Effect of Potentially Toxic Elements on Plant and Soil Biodiversity

Submitted by:

Muhammad Asad

Regn Number 328642

Supervisor

Dr. Ghulam Haider

Master of Science in Plant Biotechnology
Atta-Ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology
Islamabad, Pakistan
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Dr. Ghulam Haider
Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

Signature: ______________________

Name of Supervisor: Dr. Ghulam Haider

Dr. Muhammad Faraz Bhatti
Head of Department (HoD)
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

Signature (HOD): ______________________

Dr. Muhammad Faraz Bhatti

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of
Applied Biosciences (ASAB)
NUST, Islamabad

Signature (Dean/Principal) ______________________

Dr. Hussnain A. Janjua

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Examination Committee Members

1 Name: **Dr. Muhammad Faraz Bhatti** Signature:

Dr. Muhammad Faraz Bhatti
Tenured Professor
Head of Department (HoD)
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

2 Name: Dr. Rabia Amir Signature:

3 Name: Dr. Muhammad Bilal Khan Niazi Signature:

Prof. Dr. M. Bilal Khan Niazi
Chemical Engineering, SCME
NUST, Islamabad

Name of Supervisor: Dr. Ghulam Haider Signature:

Dr. Ghulam Haider
Associate Professor
Deptt of Plant Biotechnolog
Atta-ur-Rahman School of App
Biosciences (ASAB), NUST Islam

Date

Head of Department Date

Dr. Muhammad Faraz Bhatti
Head of Department (HoD)
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

COUNTERSIGNED

Date ____________________ Dean/Principal

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of
Applied Biosciences (ASAB)
NUST Islamabad

Declaration

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Signature of Student
Muhammad Asad
Regn Number: - 328642
Master of Science in Plant Biotechnology

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Dr. Ghulam Haider
Associate Professor
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

Supervisor
Dr. Ghulam Haider
Plant Biotechnology
ASAB, NUST

Dedication

“Dedicated to my exceptional parents, adored siblings and niece, whose tremendous support and cooperation led me to this wonderful accomplishment”

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Muhammad Asad

Table of Contents

List of Figures

Abstract

Microplastic (MPs, plastic particles <5 mm) contamination in terrestrial ecosystems has raised serious concerns due to their consistently increasing production with minimal recycling worldwide. Evidence suggests that the non-recycled plastics end up in landfills, passing through weathering and physical changes reach to micro sizes. MPs can easily leak into water bodies, sewage water/sludge, and agricultural soils. In addition, industrial effluents can also be the hotspots of MPs and other contaminants like heavy metals. Biochar, a carbon-negative technology has been proposed as a soil conditioner to improve soil properties and crop yield in less fertile soils. However, little is known about how biochar can interact in MPs and heavy metals (like chromium (vi)) contaminated soils, and secondly, if the presence of biochar can influence the effects of MPs and heavy metals on plant production and soil biology. Therefore, we investigated the effects of polyvinyl chloride (PVC) MPs, and Cr on the soil enzymatic activities and microbial community structure in a soil-plant system in the presence/absence of cotton stalk biochar. A 60-day microcosm experiment was conducted by the application of PVC-MPs (0, 0.5% (w/w)), Cr (50 mg/kg), and biochar (0, 0.5% (w/w)). Mash Bean (*Vigna mungo*) was taken as a test crop. Plant growth, physiology, soil enzyme activity, and microbial community structure were investigated using PLFA biomarkers analysis. The soil enzymatic activities (acid phosphatase (-41.30% and -42.50%), β-glucosidase (-36.53% and -44.04%), urease (-28.03% and -16.58%), and dehydrogenase (-16.83% and -43.56%)) were negatively affected in response to PVC-MPs and Cr contamination. A strong shift was also observed in microbial community structure (-8.93% and -36.43; indicated by PLFAs) in PVC-MPs and Cr treatments. However, the addition of biochar to PVC-MPs and Cr-contaminated soil significantly enhanced soil enzymatic activities and microbial community structure (0.24% and -25.87%). PVC-MPs (especially with Cr) reduced the microbial biomass carbon and nitrogen (-71.8% and -47.70%) however, biochar addition enhanced microbial biomass carbon and nitrogen (+15.69% and +9.48%). The findings presented here suggest that PVC-MPs and Cr have a significant impact on key pools and fluxes within the agroecosystem while the addition of biochar can be used as a soil amendment to improve the overall soil quality of MPs and Cr-contaminated soil. We conclude that PVC-MPs and Cr in the soil are not beneficial and therefore biochar should be added to minimize their entry into the agroecosystem and potential to transfer into the food chain.

CHAPTER 1

INTRODUCTION

Introduction

Plastics are synthetic man-made materials comprise of large molecules made up of chains of atoms. They are made from monomers which are chemical raw materials derived primarily from the petrochemical industry nowadays. Polymers are basically synthesized by adding one or more types of monomeric units to a chain of growing molecules. Carbon atoms are always present in these polymers along with other elements. Polymer chemists use only 8 of the more than 100 known elements to make thousands of different plastics (Alauddin et al., 1995) Polyethylene (PE), Polyethylene Terephthalate (PETE/PET), Polypropylene (PP), Polyvinyl chloride (PVC), and Polystyrene (PS) are some of the most commonly used plastics for different purposes. Polyethylene is a popular material that is widely used. The thermoplastic polymer is manufactured from ethylene monomers, whereas Polyethylene Terephthalate (PET) is a robust, complex synthetic polymer typically utilized to manufacture one-time-use beverage containers (Gross & Kalra, 2002). Polypropylene is made up of propylene monomers and is one of the most widely used plastics (Carniel et al., 2017). The use of polypropylene is a thermoplastic that may be fashioned into a variety of forms (Nakkabi et al., 2015). Polyvinyl chloride is the world's third most widely used synthetic plastic material after polyethylene and polypropylene. Before the addition of the plasticizers, it looks like a white breakable material. It is available in two basic forms: rigid and flexible (Vona et al., 1965). Polystyrene is a homopolymer made up of repeating styrene units. Depending on the type it can be either a thermoplastic or a thermosetting plastic. It is very inert which means it pollutes the environment whenever disposed off (Tokiwa et al., 2009). It is expected that plastic production will grow in the future due to the increase in living standards of the world's population (Awuah & Abdulai, 2022).

Plastic pollution is present all over the world and has expanded significantly in the previous 60 years from 1.5 million tons to about 335 million tons (Eriksen et al., 2014). It is expected that total plastic production will reach 34,000 metric tons (Mt) by 2050 (d’Ambrières, 2019). Plastic is made up of natural and synthetic polymers. Plastics such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) are used to make synthetic materials (Eriksen et al., 2014). Because of their low cost of manufacture, lightweight, adaptability , and durability, plastics are widely employed in a variety of sectors and all aspects of human activity (O’Connor & Lauenstein, 2006). Our dependency on plastic products is clear in this age of plastics (Thompson et al., 2009). A large amount of plastic trash is only recycled at the rate of 9%, burned at the rate of 12%, and buried at the rate of 79% (Geyer et al., 2017). In addition, there is still a large amount of plastic trash in the terrestrial environment.

Microplastics (MPs) have gotten a lot of attention in recent years because of their widespread presence in the aqueous environment; a biological threat to species and non-biodegradable capabilities (Thompson et al., 2004). The U.S. National Oceanic and Atmospheric Administration (NOAA) sponsored the first international conference on the "microplastics" problem. According to NOAA Plastic debris can come in all shapes and sizes but plastic materials having a size of less than 5 mm in diameter are specifically termed “microplastics” (Betts, 2008). These are either directly released into the environment or synthesized when larger plastic waste degrades.

