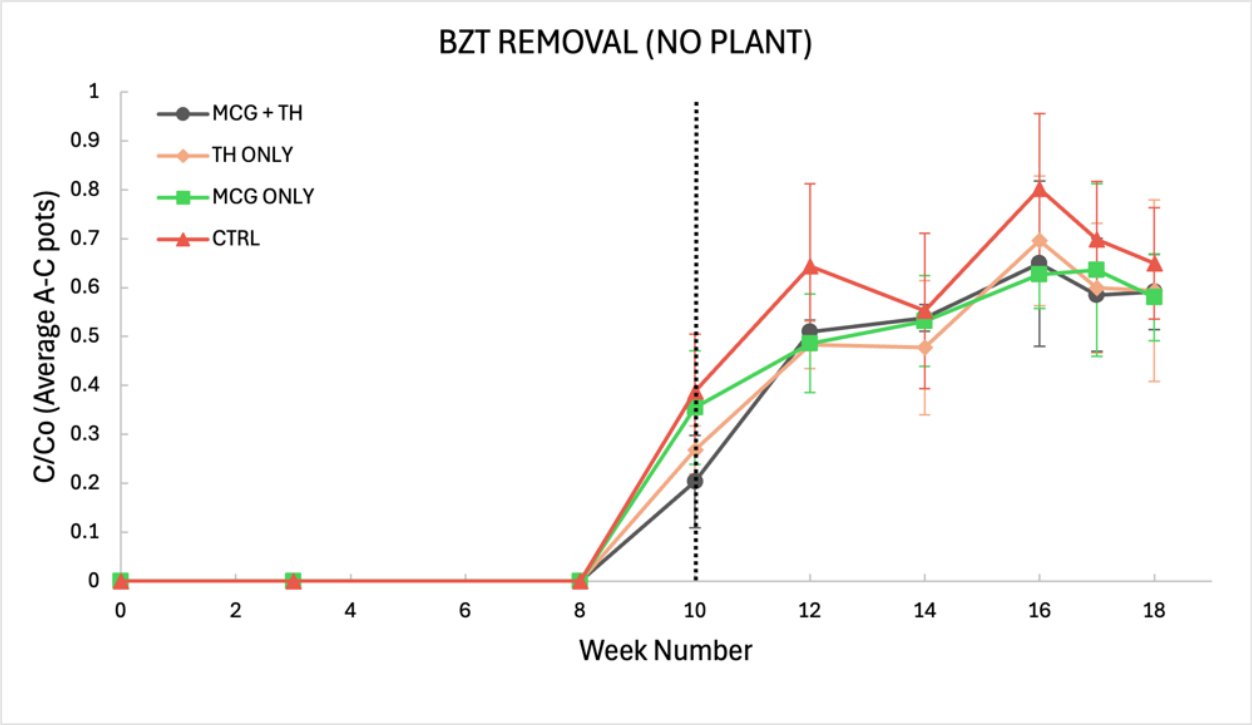


Figure 4.8: Benzotriazole removal efficiency over time in pots with no plants amended with either *TH*@MCG, *TH* only, MCG only, or no amendment (soil control). * Line at week 10 indicates contaminant concentration increase because there was complete contaminant removal prior ($C/C_0 = 0$).

ACT removal (no plants): Pots with no plants amended with *TH* only resulted in the highest removal efficiency of ACT in comparison to the other amendments. *TH*@MCG performed slightly better than MCG only when comparing removal efficiency averages from triplicate results. However, *TH* only pots once again had greater standard deviations, which can lead us to assume *TH*@MCG results in more efficient and consistent removal of ACT. Prior research explored the bioremediation potential of *TH* along with bacteria *Pseudomonas fluorescens* within dual chamber fungal microbial fuel cells and found that they achieved complete removal of acetaminophen and its by-products.[161] The findings of previous study confirm that *Trichoderma sp.* alone is capable of degrading ACT[161] and BZT,[162] which agrees with our findings. Additionally, the study found that *TH* biodegradation rate was five times higher when using bacterial-fungal biofilms (combined application of *TH* and bacteria), than *TH* alone.[161] It is likely that the existing soil microbial community established onto MCG and influenced the remediation capacity of *TH*, as prior studies have found that many microbes can impact remediation potential. Overall, removal of BZT across soil amendments in no plant mesocosms is comparable to ACT, though amendments containing biochar (MCG and *TH*@MCG) removed BZT slightly more efficiently (**Appendix 1.3**).

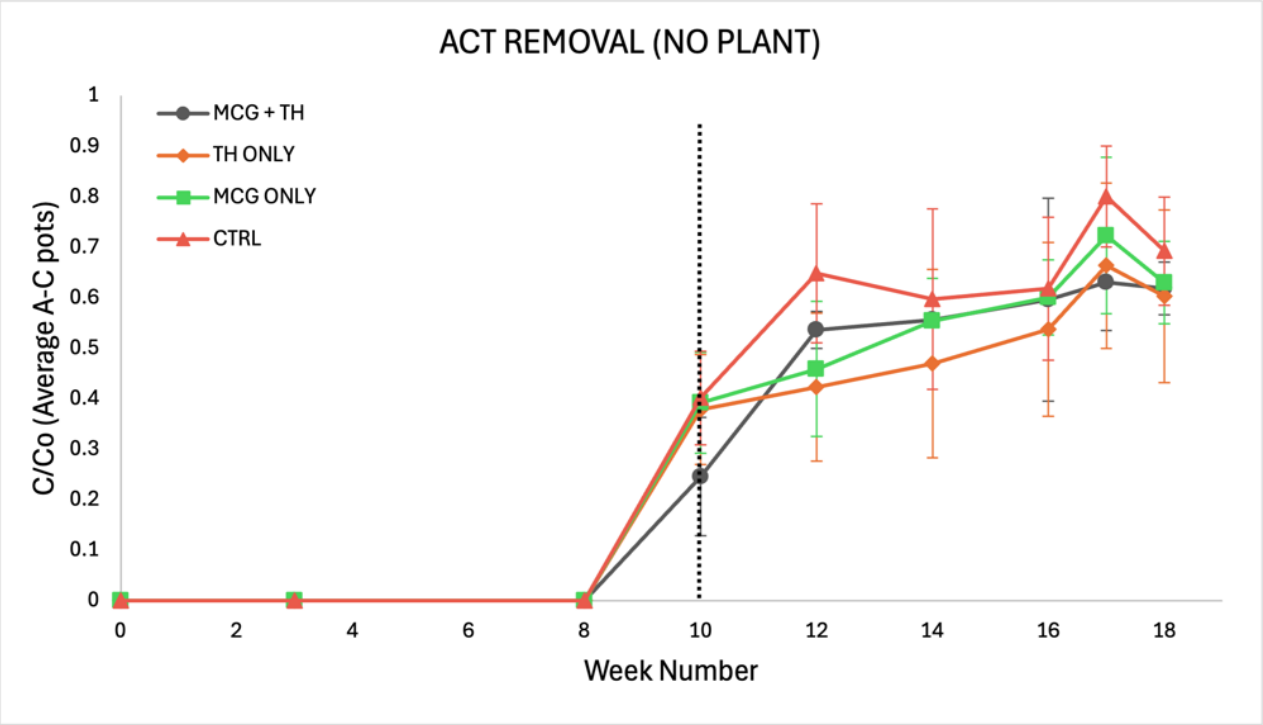


Figure 4.9: Acetaminophen removal efficiency over time in pots with **no plants** amended with either *TH*@MCG, *TH* only, MCG only, or no amendment (soil control). *Line at week 10 indicates contaminant concentration increase because there was complete contaminant removal prior ($C/C_0 = 0$).