Microplastics are classified as primary or secondary microplastics depending on how they are produced. Primary microplastics are manufactured on a micro level such as microbeads in cosmetics. While the degradation of macroplastic produces secondary microplastic (Horton, Svendsen, et al., 2017).

It has been found that microplastics are contaminating a wide range of aquatic environments but currently, there are a lot of studies that show that microplastics are also present in the soil environment (Lambert & Wagner, 2018; Zhang & Liu, 2018). The soil might represent a huge reservoir of microplastics and these microplastics may cause a danger to soil biodiversity and the functioning of the ecosystem (He et al., 2018). Soil-living organisms can easily ingest microplastics which potentially affects their fitness and survival (Scheurer & Bigalke, 2018). One important aspect influencing the UNEP’s (United Nations Environment Program) decision was the reality that soil is probably a more crucial sink for microplastics. It is predicted that plastic released annually to the terrestrial surroundings is four to twenty-three -fold more than that released to the marine ecosystem (Horton, Walton, et al., 2017). Microplastics can enter the soil through many paths for instance; amendment of soil with compost and sewage sludge, irrigation, flooding, plastic mulching, littering and deposition of atmospheres. Due to poor control practices, a huge quantity of plastic mulching residues stays in soil; degradation via ultraviolet (UV) radiation and bodily abrasion methods effects in the accumulation of microplastics in soil (Bläsing & Amelung, 2018). Microplastics in the soil may cause adverse effects in a soil environment. They may change soil physical properties, reduce soil fertility and upset local microbial populations, thus affecting the quality of soil and nutrient cycling (Wan et al., 2019).

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCHs), pharmaceuticals and personal care products (PPCPs), pesticides, perfluoroalkyl substances (PFASs), and heavy metals are just some of the many classes of potentially toxic chemicals that microplastics tend to absorb (Seidensticker et al., 2018). MPs can be harmful to human health since they impact plant growth and development in addition to soil health (Rillig et al., 2018). Changes in soil structure and plant growth are both influenced by MPs. MPs' alterations to soil physicochemical qualities will have unfavorable effects on plant root properties, growth, and nutrient absorption (Wan et al., 2019; Xu et al., 2020). Numerous studies showed that MPs induced ecotoxicity and genetic toxicity in plants, slowed seed germination, stunted plant development, etc. (Bosker et al., 2019; Qi et al., 2020).

The spatial configuration of the entire root system inside the soil is referred to as the root system architecture (Lynch, 2007). There are various root system characteristics or components that essentially constitute a specific root system architecture. The primary root branch, root density, lateral root branch, root length, root diameter, root angle, etc. are some of these components (Kuijken et al., 2015). The root architectural features respond and operate under the needs of the plants and the soil microenvironment, and they are modified. Therefore, root system architecture under the effect of abiotic stress plays a significant part in the adaptation of the plant to the environment. (Lynch, 2007) contends that to boost a crop's ability to adapt to a variety of stressful circumstances and hence increase crop yield, greater focus should be placed on changes to the root system. When a crop is subjected to drought and a nutrient-poor environment, altering its root system design can significantly enhance production (Wasson et al., 2012).

Heavy metals are high-density metals or metalloids known for their potentially toxic effects on the environment. Although these heavy metals are naturally present on Earth, they are enriched by human activity and take entry into plants, animals, and human tissues by diet, inhalation, and manual handling (Gill, 2014). The toxicity of heavy metals in plants depends on the type of plant, specific metal, concentration, chemical form, soil composition, and pH, as many heavy metals are considered essential for plant growth (Yaashikaa et al., 2022). Chromium is highly toxic to plants and inhibits various morphological, physiological, and metabolic activities of plants, and can even cause complete damage (Shanker et al., 2005). Plants infected with chromium causes ulcer, liver, and kidney cancer in humans (Zaheer et al., 2020). Many researchers have noted that chromium stress hurts root cells and young leaf yellowing which reduces the rate of photosynthesis and decreases biomass (Javed et al., 2021).

Biochar is a by-product of biomass pyrolysis in a low oxygen atmosphere. It contains a porous carbonaceous structure and many functional groups (Major et al., 2009). Biochar is a promising resource for soil fertility management because of its economic and environmental benefits. In addition, it could be also proposed that biochar loaded with ammonium, nitrate, and phosphate act as a slow-release fertilizer to enhance soil fertility (Kammann et al., 2015; Schmidt et al., 2015). It has been reported that biochar improves not only the chemical and physical properties of the soil but also the microbial properties of the soil. Numerous scientific investigations have demonstrated that incorporating biochar into soil improves soil structure, porosity, bulk density, aggregation, and water retention (Baiamonte et al., 2015). Biochar also increases the composition of the soil biological community (Grossman et al., 2010).

Mash bean (*Vigna mungo*) is a popular legume in Pakistan due to its importance in the diet (Bhattacharya et al., 2004). The root of mash bean has a sedative and diuretic effect and is used to treat nostalgia, abscesses, bone pain, dropsy, headaches, and inflammation while the seeds are useful in treating scabies, aches and pains, nosebleeds, asthma, heart attacks, constipation, nervous breakdowns, partial paralysis, facial paralysis and memory weakness (Anitha et al., 2012; Battu et al., 2011). Pakistan's agricultural sector relies heavily on mash beans, which are planted on an annual area of 27.6 thousand hectares, yielding 13,6000 tons with an average yield of 493 kilograms per hectare (Ahmed et al., 2012). It is grown all over the country, but cultivation is mainly concentrated in Punjab, the main province that produces mash. It is the least researched of all the crops in Pakistan, as evidenced by its very limited literature and therefore its area of cultivation and production is decreasing (Achakzai & Taran, 2011). The study involves the following main objectives:

- Identification of microplastics and trace metals effects on plant and soil biodiversity.
- To determine, if the combination of heavy metals with microplastics synergies the negative effects (if any) on plant and soil biodiversity?
- If biochar amendment in MPs and trace metals contaminated soils can reduce the negative effects on plant and soil biodiversity

CHAPTER 2

LITERATURE REVIEW

Review of Literature

Plastics

The long chain-like molecules that make up plastics have a very high molecular weight, making them a sort of synthetic organic polymer. Hydrocarbons are the primary component of many popular plastics, and they are often sourced from fossil fuel feedstocks (Jambeck et al., 2015). There are more than fifty different families of plastics and hundreds of different plastic goods available today, making plastic a rather general term. Plastics are an extensively utilized material in both industry and everyday life because of their low production cost, versatility, and ease of use (Rosato et al., 2004). Plastics as a readily available, cost-effective, and convenient material, are widely used in industries and our daily lives and they provide a great deal of convenience to humanity. Along with its practical application, it has recently been identified as a global environmental threat (Ali et al., 2021; Cheng et al., 2021). Plastic production has increased rapidly in the last 60 years, reaching 288 million tonnes annually, and is expected to reach 33 billion tonnes by the end of 2050 (Browne et al., 2013). A considerable amount of plastics have accumulated in the environment, which is probably caused by the increasing demand and use of plastic items (Kumar et al., 2021). Because of its widespread distribution and associated environmental and ecological consequences, the problems posed by plastic debris have raised global concerns (Obbard et al., 2014; Sun et al., 2018). Plastic waste pollutes the environment and will not degrade in the natural environment even in 100 years (Ricardo et al., 2021). By 2025, 11 billion tonnes of plastics are expected to accumulate in the environment according to reports. Plastics on the other hand have a recovery rate of less than 5% at present (Brahney et al., 2020).

Types of plastics

Thermoplastics and thermosets are two different types of plastics.