SMX removal (no plants): Mirroring our batch isotherm study results, the extent of SMX removal was the lowest in mesocosms without plants. As stated, this may be attributed to sorption site competition, molecular size, and presence of DOM. By week 16, there was almost complete breakthrough of SMX in all greenhouse pots (without plants) which indicates that there was little to no removal of SMX across media. Additionally, control pots (no soil amendments or plants) performed better than *TH* only, signaling that the fungal amendment alone may have contributed to accelerated breakthrough of SMX. Nonetheless, MCG and *TH@MCG* were able to maintain comparable efficiency to their respective BZT and ACT removal rates up until week 14. The drastic reduction in contaminant removal between week 8 to 12 may signify that the media amendments cannot remediate large fluxes of SMX without subsequent breakthrough of the contaminant, which does not seem to be the case for BZT or ACT. Without plants, it appears that no amendment system is significantly more efficient at remediation; however, these amendments prove to show promise for removing contaminants at environmentally relevant concentrations.[148,149]

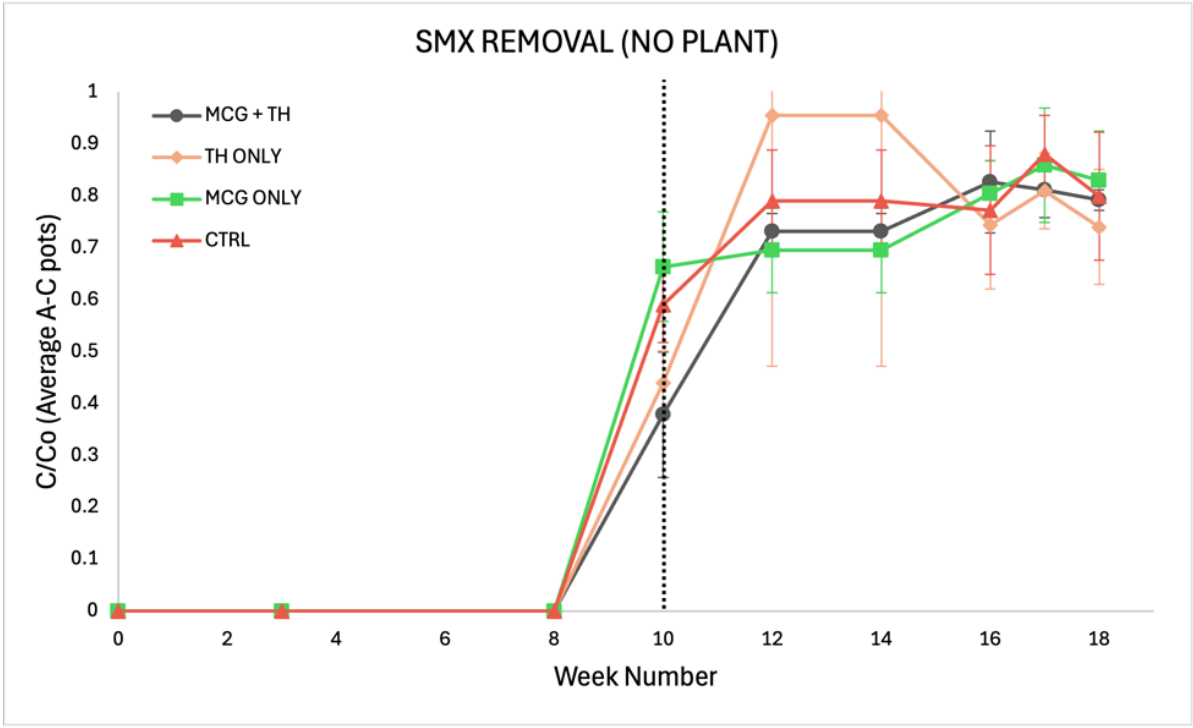


Figure 4.10: Sulfamethoxazole removal efficiency over time in pots with **no plants** amended with either *TH*@MCG, *TH* only, MCG only, or no amendment (soil control). *Line at week 10 indicates contaminant concentration increase because there was complete contaminant removal prior ($C/C_0 = 0$).

4.4.2. Mesocosms with plant Juncus patens

SEM IMAGING

SEM imaging was performed to visually assess changes to MCG due to the experimental conditions and *TH* inoculation. **Figure 4.7** presents images of *TH@MCG* and MCG-only amended mesocosms planted with *JP*. The incorporation of *TH@MCG* in mesocosms planted with *JP* appear to sustain microbial colonization on MCG due to its comparable qualities to the images of *TH@MCG* imaged after two weeks of incubation within the laboratory **(Figure 4.2)**. Images of *TH@MCG* within pots without plants do not seem to have the same biofilm formation on the biochar surface. Previous studies report that biochar increases organo-mineral association and fungal hyphae length, which are two important factors for soil aggregation and structure. Therefore, the colonization of *TH* on MCG holds promising benefits for soil and plant health.[2,53,88,138] In planted pots containing MCG only, it appears the surface texture has changed, likely by the thin plant roots growing along the surface of the sorbent. Prior studies have found that some plant root systems can interact with biochar particles within soils and root hairs can enter macropores or bond onto the biochar surface.[88,106,163] Plant roots have been found to explore sites within soils that have biochar particles, and plant morphology (root length, surface area, tip numbers) is subsequently enhanced by biochar amendments.[55,164] The addition of biochar allows improves nutrient sorption to plants, allowing their roots to reach depths they normally cannot access.[4,88]

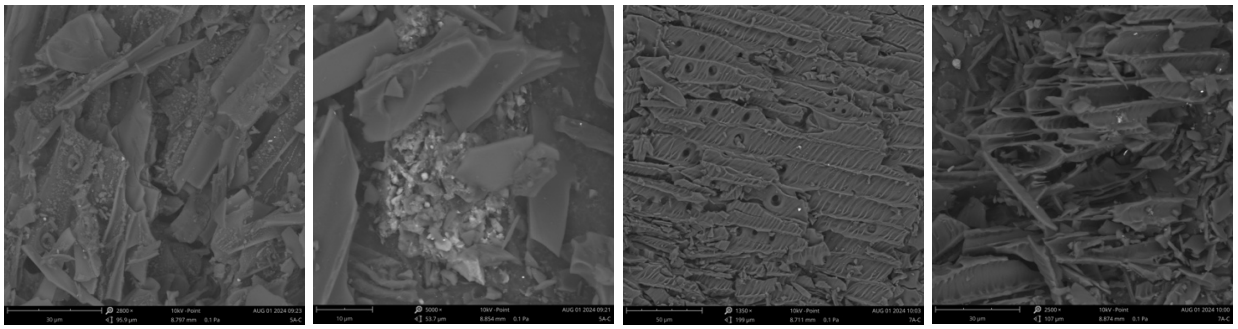


Figure 4.11: (A) and (B) exhibit SEM images of *TH@MCG* biochar taken from mesh baggies buried within planted mesocosms at the end of the study at 30- and 10-micron scale; (C) and (D) exhibit SEM images of MCG only planted mesocosm biochar at the end of the study from the mesh baggies buried within the pots at 50- and 30- micrometer scale.

HPLC DATA

BZT removal (with plants): In planted mesocosms, BZT was removed most efficiently by *TH*@MCG, and standard deviations between triplicates remained consistently low in comparison to other tested amendments. *TH*@MCG maintained 70-80% removal efficiency the final two weeks of the study, signaling that the media had not reached sorption capacity yet. These findings hold promise for the longevity of this potential bioretention amendment, as many concerns surrounding biochar relate to fouling of the media. When comparing removal rates of *TH*@MCG with and without the addition of *JP*, it appears that the incorporation of the plant improved removal efficiency by *TH*@MCG of BZT by 30-40%. Planted mesocosms amended with MCG only resulted in 10-20% improved remediation capacity of ACT after week 13 in comparison to MCG only pots without *JP.* These results may further confirm that *JP* and MCG were interacting to potentially enhance microbial remediation, contaminant immobilization by MCG, or plant uptake of contaminants. Control mesocosms planted with *JP* resulted increased removal efficiency of BZT by 20-30%. *TH* only planted pots performed worse than the control planted pots at removing BZT, and *TH* only remediation capacity varied widely between pots. All systems performed better at remediation of BZT with the incorporation *JP* except *TH* only pot, which only slightly improved (5-10% after week 15).