Thermo plastics

When heated, this type of plastic softens but then hardens again. This class includes over 80% of all plastics. HDPE is used in things like detergent bottles, food packaging, plastic toys, and plastic pipes. Cling film, flexible containers, and trash can liners are all examples of low-density polyethylene (LDPE). "PET" stands for "polyethylene terephthalate," Polypropylene (PP) helps make milk crates, margarine pots, motive parts, fibers, etc., Polyvinyl chloride (PVC) is made from salt and oil and is used in medical products, pipes, plastic flooring, window frames, wall papers, etc. (Gross & Kalra, 2002).

Thermosets

This is a type of plastic that hardens during the curing process but cannot be remolded or remelted. They account for 20% of all plastics manufactured. An example includes. Polyurethane (PU) is used in vehicle seating, mattresses, various finishes, coatings, and so on., Epoxy is most used for adhesive purposes, but it is also used to make sports fixtures, boat castings, and auto parts and Circuit electric boards, oven sealing covers, and auto parts are among the most common uses of phenol (Gross & Kalra, 2002).

Human activities are responsible for a significant loss of biological diversity around the world, and the problem is so serious that the combined effects of human activities could have accelerated current extinction rates to 1000–10,000 times the natural rate (Reaka-Kudla, 1997). Plastics are widely used in a variety of industries and all aspects of human activity due to their low manufacturing costs, lightweight, adaptability, and durability (O'Connor & Lauenstein, 2006). Plastic pollution is found all over the world and its volume has increased dramatically over the last 60 years, from 1.5 million tons to around 335 million tonnes. Synthetic materials are made from plastics such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) (Eriksen et al., 2014). By 2050 overall plastic manufacturing is predicted to reach 34,000 metric tonnes (Mt) (d'Ambrières, 2019). Because they are buoyant, a growing amount of plastic debris is being dispersed over long distances and once they settle in sediments they may last for centuries (Debris, 1990; Goldberg, 1997; Goldberg, 1995; Ryan, 1987). Globally, 280 million tonnes of plastic were produced in 2012. Only about half of it was disposed of in landfills or recycled. Some of the remaining 150 million tones may still be in use, while the remainder litters continents and oceans (Rochman et al., 2013). Between 1950 and 2015, a total of 6300 million metric tonnes (Mt) of recycled plastic waste was generated. Approximately 800 Mt (12%) of plastics were incinerated, and 600 Mt (9%) were recycled, with only 10% being recycled numerous times. Around 4900 Mt of plastics were discarded, accounting for 60% of all plastics ever produced, and are now accumulating in landfills and in the natural environment.

Microplastics

Most of the trash in our oceans and Great Lakes is plastic. According to National Oceanic and Atmospheric Administration (NOAA), microplastics are defined as plastic trash that is five millimeters or less in length (or roughly the size of a sesame seed). Richard C. Thompson first proposed the concept of microplastics in 2004 by defining microplastic as tiny plastic debris with a diameter of less than 20 mm (Thompson et al., 2004). Subsequently, in 2009 the size of microplastics was defined as less than 5 mm (Ryan et al., 2009). Microplastics are found all over the globe from the soil to the water and the atmosphere (Amélineau et al., 2016; La Daana et al., 2018; Nor & Obbard, 2014; Peeken et al., 2018; Su et al., 2016). Microplastic distribution and risk assessment in the environment has also been documented (Y. Zhou et al., 2020). Chemical pollutants and pathogens can spread through microplastics (Caruso, 2019; Wu et al., 2019). Microplastics can persist in nature for a long time due to slow degradation rates causing severe environmental pollution (Lithner et al., 2011; Wright & Kelly, 2017). Inflammation of the digestive system reduced nutrient absorption, and effects on growth and reproduction are all potential hazards of microplastics to organisms (Hurley et al., 2017; Sussarellu et al., 2016; Von Moos et al., 2012). are readily absorbed by biota and can bind to pollutants on their surfaces. MPs are being consumed by marine life including mammals, fish, and invertebrates according to recent studies on their environmental effects (Cole et al., 2011; Lusher et al., 2013; Setälä et al., 2014). Many studies on microplastics have been conducted to better understand their impact on the natural environment and human health (Anik et al., 2021; Ge et al., 2021; Guo et al., 2020; Koutnik et al., 2021; Ragusa et al., 2021; Ren et al., 2021; Sharma et al., 2021; L. Yang et al., 2021). The sources, movement, and toxicology of microplastics in the environment, for example, have all been studied by (Guo et al., 2020).

Sources of microplastics

Microplastics can be categorized in a variety of ways. They are classified as primary or secondary microplastics depending on their origins. Primary microplastics are plastic microbeads that are directly used in cosmetics and other items and then discharged into the aquatic environment because of human activity. Secondary microplastics, on the other hand, are plastic pieces created by larger plastic waste fragmentation and volume decrease due to degradation processes. Biodegradation, physical degradation, photodegradation, and chemical degradation all produce secondary microplastics from plastic deterioration (Corcoran et al., 2019; Da Costa et al., 2018; Klein et al., 2018; Pathak, 2017). Primary MPs are released in their original plastic state from products that contain MPs, such as clothing, toothpaste, cosmetics, and so on, usually in the form of microfibers, beads, and pellets (Conkle et al., 2018; Wang et al., 2019). Large-scale plastic disintegration or degradation, such as natural weathering, mechanical decomposition, oxidation, and degradation of manufactured plastic items while being used and recycled results in secondary MPs (Rezania et al., 2018). MPs have been found in shower gels, eye shadows, peelings, foundation creams, bath lotion, nail paint, sunscreen, insect repellents, hair color, and blush powder according to various studies implying that the personal care market is the primary source of primary MPs (Malankowska et al., 2021; Vickers, 2017). Face cleaning scrubbers, air blasting media, and cosmetics all contain the primary MPs particles. There are a variety of products based on primary MPs, such as plastic pellets and drug vectors (Isobe et al., 2014).

Microplastics in soil environments

MPs are found in large quantities in the soil. Plastic film mulching, sludge utilization, organic fertilizer application, wastewater irrigation atmospheric deposition, and other sources of soil MPs have all been reported in numerous studies (Allen et al., 2019; Ng et al., 2018; Sintim & Flury, 2017; Weithmann et al., 2018).

Plastic mulching and microplastics

Plastic films have been reported to cover more than 128,652 km2 of farmland around the world (Gao et al., 2019). The volume of MPs in mulching cropped soils was more than twice as high as in non-mulching cropped soils according to a survey of agricultural soil samples (B. Zhou et al., 2020). The use of sludge as a source of soil MPs has been studied and it was discovered that about % of MPs in WWTPs (wastewater treatment plants) are retained in the sludge (Ziajahromi et al., 2016). The use of plastic mulch in agriculture is a useful exercise that increases crop yield by enhancing soil moisture, temperature, and pest assault (Liu et al., 2022). Polyethylene, low-density polyethylene, and high-density polyethylene make up the majority of plastic mulch (Xiong et al., 2020). In China, 1.4 million tons of polyethylene plastic film, covering 15% of the agricultural land, were utilized in 2018 (Huang et al., 2020). Despite the positive aspects of using plastic mulch, landfill disposal or plowing mulching are not environmentally friendly disposal methods (Havstad, 2020). Consequently, the continued addition of plastic residues to agricultural land causes a significant formation of microplastics (Liu et al., 2017). MPs concentrations were higher in mulched soil (754 ± 477 items kg–1), compared to non-mulched soil (376 ± 149 items kg–1), and MPs in mulched soil increased over time (Zhang et al., 2021).