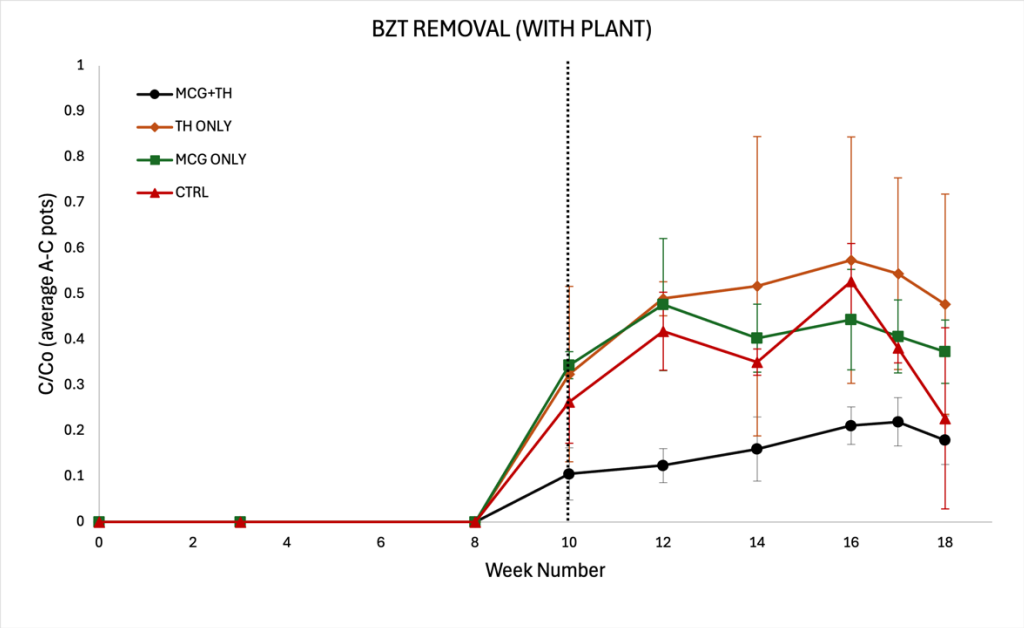


Figure 4.12: Benzotriazole removal efficiency over time in planted mesocosms amended with either *TH*@MCG, *TH* only, MCG only, or no amendment (soil control). * Line at week 10 indicates contaminant concentration increase because there was complete contaminant removal prior ($C/C_0 = 0$).

ACT removal (with plants): Like BZT, the removal efficiency was drastically improved by the incorporation of *JP* in pots amended with *TH*@MCG with minimal variation between pots. The last two weeks of the study, *TH*@MCG maintained 70-80% removal efficiency of ACT. In comparison to mesocosms that were not planted, ACT removal capacity by MCG was improved by 10-20% and ACT removal within control pots improved 20%-30%. ACT removal rates were worse in planted mesocosms compared to unplanted *TH* only pots, signaling that the incorporation of *JP* did not enhance remediation of ACT in soils amended with *TH.* Because removal efficiency of ACT was improved by *JP* within control mesocosms, these results may imply that *TH* may deter the remediation potential of *JP.* While many studies have proved *Juncus sp.* is capable of phytoremediation, further research is necessary to confirm whether *JP* is conducting remediation, or if its root system is solely immobilizing contaminants like ACT within the soil.[109,110] The inefficient removal capacity of BZT, ACT, and SMX by *TH* only systems may also indicate that applying liquid fungal cultures to bioretention soils may not be effective at sustaining *TH* colonization because of the lack of a fungal substrate, or the substance that fungi grow through and use for nutrition, energy, and structure, like biochar.[124] Additionally, this may have induced competition for nutrients and resources between *JP* and *TH*, reducing their capacity for remediation.[106]

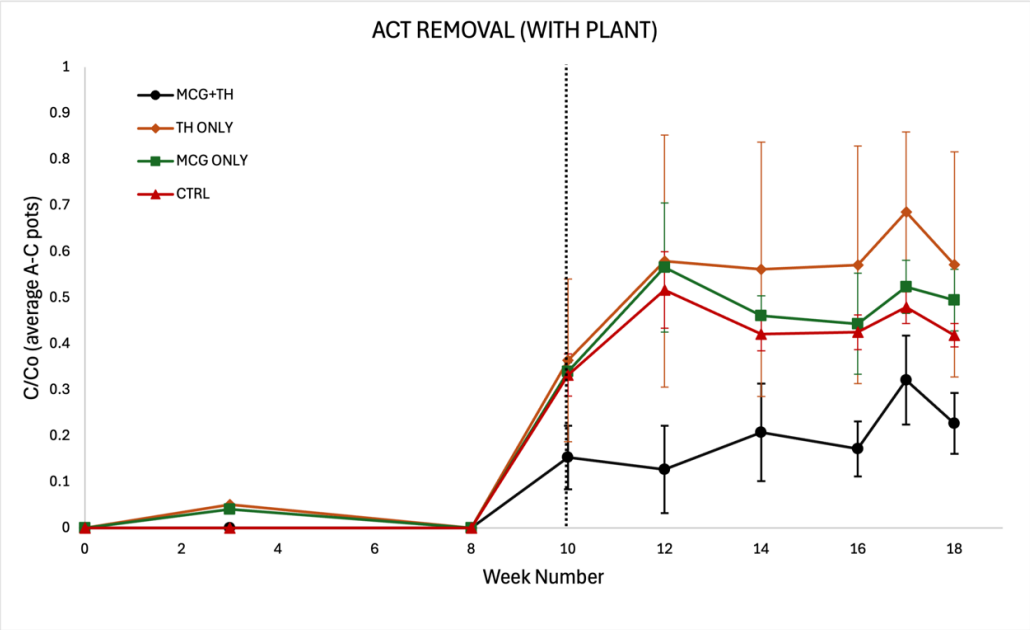


Figure 4.13: Acetaminophen removal efficiency over time in planted mesocosms amended with either *TH*@MCG, *TH* only, MCG only, or no amendment (soil control). Detected concentration at week 3 likely due to analytical error. *Line at week 10 indicates contaminant concentration increase because there was complete contaminant removal prior ($C/C_0 = 0$).

SMX removal (with plants): Removal efficiency of SMX improved by 20-30% in pots amended with *TH@MCG*, in which planted pots maintained around 40-50% removal after week 10. In comparison to our other treatments, *TH@MCG* had the highest removal of SMX, as well as BZT and ACT. When comparing our *TH* only amended mesocosms, *JP* incorporation only improved SMX removal by 15% between weeks 12-14. Additionally, MCG only planted mesocosms performed worse at removed SMX in comparison to MCG only mesocosms without *JP*. Further, removal efficiency of SMX in planted control mesocosms shows negligible differences in comparison to that of the control mesocosms without plants. This may indicate while *JP* likely supported fungal growth and the soil microbiome, it may not have contributed significantly to the remediation of SMX. Both in batch sorption studies and in the greenhouse study, MCG and *TH@MCG* maintained low removal rates of SMX, signaling that these media may not be adept to remove high concentrations of SMX. Nonetheless, *TH@MCG* maintains removal capacity beyond environmentally relevant concentrations of SMX for a prolonged period. As mentioned, MCG performance at removing SMX may have been impacted by competition among contaminants for MCG adsorption site.[165] The size and structure of SMX likely impacted removal efficiency of both MCG and *TH@MCG*.[148,149] Because *TH* appeared to improve removal efficiency of SMX in batch studies as well, it is probable that the use of MCG as a substrate for *TH* played a more influential factor in enhancing remediation of SMX than the incorporation of *JP*.[148,149] Further research is necessary to determine what factors are inhibiting higher removal rates of SMX, which can support the removal of other antibiotics and organic compounds.

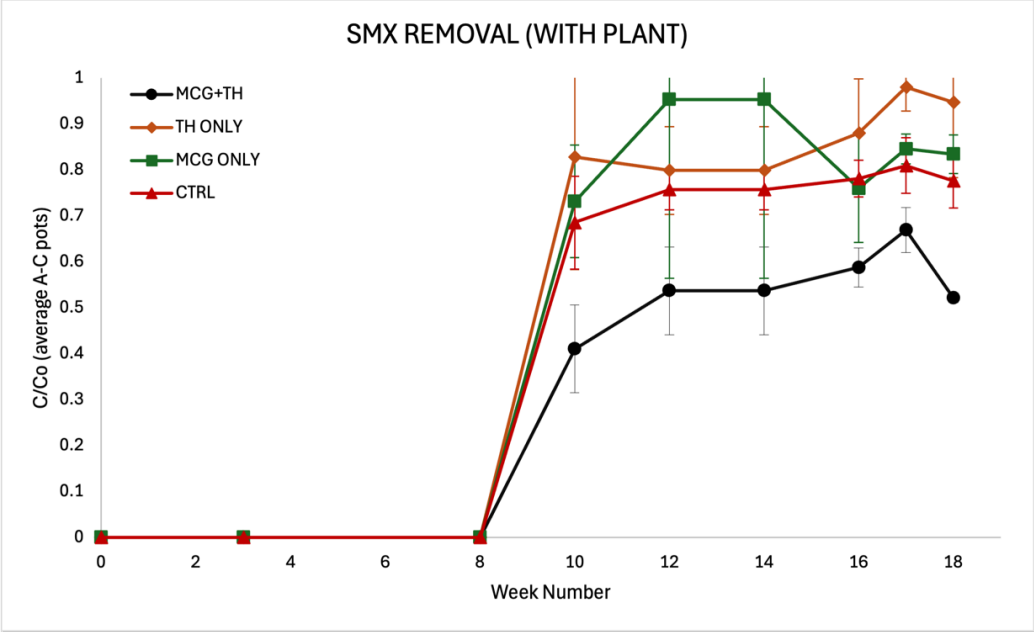


Figure 4.14: Sulfamethoxazole removal efficiency over time in planted pots amended with either *TH*@MCG, *TH* only, MCG only, or no amendment (soil control). *Line at week 10 indicates contaminant concentration increase because there was complete contaminant removal prior ($C/C_0 = 0$).