Microplastics in wastewater

Irrigated agriculture frequently uses domestic wastewater as a source of water. Untreated wastewater, on the other hand, contains a lot of MPs. It was testified that washing 1 kg of laundry in a washing machine releases 308 mg of MPs with the relating MPs content in the discharged sewage reaching 1.5 million (De Falco et al., 2019). Common practice is using wastewater for agricultural land, especially in arid regions where fresh water is insufficient to supply the fields (Ashraf et al., 2018). Primary and secondary microplastics from industrial and domestic waste that are brought to wastewater treatment plants are present in untreated wastewater (Sol et al., 2021). Soil MPs contamination can be caused by several sources, including the widespread manufacture and use of plastic dinnerware, the washing and wearing of automobile tyre, and microbeads in face cleansers and body shower gel products (Bläsing & Amelung, 2018). As MPs collect, they will have both direct and indirect effects on soil qualities. The hydrophobic nature of most MPs has implications for soil water retention, movement, and availability (de Souza Machado, Lau, et al., 2018). Environmental problems are exacerbated because smaller MPs are more easily absorbed by soil organisms and can obstruct soil micropores (Choi et al., 2021).

In recent years, attention to MP contamination has shifted from the sea to the terrestrial environment (de Souza Machado, Kloas, et al., 2018). Approximately 80% of the plastic waste found in the ocean comes from land. As a result, soil could be a large sink for MPs from a variety of sources including wastewater irrigation, bio solid utilization, atmospheric deposition, sewage sludge land use, and plastic film mulching (Andrady, 2011; Wang et al., 2021).

Organic fertilizers and microplastics

The best way to improve soil fertility, carbon sequestration, crop nutrition, crop yield, and organic matter is by using compost (Amanullah et al., 2019). Manure, plant residues, municipal waste, and sewage sludge can all be mixed to produce compost (Brock et al., 2021). Industrial and household waste contain plastic that mechanically and physically decomposes into microplastic, which is unfortunately added to the composting process and used as organic fertilizer in agriculture (Liu et al., 2018). When it comes to compost, macroplastic has previously received more attention than microplastic, which is more prevalent. Even though many composting facilities pretreat waste to reduce plastic debris, there is still a significant amount of plastic in the compost (Gui et al., 2021).

Effect of microplastics on soil

Food, wood raw material for infrastructure, prevention from flood nutrient cycling, carbon sequestration, and biodiversity are just a few of the many ecosystem services that soil provides to humans (Kopittke et al., 2019). However, anthropogenic activities like industries contribute to an increase in soil contamination that harms living things (Ye et al., 2019). The MP contamination that has a significant impact on the soil's function and biodiversity confronted the soil, which is a crucial component of the terrestrial ecosystem(de Souza Machado, Lau, et al., 2018). Microplastic contamination can alter soil structure, so it's important to comprehend how MPs affect the chemical, physical, and biological characteristics of soil (Liang et al., 2019).

Effect of microplastics on microbial communities

Mycorrhizal fungi and symbiotic bacteria are two examples of microorganisms that oversee providing benefits to agriculture (Jacoby et al., 2017). The enzymatic activity and types of microorganisms determine 80–90% of biological activity (Furtak & Gajda, 2017). Numerous soil nutrients are provided by microorganisms, which also improve soil fertility, assist in the recycling of organic waste, and shield the soil from pathogens (Mengqi et al., 2021). Soil microorganism management is crucial for both sustainable agriculture and the safety of our food supply (Bertola et al., 2021). However, anthropogenic activities hurt the diversity of soil microbes, which may negatively affect the health of the soil (T. Yang et al., 2021).

Microplastic interacts with microbial communities and influences their biological activity, according to recent studies (Sun et al., 2022; Wang et al., 2020; Zhang et al., 2019). Polyethylene and polypropylene had a larger impact on bacterial communities than polystyrene, while MPs with various sizes, shapes, and polymers had different effects on bacterial communities (Sun et al., 2022). Additionally, microbial communities are affected by soil physiochemical characteristics such as soil aggregate. The shape of MPs is connected to the physical characteristics of the soil, film, fiber, and foam due to which MPs have a detrimental effect on the bacterial communities (Sun et al., 2022). According to research, soil physical characteristics such as bulk density, porosity, evaporation, and soil moisture are related to the abundance and depletion of MPs (M. Huang et al., 2019). The diverse bacterial phyla were enhanced by MPs included in some members of the bacterial phylum bacteroidetes that can infect plants with hoof rot diseases, which poses a hazard to the agricultural ecosystem (Wang et al., 2019; Zhang et al., 2019).

Effect of microplastics on soil enzyme activity

The breakdown of organic matter and the cycling of nutrients are both significantly influenced by soil enzymes. Some enzymes, such as hydrolase and glucosidase, only assist in the breakdown of organic matter, whereas other enzymes are involved in the mineralization of nutrients (e.g., amidase, urease, phosphatase, sulphates) (Adetunji et al., 2017). The synthesis of soil enzymes is crucial for nutrient cycling and energy flow (Chen et al., 2018). Indicators of soil health include microbial activity, catabolism of organic molecules in the soil, and soil physical characteristics. Soil enzymes play a significant role in soil health (Farooq et al., 2021).

Depending on the type and concentration of the MP's, soil enzyme activity may be increased (Fan et al., 2022). The addition of polyamide beads, polyacrylic fibers, polyethylene fragments, polyester fiber (de Souza Machado, Kloas, et al., 2018), and polypropylene MPs increased the FDAse activities (Liu et al., 2017). The addition of polyethylene MPs increased the activity of the enzymes i.e., urease and catalase, but only a slight increase occurred in the activity of the enzyme invertase (Y. Huang et al., 2019). Alkaline phosphatase in polyethylene increases by about 20.8%, catalase by about 38%, and urease by about 63.2% when polystyrene MP is added (Fan et al., 2022). However, the activity of catalase, phenol oxidase, urease, manganese peroxidase, laccase, and -glucosidase is reduced by polyethylene MPs (Karaca et al., 2010).

Effect of microplastic on plants

Microplastics have an impact on plant physiology and growth. After 48 hours of exposure to microplastics, a significant reduction in growth was seen in the *Vicia faba* plant. MP caused a decrease in plant biomass and the catalase enzyme, but an increase in the peroxidase and superoxide dismutase enzymes. MP causes *V. faba* to be genotoxic and to suffer from oxidative damage (Jiang et al., 2019). The smaller MP became more toxic as its size got smaller. 0.2 μm microbeads can easily translocate to stem and leaves through the apo plastic pathway, according to some studies on the translocation and accumulation of MP beads in wheat plants (Li et al., 2019). Through the apoplastic pathway, MPs and NPs can quickly enter plant tissues and move toward various parts of the plant (C. Li et al., 2020; Liu et al., 2021). Compared to larger microplastic, the 0.2 micrometer microplastic translocates effectively in the wheat, carrot, and lettuce plants (C. Li et al., 2020). PSMP of sizes 500 and 700 nm accumulated in plant aerial parts such as the cucumber plant's stem, leaf tissue, calyx tissue, and fruit. There were no traces in the leaves, but there was an accumulation of large MPs (one micrometer) in the carrot stems (Dong et al., 2021).

The MPs' component can lessen the density of root tissue (de Souza Machado et al., 2019). Low-density polyethylene has a less negative impact on the root, shoot, and fruit biomass in the *Triticum aestivum* than biodegradable microplastics from the mulch (Qi et al., 2018). Different sizes of microplastic on Lepidium sativum reduce the length of the plant's roots, and MPs that accumulate in the pores of the plant's seed coat can hinder its ability to absorb water (Bosker et al., 2019). The component of the MPs can reduce the root tissue density in *Allium fistulosum* (de Souza Machado et al., 2019). With the inoculation of the 300-500nm polystyrene plastics, the cucumber plant's root diameter decreases (Z. Li et al., 2020). As the concentration of polystyrene MPs and arsenic increases, the biomass of the root and leaf of the Daucus carota carrot decreases (Dong et al., 2021).