4.4.3. Benzotriazole Bi-Product Formation

As seen in Figure 4.12, removal efficiency appears to be improving towards the end of the study (week 14-18), which may be attributed to biotransformation produced from BZT within mesocosms. At week 12, HPLC chromatography revealed the presence of a new compound (or additional peak) within samples that was not present prior to week 12. The appearance of this compound was also not detected in *TH@MCG* or MCG mesocosm effluent samples. Additionally, the concentration (or peak area) grew over time within the HPLC-UV wavelength used to measure BZT and SMX concentrations. Because removal was poor across treatments for SMX and because the ACT chromatography wavelength did not detect this compound significantly, it can be assumed to be a BZT transformation product. Furthermore, the wavelength the instrument assigned to this additional compound was different from the BZT and SMX wavelength, meaning that this was a separate compound from our contaminants.[127] This transformation product had the highest concentration within control pots and *TH* only pots. Comparing control mesocosms, the concentration of this additional compound was greater in the planted systems.

The presence of this additional peak was also slightly greater for planted *TH* only mesocosms. Increased concentrations of this additional compound in planted systems could signal that *JP* is likely supporting microbial degradation of BZT. While it has been confirmed that other species within *Juncus* genus are capable of bioaccumulating benzotriazole and its transformation products,[121] further analysis is needed to determine whether *JP* triggered the biotransformation of BZT or if the rhizosphere of *JP* supported biodegradation conducted by existing soil microbes. Because biodegradation of BZT appeared in control pots without *JP*, it is possible that the root system of *JP* supported microbial degradation of BZT by, for example, producing root exudates

which can act as a nutrient source for pollutant degrading microbes.[166] *TH* only mesocosm effluent also contained this additional compound, though it is uncertain if this biotransformation can be attributed to *TH* because it occurred in control pots as well. The soil used for this study was sourced from the University of Washington Center for Urban Horticulture, and characterization of the soil microbial community may help determine whether existing bacterial species conducted bioremediation of BZT.[119] Further, existing studies have confirmed that BZT is biodegradable by soil microbes, so it is likely the existing microbial community contributed to BZT biotransformation.[25,121] It is also important to note that the lack of detection of this additional compound in *TH@MCG* and MCG mesocosms highly indicates that MCG can support not only the removal of contaminants, but also transformation products within bioretention systems. This finding is very useful when considering contaminants with toxic or environmentally harmful transformation products.[156] A large concern surrounding bioremediation and mycoremediation is the production of toxic byproducts, and MCG has the potential to reduce their impact.

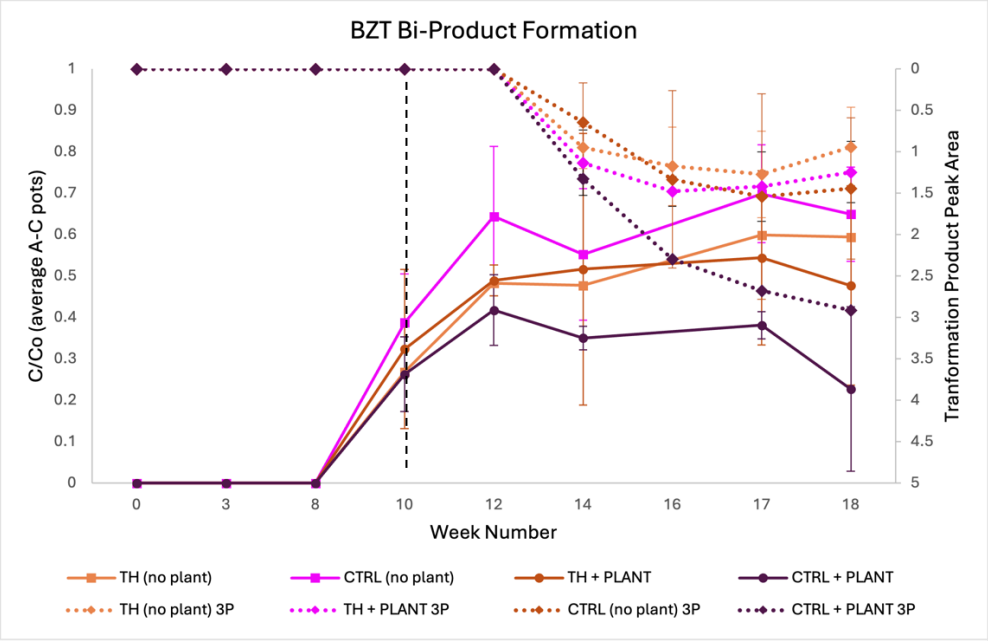


Figure 4.15: Greenhouse mesocosms were sampled weekly to measure effluent concentrations of BZT over the duration of the study, and the concentration introduced to the system of BZT was increased at week 10 (dotted line). HPLC results revealed a transformation product in *TH* only and control mesocosms (planted and not planted) at week 12, which grew in concentration (peak area) over time. The left-hand y-axis quantifies removal efficiency of BZT during the duration of the study, whereas the right-hand y-axis measures the transformation product peak area (or concentration within pot samples) over the study. Improved removal efficiency at the end of the study may be attributed to bi-product formation (secondary y-axis).

4.4.4. Final Plant Biomass (Height and Weight)

Final plant measurements of *JP* revealed that while the incorporation of *TH@MCG* improved plant height (Figure 4.16), it did not significantly increase leaf or root biomass. Prior studies have reported similar findings as well when comparing compost-mulch bioretention media to biochar-amended soils.[55,61] However, MCG did result in increased root mass, which could be beneficial when attempting to improve the phytoremediation potential of plants that uptake contaminants

using their roots. Amendment influence on plant physiology can be used to inform what plants are incorporated to MCG or *TH@MCG* amended bioretention systems, and potentially accelerate phytoremediation.[4,87,104] For instance, bioretention systems planted with native plants that can bioaccumulate contaminants in their leaves may benefit from *TH@MC* amended soils, as this amendment increased plant height notably. An existing study found that amending Cd and Zn contaminated soils with the bamboo biochar and the perennial *Sedum alfredii* increased accumulation 129.16% and 196.61% respectively, while also increasing plant biomass.[167] This improvement was attributed to the increased concentration of nutrients biochar provided, including N, P, and K for the rooted soil region as well as increase in organism-produced degradation enzymes (phosphatase, urease, protease and invertase).[167]

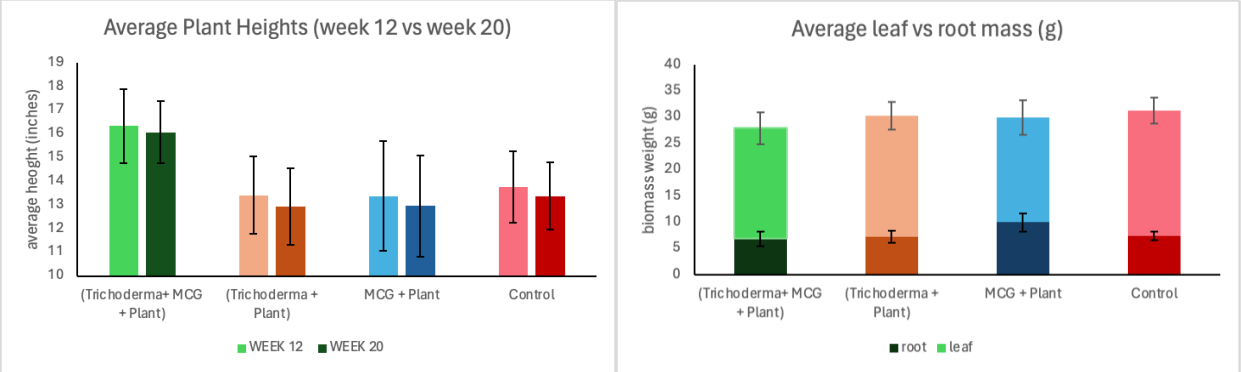


Figure 4.16: (A) Mesocosms were planted with *Juncus patens* and plant heights were recorded week 12 and week 20. (B) Upon completion of the study, plant biomass was washed, dried, and weighed to assess root biomass (darker color) and shoot biomass (lighter color) changes between amendments.

Additionally, *TH@MCG* and MCG amended mesocosms exhibited the significantly less yellowing throughout the study (in comparison to *TH* and control pots). Thus, while these amendments did not result in significant plant biomass improvement, they appeared to support the health and resilience of *JP* despite the high volume of contaminants introduced to the system. Moreover, *Trichoderma sp.* is known for its ability to form relationships with plants to deter formation of plant pathogens and enhance plant health, as well as its ability to perform mycoremediation.[158] However, plant biomass and yellowing data does not correlate with observations of *TH* amendments in our study as the TH only pots experienced the most yellowing. Some studies have found that the mycoremediation efficiency of *Trichoderma sp.* and their ability to support plant health are often dependent on nutrient availability (N:C ratio).[168] Future research exploring if MCG provides the added nutrients to support the bioremediation synergy between *TH* and *JP* is needed.

5. Conclusion

This study aimed to investigate how bioaugmented biochar impacts stormwater runoff remediation through laboratory and greenhouse scale studies. Previous research has explored inoculation of soils with fungi and biochar. However, the research exploring the inoculation of biochar with fungi to support biochar sorption, mycoremediation, and phytoremediation is limited. Therefore, the **first** objective of this study was to evaluate the contaminant removal capacity of MCG biochar in comparison to *Trichoderma harzianum* inoculated biochar, *TH@MCG.* The **second** objective was to determine whether native wetland grass *Juncus patens* is capable of phytoremediation, and if so, whether *T. harzianum*-inoculated biochar enhances the phytoremediation capacity of the *Juncus patens*. The findings our study in relation to our objectives are as follows:

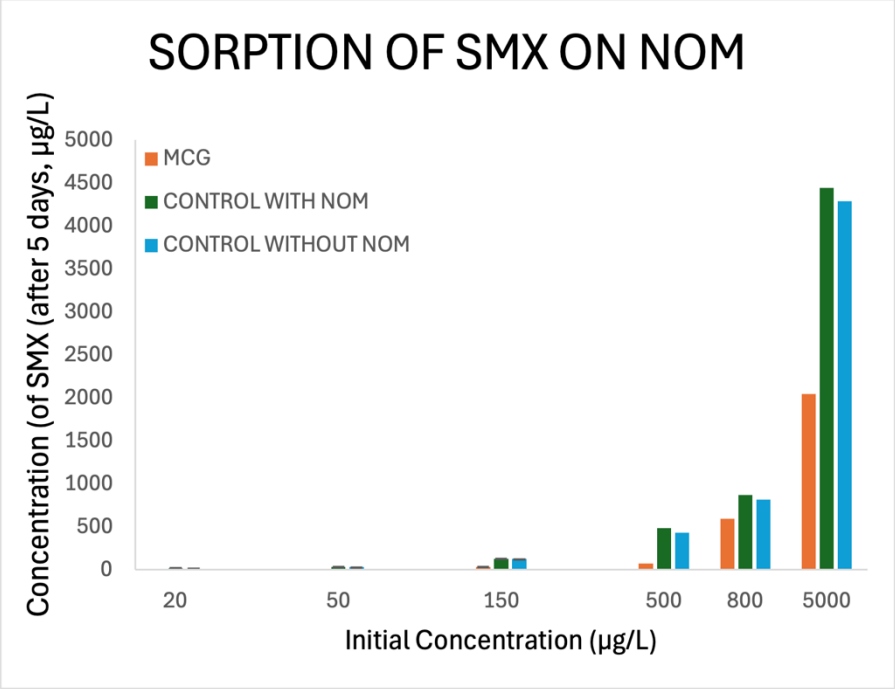
In relation to our **first** objective, batch isotherm sorption studies were conducted to effectively quantify MCG and *TH@MCG* contaminant removal capacity and model its sorption capacity using Langmuir fittings. MCG achieved higher sorption of ACT over BZT and SMX, and maintained 100% removal efficiency of BZT and ACT up to 50 µg/L in a mixed contaminant system (BZT, ACT, SMX in synthetic stormwater matrix). The inoculation of MCG with *Trichoderma harzianum (TH@MCG)* resulted in greater sorption of BZT over ACT and SMX. *TH@MCG* was also capable of achieving 100% removal of higher concentrations of all three contaminants (up to 250 µg/L each). These results indicated that *Trichoderma harzianum* enhanced removal capacity of MCG and *TH@MCG* had a greater affinity for removing BZT in comparison to MCG alone. Poor removal efficiency of SMX can be attributed to molecular structure of SMX, or unfavorable conditions for removal (e.g., NOM presence, pH, intermolecular interactions). Further research is required to determine the biological mechanisms *Trichoderma harzianum* utilized to enhance remediation of mixed contaminant synthetic stormwater (e.g., immobilization of contaminants via hyphal network, degradation of contaminants via enzymatic activity). Furthermore, our hypothesis that *T. harzianum*-inoculated biochar would improve removal efficiency of MCG biochar across contaminants was correct.

The **second** objective of this thesis was to determine whether native wetland grass *Juncus patens* could perform phytoremediation, and if so, whether *TH@MCG* enhanced the phytoremediation potential of the *Juncus patens*. A greenhouse mesocosm study was conducted to evaluate the efficiency of MCG only*, TH* only, and *TH@MCG* amended pots to mimic stormwater bioretention systems. Mesocosms (both planted and not planted) were subjected to weekly synthetic storm events spiked with BZT, ACT, SMX for 18 weeks, during which contaminant concentrations were increased from 100 µg/L to 10 mg/L at week 10. Across contaminants, the incorporation of *JP* significantly improved remediation efficiency in pots amended with *TH@MCG* throughout the study. Removal efficiency of ACT in planted control pots improved 20-30% with the incorporation of *JP,* whereas the removal efficiency of BZT in planted control pots improved 20-40% with the incorporation of *JP.* The incorporation of *JP* did not affect the removal of SMX in control pots. Because environmentally sourced soils were used for the mesocosms, improved removal of BZT and ACT can be attributed to either phytoremediation or microbial degradation enhanced by the rhizosphere of *JP.* Further analysis on *JP* biomass to accurately determine whether contaminants were bioaccumulated within the plant, or if removal in control systems were primarily due to soil sorption and microbial degradation. Further, *TH@MCG* improved plant height and reduced

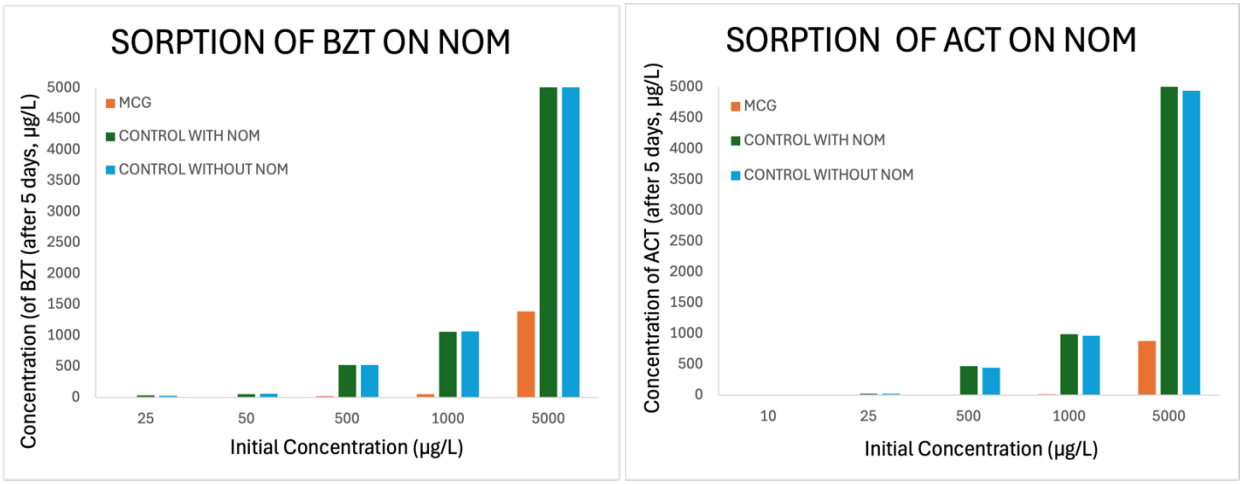
phototoxicity in comparison to control and *TH* only planted mesocosms. Furthermore, the mesocosms planted with *JP* only resulted in improved removal for *TH@MCG,* and not for *TH* only systems. Results signaled that *TH* only systems were less effective at removing contaminants with the incorporation of *JP.* This could indicate that the accumulation of contaminants and depletion of nutrients over time may have induced competition within the soil microbiome, reducing efficiency of *Trichoderma harzianum* (or *JP)* to degrade or sorb contaminants. *Juncus patens* may have also changed soil chemistry, structure, or released compounds that ultimately inhibited fungal growth or degradation capacity. Due to this, it likely that the inoculation of biochar with *Trichoderma harzianum* enhances soil conditions and encourages a synergy between *TH* and *JP* that promotes the removal of organic contaminants. Our results validated our hypothesis that *TH@MCG* improved plant health and growth but leaves room for exploration for the phytoremediation potential of *JP.*