The chlorophyll absorbs the scattered sunlight and radiation, which is a crucial part of photosynthesis (Kume et al., 2018). However, in the *Lolium perenne* ryegrass, MPs have an impact on the ratio of chlorophylls a and b (Boots et al., 2019). In Vicia faba, an accumulation of MPs can result in a blockage of the cell connection, which affects the transport of minerals through the water. Additionally, 100 nm fluorescent polystyrene MPs have higher toxicity and oxidative damage than 5 mm PS-MPs (Jiang et al., 2019). Rubisco's activity declines when arsenic and PS microplastics are present (Dong et al., 2021). In response to antibiotic stress, plants release a variety of antioxidant substances (Zhang & Liu, 2018). When contaminated with polystyrene MPs, the malondialdehyde and proline content of the cucumber Cucumis sativus increases (Z. Li et al., 2020).

Another study in *Zea mays* maize found that the coexistence of heavy metal and MPs had an impact on plant function and the symbiotic relationship between the plants and arbuscular mycorrhiza (Wang et al., 2020).An increase in the growth of the *Hieracium* could result in a reduction in the growth of the *Festuca* species under drought conditions due to the allelopathic nature of *Hieracium* as the allelopathic species can reduce the growth and performance by about 25%, according to a community study of plants (Zhang et al., 2021). MPs particles in the apple and carrot may be caused by their complex root systems and high levels of vascularization, but more research is needed to confirm this (Conti et al., 2020).

Impact of microplastics on soil physical properties

The shape of the MP's, which comes in a variety of forms including fragments, fibers, spherical, column, and foam, is the key aspect that influences the soil's physical characteristics (Wang et al., 2022). Fibers are the most prevalent MPs in terms of morphology, and because of their linear shape, they can have an impact on how soil aggregates (Zhang & Liu, 2018). Soil aggregate stability is the main soil physical property that aids in the prevention of erosion, providing aeration, soil fertility, and soil aeration (Liang et al., 2019; Liu et al., 2017). water stable soil aggregates are crucial to quantify the soil structures and examine the behavior of MPs that these contaminants interact with the soil aggregates (Zhang & Liu, 2018). MPs fiber participates in the aggregation of the soil and acts as the binding agent (Gao et al., 2019). According to (Zhang & Liu, 2018) 72% of MPs participate in the soil aggregation, with 16% contributing micro aggregates, 56% contributing macro aggregates, and the remaining 28% in a desperate situation.

MPs may have a deleterious impact on soil aggregation (de Souza Machado, Lau, et al., 2018; Lozano et al., 2021; Zhao et al., 2021). According to morphology, the MP fiber reduced soil aggregation by about 29%, films by 20%, and 27%, whilst LPDE foams reduced polypropylene by 32% and polycarbonate by around 31% (Lozano et al., 2021). MPs have a detrimental impact on the aggregation of the soil, according to a meta-analysis study(Z. Li et al., 2020). Additionally, a different study found that the aggregation of soil was negatively impacted by the presence of microplastics in organic matter such as wheat straw and *Plantago* (Liang et al., 2019).

Impact of microplastics on soil chemical properties

Numerous studies suggested that MPs could have an impact on the chemical characteristics of the soil, including its pH, organic matter (Liu et al., 2021), and nutrients (Z. Li et al., 2020). The pH of the soil was often raised by MPs. (Lozano et al., 2021) discovered a rise in pH when 0.4% w/w MPs were used to inoculate the soil. Some research, however, showed low pH or no appreciable impact on pH (Boots et al., 2019). The pH was lowered by high-density polyethylene MP’s but was unaffected by PLA MPs. Other variables are also to blame for pH changes. For instance, when MPs degrade under light, they release chemical additives that change the pH of the soil (Bandow et al., 2017). Additionally, the MPs alter the microbial communities that affect the soil's pH (Cheng et al., 2021).

For the health of the soil, organic matter in the soil is important. Results regarding the impact of MPs on soil organic matter are conflicting. In soils that are alkaline rather than acidic, MP has a greater impact on soil organic matter, and this effect grows as MP concentration rises (Liu et al., 2021). Soil organic matter increased in the presence of MP because the MP itself is the carbon source and released carbon in the soil (Lee et al., 2021). Bacteria that break down plastic in soil accelerate MP's breakdown and increase soil carbon content (Zhang et al., 2019). However, some research revealed that MP-injected soil had little SOM or a considerable impact (Dong et al., 2021; Ren et al., 2021).

In the presence of MPs, soil nutrients may be increased, decreased, or unaffected. For instance, PVC-MPs reduced the available nitrogen and phosphorus in the rice field by roughly 13% and 30%, respectively (Yan et al., 2021). Under low carbon circumstances, PLFA MPs increase NO3-N and NO2-N (Chen et al., 2018). Additionally, in a different study, the phosphorus level rose in plasticized PVC and fell in non-plasticized plastic (Yan et al., 2021). Furthermore, it was discovered that the high dibutyl phthalate level in PVC had a significant impact on the nitrogen characteristics of soil (Zhu et al., 2022) indicates that plasticizers may have a significant impact on nutrient availability as MP can affect the AMF community, which plays a role in the availability of P, other factors can also have an impact on the availability of nutrients.

Interaction of microplastics with heavy metals

Chromium (Cr), cadmium (Cd), nickel (Ni), arsenic (As), copper (Cu), and mercury (Hg) are heavy metals that are frequently referred to as metalloids. However, when these metals exceed the legal limit, they might affect the soil's characteristics and plant growth (Alengebawy et al., 2021). Heavy metals pose a severe hazard to the environment since they are persistent and irreversible contaminants (Z. Li et al., 2020). Through anthropogenic operations (Chen et al., 2015) including mining, applying pesticides and fertilizers, smelting, and burning fossil fuels, heavy metals can contaminate agricultural soil (Li et al., 2019). 500 million acres of land worldwide have 500 million places where the soil has more heavy metal contamination than is allowed (Liu et al., 2018).

The interaction of MP with heavy metals has only been the subject of a few investigations. (Pinto-Poblete et al., 2022) show that after being inoculated with the MP, the Cd concentration in the soil and roots rose. The distribution of heavy metals is also influenced by the soil aggregates (Jiang et al., 2019). Additionally, soil aggregation is impacted by microplastic pollution. The size of the microplastic (MP) affects its capacity to absorb the heavy metal, with smaller MPs having a higher capacity than bigger ones (Gao et al., 2019). The MP increases soil desorption capacity and decreases soil desorption capacity, both of which improve the bioavailability of metals. It has been suggested that the functional group of the MP and pH affect the adsorption capacity of cadmium on MPs, which is why microplastic acts as a carrier for heavy metals (Z. Li et al., 2020).

Interaction of microplastics with biochar

The production of biochar at low temperatures with little oxygen can enhance soil organic carbon and aid in the sequestration of carbon in the soil (Shi et al., 2022; Wang et al., 2019). Gram-positive, gram-negative, and fungus populations all rise thanks to biochar's provision of habitat for microbial communities (Wang et al., 2020; Ye et al., 2019; Zhang & Liu, 2018). The enzyme activity in the soil is increased by biochar. According to numerous research, applying biochar helps remove a variety of contaminants (Diao et al., 2020; Yang et al., 2020).

There isn’t much research that look at how MPs and biochar interact. The co-application of biochar and MPs for the remediation of the MPs and heavy metal is studied by (Li et al., 2021; Wang et al., 2021). A study found that microplastic spheres can become caught, imprisoned, and entangled in biochar(Wang et al., 2020). (Li et al., 2022) investigated whether metal-loaded PVC decreased the amount of bioavailable metal in biochar. Additionally, biochar boosted the bacterial population and enzyme activity (Dissanayake et al., 2022; Palansooriya et al., 2019).