Overall, *Juncus patens* planted in soils amended with *TH@MCG* holds a great amount of promise for the future of bioretention systems and stormwater runoff remediation. Both the batch tests and the greenhouse mesocosm study validated the enhanced removal capacity of *TH@MCG* across commonly found stormwater runoff contaminants. *TH@MCG* and MCG amended systems also maintained soil pH levels (between 7-8) without drastic changes, signaling suitable conditions for microbial activity and contaminant sorption (**Appendix 1.4**). Total organic carbon levels of mesocosms were relatively high across amendment types; however, this is likely due to the high volume of organic contaminants being introduced to the mesocosms every week. Additionally, systems containing *TH* and *JP* likely maintained higher total organic carbon levels due to microbial activity, release of root exudates, or decaying plant material. Because MCG and *TH@MCG* did not appear to reach their sorption capacity across contaminants, increased total organic carbon levels due to fouling of MCG seems less likely. Considering the improved remediation capacity and improved plant resilience within soils amended with *TH@MCG,* fungus inoculated biochar should be explored further to support the protection of human health, aquatic systems, and urban ecosystems. Fungal inoculation of biochar paired with plants capable of phytoremediation provides a cost-effective, sustainable solution to remediating contaminated stormwater runoff. Future studies should continue to explore the remediation potential of native plants and fungi, as opposed to introducing non-native and potentially invasive species to urban environments. Additionally, more in-depth research should be conducted to assess bi-product formation pathways of contaminants to ensure environmental safety and prevent further contamination.

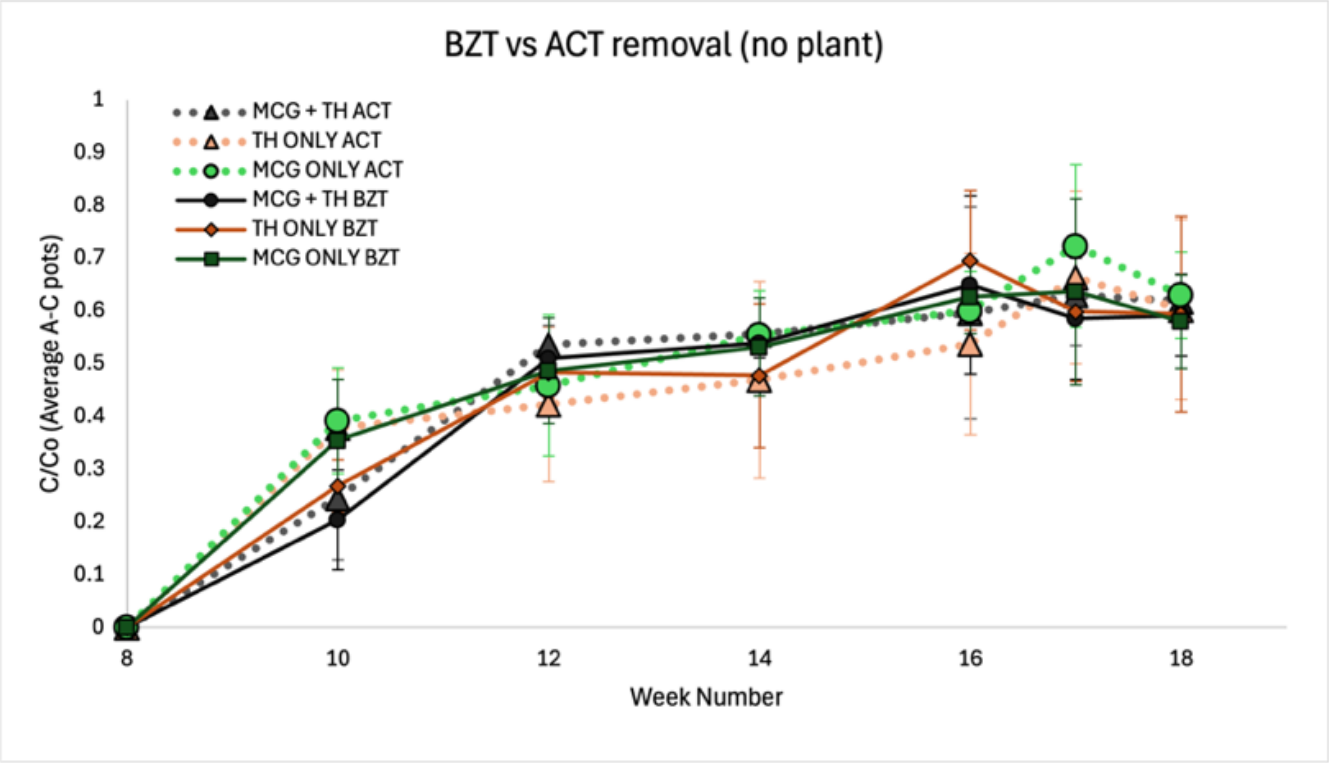
6. Appendix



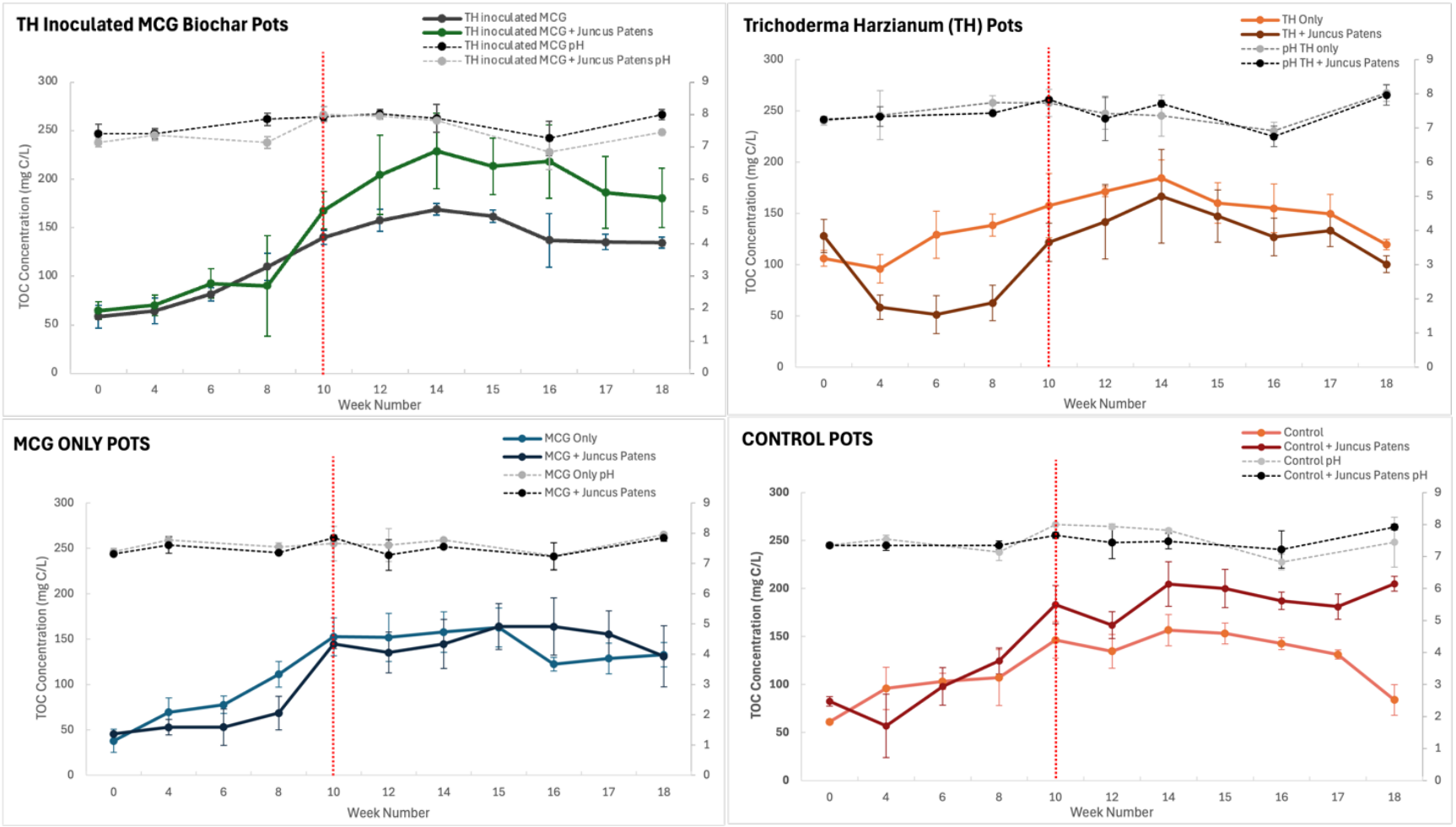
Appendix 1.1: Increased sorption of SMX by SRNOM as concentration in synthetic stormwater matrix increases (individual contaminant batch results).