CHAPTER 3

METHODOLOGY

Materials and Methods

Properties of soil, biochar and microplastics

Properties of soil

Soil used in the experiment was collected from National Agriculture and Research Centre Islamabad, Pakistan. Soil samples were collected from ten, 2 x 5 m plots in a gross plot of 20 x 80 m, at 0-15 cm depth with the help of shovel. Soil was alkaline in nature. And the texture of soil was silt loam. All the samples were mixed thoroughly for further physiochemical analysis. The sample was then air dried, cleaned from stem and roots residues of crops, and sieved through 2mm mesh. Samples were stored at a temperature of 20±2ºC in the glasshouse.

Properties of biochar

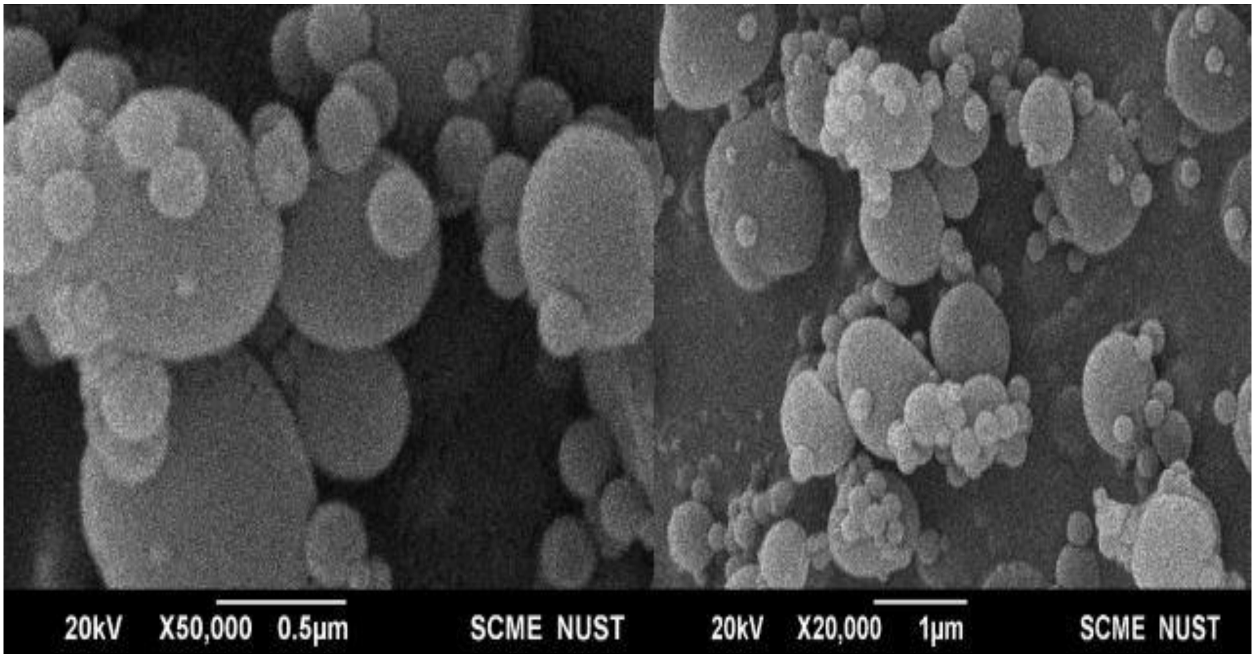
Cotton stalk was used to produce biochar, which was then manufactured on a small scale utilizing the Kon Tiki Flaming Curtin Pyrolysis Kiln. Biochar is produced in kilns at a specific temperature of 650–750 °C. Eurofins Umwelt Germany conducted a complete physical and chemical analysis of the produced biochar. the particle size and surface morphology of biochar were evaluated using a scanning electron microscope (SEM, JEOL JSM-6490A, Analytical Scanning Electron Microscope) at 20 kV, X20,000, and X50,000.

Table 3.1: Elemental composition, chemical characterization, and nutrients content of biochar.

Properties	**Values**	**Unit**
Hydrogen (H)	0.7	w/w %
Oxygen (O_2)	0.79	w/w %
Carbon (C)	54.5	w/w %
Nitrogen (N)	0.79	w/w %
Phosphorous (P)	1.8	w/w %
Potassium (K)	10.0	w/w %
Calcium (Ca)	8.1	w/w %
Magnesium (Mg)	3.8	w/w %
Sodium (Na)	1.7	w/w %
Iron (Fe)	4.4	w/w %
Zinc (Zn)	25	g / metric ton
Copper (Cu)	55	g / metric ton
CEC	6.4	cmol (+) kg^{-1}
pH	9.6	$CaCl_2$ method

Properties of microplastic

In this study Polyvinyl chloride (PVC-MP) was used as it is an emerging contaminant wastewater that is used in agricultural land for irrigation. PVC-MPs were in a white powder with an average Mw ~ 48,000 purchased from Sigma Aldrich. Morphological analysis of PVC-MPs was done by using Scanning Electron Microscope (SEM, JEOL JSM-6490A, Analytical Scanning Electron Microscope) at 20 kV, X20,000, and X50,000. The result showed that the particle's shape was round and spherical and have different sizes ranging between <0.5 µm – 10 µm.



Experimental design

Mash bean (*Vigna mungo*) was chosen for the study because of its economic importance in Pakistan. An experiment was done under controlled glasshouse conditions at the plant Biotechnology Department, at the National University of Science and Technology. In this study experimental treatments consist of PVC-MP (i) control and (ii) 0.5 %, (w/w)) a cotton stalk biochar (i) control and (ii) 0.5 % (w/w) and Chromium 50 mg per kg. The PVC-MPs concentration was based on the previous studies of (Z. Li et al., 2020) and Dong et al.,2021 (Dong et al., 2021). Biochar concentration was selected from the Study of (Haider et al., 2015) as the study concluded that biochar at low concentration provides better crop response. High Cr concentrations were adopted from (Arshad et al., 2017). All the treatments had 4 replicates which were used in an experiment for growth treatments and physiology.

The mash bean (*Vigna mungo*) seeds were obtained from National Agriculture Research Centre in Islamabad, Pakistan. Seeds were rinsed with distilled water and transferred to glass petri dishes to germinate for 2 days in darkness at 25±1ºC. At complete germination 5 synchronized seeds were selected and shifted into the pots containing experimental soil. Plants were grown in the glass house with controlled conditions: 25-35ºC temperature, 12/12 day/night photoperiod, and 65% relative humidity. After 7 days seedlings were thinned to three plants in each pot. The pot position changed after every three days for uniform environmental conditions. Boxes were irrigated regularly according to field capacity. Plants were grown for 65 days from 21st January 2022 to 27th March 2022 after germination and then harvested.

The soil used for growing plants was prepared for eight treatments. PVC was used as microplastic. Chromium was used as a heavy metal while biochar was used as a soil amendment. T1 was Control. T2 contains 0.5% PVC MP. T3 contains Cr. T4 contains 0.5% Biochar. T5 contains a combination of 0.5% PVC MP and Cr. T6 contains a combination of 0.5% PVC MP and 0.5% Biochar. T7 contains combination of 0.5% Biochar and Cr and T8 contains combinations of 0.5% PVC MP, 0.5% Biochar and Cr. All treatments are illustrated in the given table given below. Each batch consisted of four replicates (labeled) and soil per pot was 1kg.