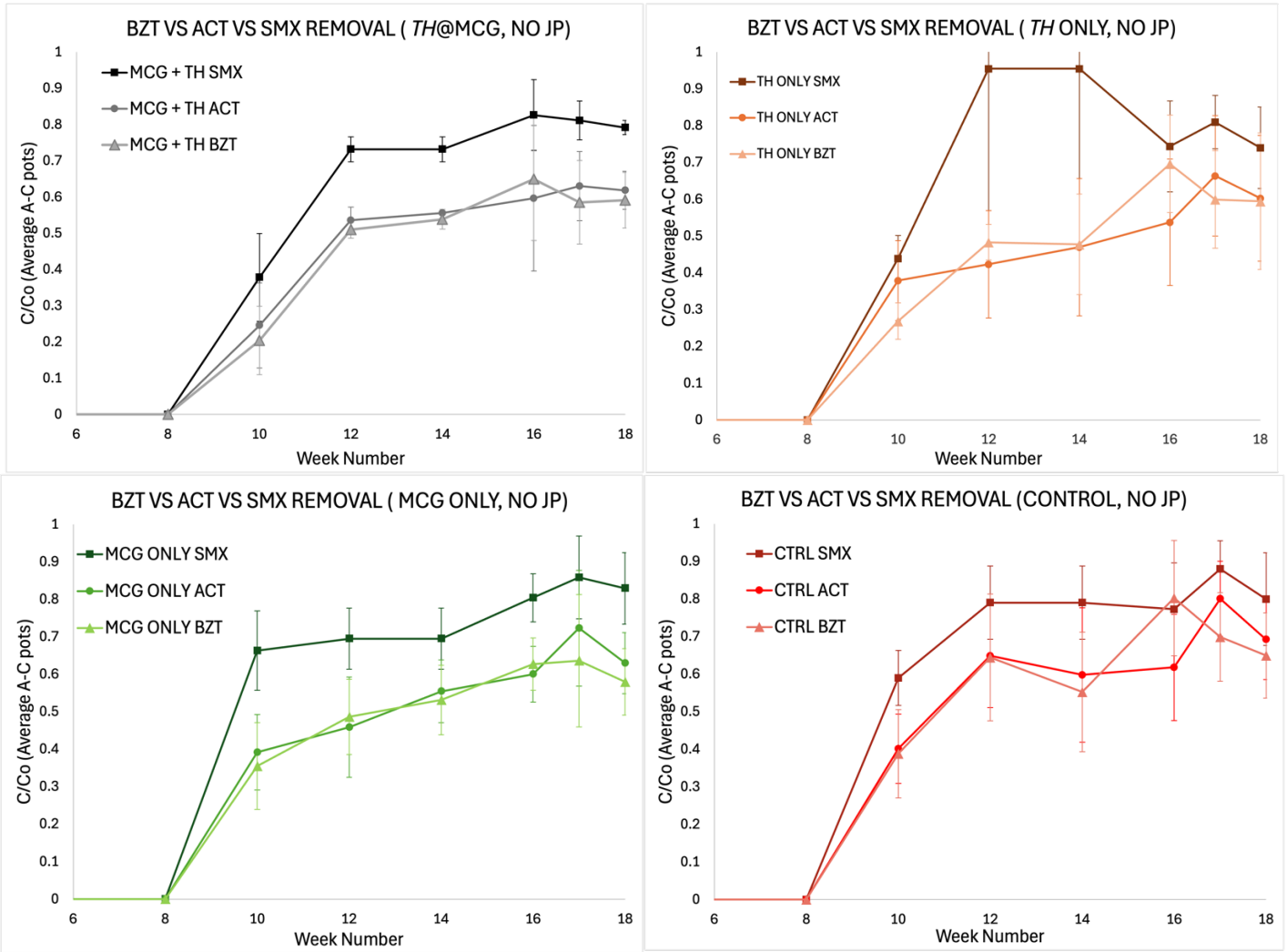
Appendix 1.2: Sorption removal of BZT and ACT by NOM as shown in figure, minimal removal across concentrations when comparing the batch test controls with NOM and without NOM.



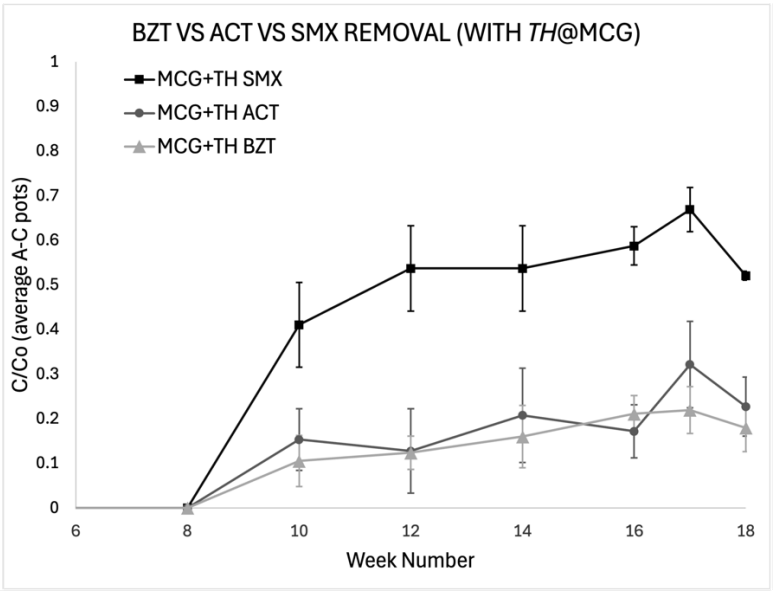
Appendix 1.3: Comparison of BZT and ACT removal efficiency over the duration of the greenhouse experiment in pots without plants, exemplifying the lack of significant differences in removal efficiency between amendments when *Juncus patens* is not present.