Water holding capacity

For calculating the water holding capacity of the soil media, we took 3 pots for simple soil and 3 pots for soil containing 0.5 % biochar. We did 5 holes in each pot in the bottom of the pots and Put tissue inside each of them. Then soil and soil having 0.5 % biochar were added into the pots having space of one inch above in all pots. Then three pots having only soil were placed in one water tube and the other three pots had 0.5 % biochar in the other water tub. water was added to both tubs up to the level of soil in the pots. After 8 hours all pots were placed on inverted sieves for water drainage for an equal amount of time. The weight of the pots was measured which is W1. Then soil was removed from the pots covered in aluminum foil and dried in a drying oven for 4 to 5 hours at 105 C. soil weight was measured after drying (Wa). Pots were dried at room temperature weight was measured (Wb). We got values for W2 which is W2= Wa +Wb and water holding capacity was measured according to the given formula (Wang et al., 2014).

100 % Water holding capacity = [W1-W2) /W2] * 100

But I watered plants at 65% water holding capacity.

Heavy metal content in the plant

To eliminate the dust, the collected plant parts were gently rinsed with tap water, twice to three times, and then dried in an oven at 60 to 70 °C for 48 hours. Mash bean tissues were ground into a fine powder using an electric mill. To measure the Cr concentrations by AAS, the plant tissue subsamples (0.20 g) were digested with a solution of H2SO4/HClO4 (2:1 ratio v/v).

Measurement of soil enzyme activities

The soil dehydrogenase activity has been used for overall microbial activity (Casida Jr, 1977), and it plays a crucial role in the soil carbon cycle. The dehydrogenase activity was measured by spectrophotometer (Alef, 1995). The soil (5g) was taken in the tube and incubated (for 96 hours at 30 ºC) with 5ml TTC (2,3,5 triphenyl tetrazolium chloride) to reduce TTC into TPF (triphenyl formazan). After incubation acetone was added and shaken thoroughly while control tubes only contain Tris buffer (without TTC). After filtration, the optical density of the soil extract was analyzed at 546 nm after filtration on a 3500 UV-VIS spectrophotometer (Agilent technologies). The dehydrogenase activity (TPF µg g^{-1} dry weight of soil) was calculated as TPF (µg ml^{-1}) x 45/dwt/5 (Alef, 1995).

To determine the urease activity moist soil collected from the experimental pots was sieved from a 2mm sieve and saved in the polyethene bag at 4°C. Five-gram soil was taken in the flask and urea solution was added. The solution was incubated at 37 °C for 2h and then added 20ml KCl was. After the filtration process ammonium content was measured at the optical density of 690 nm (Kandeler & Gerber, 1988).

The protocol suggested by (Tabatabai, 1994), which entails incubating the soil sample with the colorless substrates specific to each enzyme along with p-nitrophenyl, was used to assess the activity of -glucosidase and acid phosphatase. The amount of p-nitrophenol produced after the incubation period is then calculated and expressed in mg of PNP per kilogram of soil per hour (g p-nitrophenol per kilogram of dry soil per hour).

Microbial biomass carbon and microbial biomass nitrogen

5 g of soil was fumigated at 25ºC with $CHCl_3$ (ethanol-free chloroform) for 24 hours, and the samples were extracted using 0.5M K_2SO_4. The same steps were taken for the nonfumigated soil. The MBC was calculated after titration according to (Wu et al., 1990).

$$MBC = (Cabon\ fumigated - Carbon\ nonfumigated) \text{ x } 2.64$$

After Kjeldahl digestion, total nitrogen in the sulfated potassium extract was measured. Digestion mixture ($FeSO_4$ 10: $CuSO_4$-1: Se-0.1) and concentrated H_2SO_4 (4.5 mL) were added after cooling, and further digestion was carried out for 3 hours. 20 mL water and 25 mL 10 M NaOH were added. 10 M NaOH and 2% H_3BO_3 were used for digestion and then moved into the distillation chamber of Kjeldahl. The distillate was collected and titrated to a bluish-red endpoint in 50 mM sulphuric acid (Brookes et al., 1985) and calculated by the given formula.

$$MBN = (Nitrogen\ fumigated - Nitrogen\ nonfumigated) \text{ x } 1.4$$

Phospholipid fatty acid analysis

Five grams of soil were placed in a 25 ml centrifuge tube and phosphate buffer, methanol, and chloroform were added. Tubes were sonicated for 10 min and rotated for 2 h at room temperature. Then centrifuge at 2500 rpm for 10 minutes, and the liquid phase was transferred into a separated test tube. Chloroform and water were added and left overnight for separation; Organic phase was separated under 20°C. Solid-phase extraction chromatography was used to separate lipids in the organic phase. Phospholipids were eluted in 5ml methanol. Phospholipids were stored at 20°C.

Methanol and toluene (1ml of 1:1) and methanolic KOH were used to transesterify the fatty acids at 37°C for 15 min. Hexane, acetic acid, and H2O were added and vortexed until phase separation. The organic phase was removed and stored at 20 C until further gas chromatography (HP6980-Hewlett Packard; Wilmington, Del.) with FID (Flame Ionization Detector) and the HP3365-Chem-Station and MIDI Sherlock. Single PLFA were allocated to taxonomic groups according to following pattern: Arbuscular mycorrhizal fungi (AMF): 16:1ω5c; general bacteria: 14:0, 15:0,16:0, 17:0, 18:0; gram-positive bacteria: i14:0, a14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0; gram-negative bacteria: 16:1ω7c, cy17:0, 18:1ω7c, cy19:0; *pseudomonas* 16:00, 18:1 w9t Alcohol (Frostegård et al., 1993; Steinberger et al., 1999; Zelles et al., 1992).

Statistical analysis

One-way ANOVA was performed. Data was arranged, organized, and compared using Microsoft® office 365 Excel. Inferential statistics were applied to calculate the significance of collected data by using GraphPad Prism® version 5.01, USA. Difference between the treatment were compared through the Tukey's test at $p < 0.05$ and 0.05 p-value were considered to significant in the given analysis.

CHAPTER 4

RESULTS

Results

4.1. PVC-MP and biochar effect on soil enzyme activity

The PVC-MPs and cotton stalk biochar were used to investigate their effects on soil enzyme activity, urease, dehydrogenase, acid phosphatase, and β-glucosidase. The urease activity significantly decreased with the application of 0.5% PVC-MPs and combination of 0.5% PVC-MPs with chromium as compared to control. However, with the addition of biochar with respect to the control a slight increase in the urease activity was observed but the effect was non-significant. And combination of 0.5% PVC-MPs and chromium with biochar significantly increased the activity of urease as compared to combination of 0.5% PVC-MPs and chromium without biochar.

Dehydrogenase activity reduced significantly in chromium contaminated soil and soil having combination of 0.5% PVC-MPs and chromium as compared to control. However, with the addition of biochar to soil having combination of 0.5% PVC-MPs and chromium and chromium sole, a significant increase was observed in the dehydrogenase activity.

The activity of acid phosphatase increased significantly in PVC contaminated soil as compared to control. There was not any significant change observed in soil having chromium as compared to control, but the acid phosphatase activity significantly reduced in soil having combination of 0.5% PVC-MPs and chromium as compared to control. However, with the addition of biochar to a significant increase was observed in acid phosphatase activity as compared to control. And same trend was observed in combination of chromium with biochar and PVC-MPs and chromium with biochar as compared to chromium sole and combination of 0.5% PVC-MPs and chromium respectively.

β-glucosidase activity significantly increased in biochar containing soil as compared to control having no biochar. PVC with biochar also increased the activity of β-glucosidase significantly as compared to sole treatment of PVC. Combination of 0.5% PVC-MPs and chromium with biochar significantly increased β-glucosidase activity as compared to combination of 0.5% PVC-MPs and chromium.