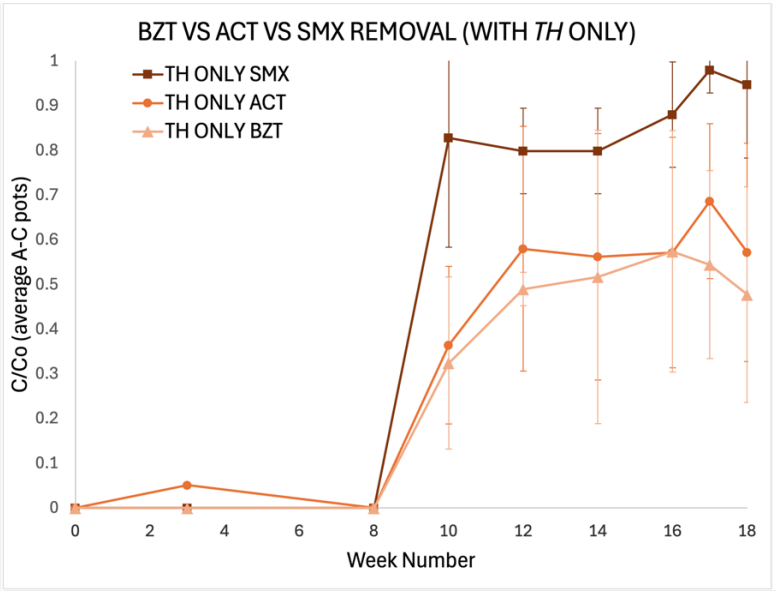


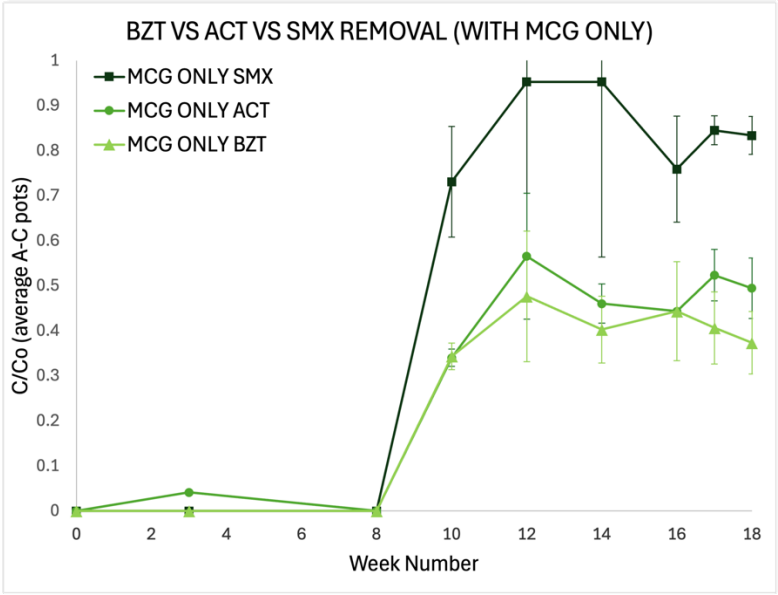
Appendix 1.4: Total organic carbon levels from pot effluent samples over the duration of the greenhouse pot study, separated by amendment type. The secondary, right-hand y-axis portrays pH levels over the duration of the study, and a red dotted line was used to indicate contaminant concentration increase at week 10.

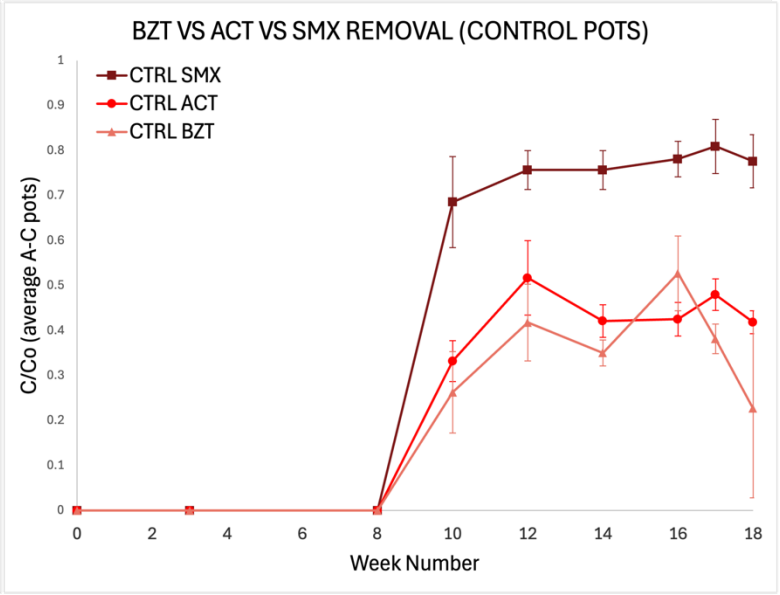


Appendix 1.5: Greenhouse pot study HPLC results from mesocosms **WITHOUT PLANTS**, separated by amendment type. Removal efficiency is plotted as a function of week number, allowing for comparative analysis of removal efficiency between contaminants introduced into each system.

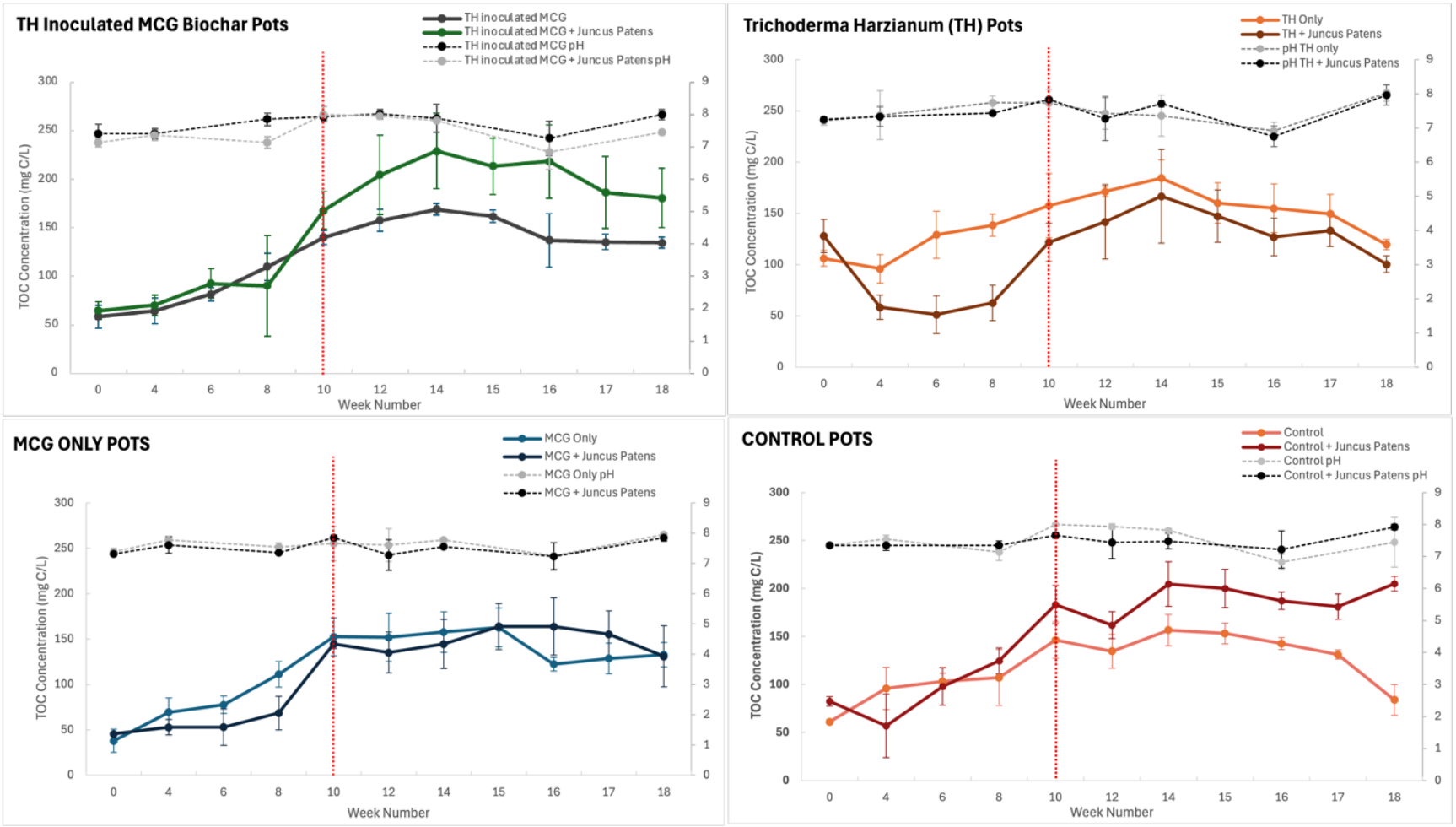

BZT VS ACT VS SMX REMOVAL (WITH *TH*@MCG)
MCG+TH SMX
MCG+TH ACT
MCG+TH BZT
C/Co (average A-C pots)
Week Number


BZT VS ACT VS SMX REMOVAL (WITH *TH* ONLY)
TH ONLY SMX
TH ONLY ACT
TH ONLY BZT
C/Co (average A-C pots)
Week Number


BZT VS ACT VS SMX REMOVAL (WITH MCG ONLY)
MCG ONLY SMX
MCG ONLY ACT
MCG ONLY BZT
C/Co (average A-C pots)
Week Number


BZT VS ACT VS SMX REMOVAL (CONTROL POTS)
CTRL SMX
CTRL ACT
CTRL BZT
C/Co (average A-C pots)
Week Number

Appendix 1.6: Greenhouse pot study HPLC results from **PLANTED** mesocosms, separated by amendment type. Removal efficiency is plotted as a function of week number, allowing for comparative analysis of removal efficiency between contaminants introduced into each system.



Appendix 1.7: Total organic carbon levels from pot effluent samples over the duration of the greenhouse pot study, separated by amendment type. The secondary, right-hand y-axis portrays pH levels over the duration of the study, and a red dotted line was used to indicate contaminant concentration increase at week 1.

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