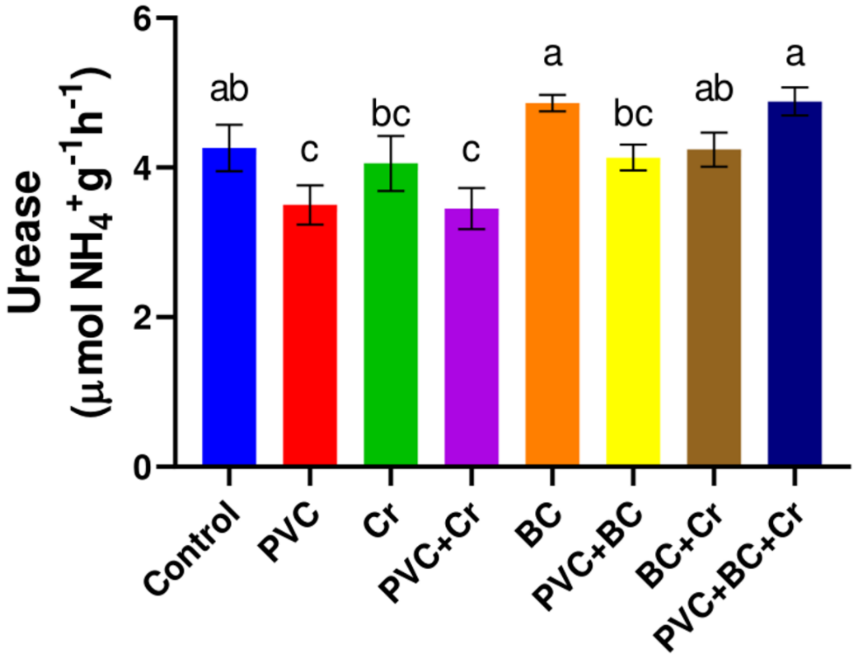


Figure 1. Effect of PVC-MP (control = 0%, 0.5%, on dry weight basis (w/w)) and biochar (control = 0%, 0.5%, on dry weight basis (w/w)) on urease activity. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

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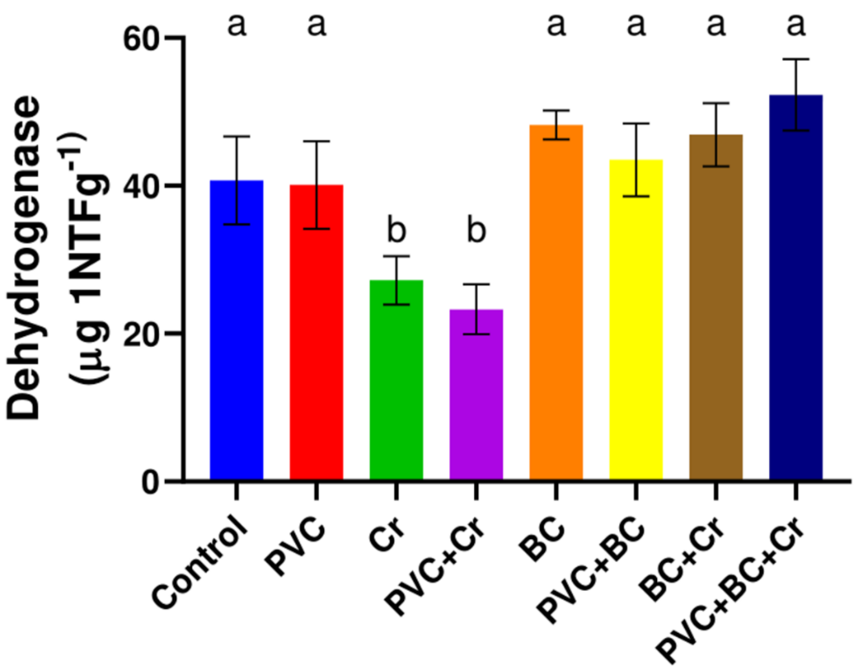

Figure 2. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on dehydrogenase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

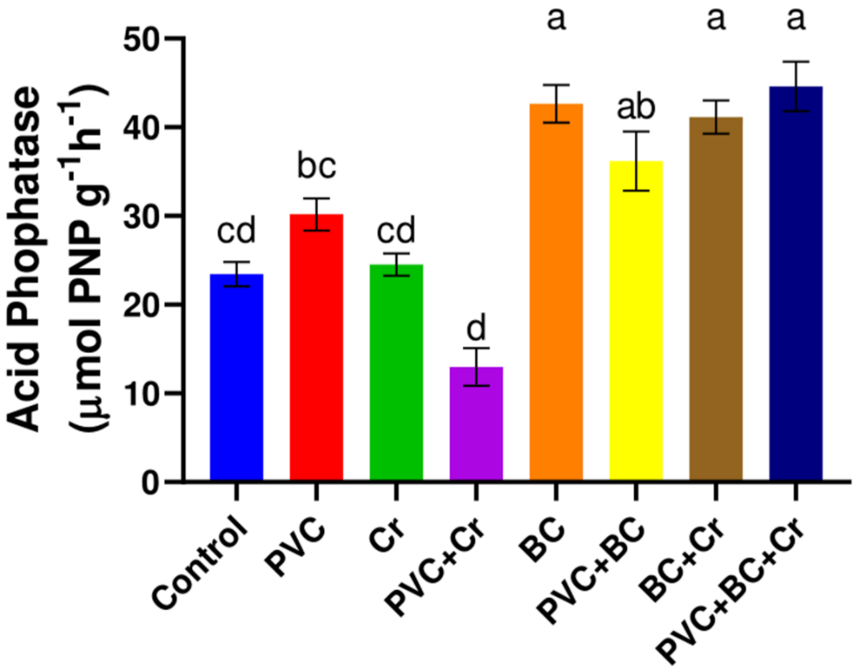

Figure 3. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on acid phosphatase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

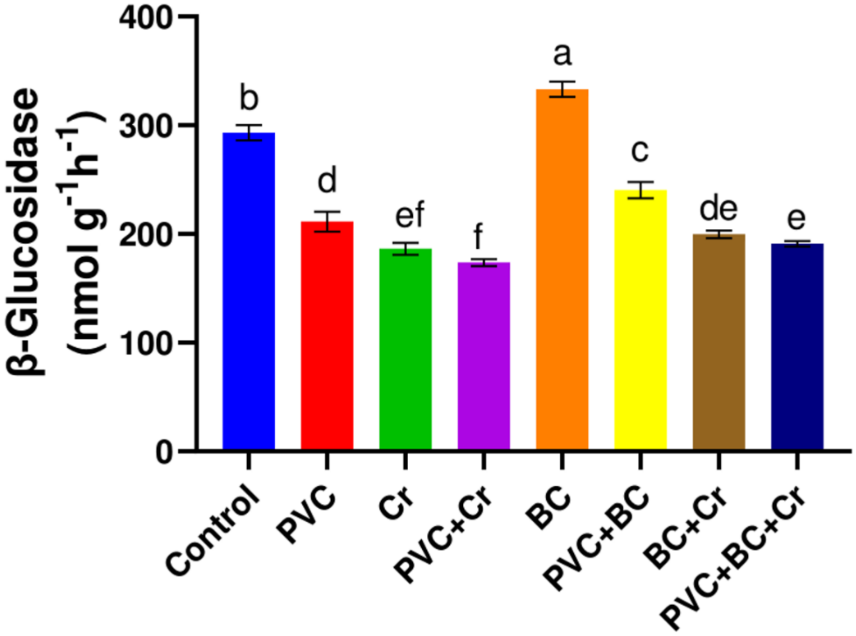

Figure 4. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on β- glucosidase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

4.2. PVC-MP and biochar effect on microbial biomass carbon and nitrogen

The PVC-MPs and cotton stalk biochar were used to investigate their effects on soil organic carbon and nitrogen. Generally, biochar application significantly increased overall MBC compared to control soil without any biochar and same results were observed in combination of PVC-MPs and chromium with biochar. Chromium alone and combination of chromium and PVC-MPs significantly reduced the MBC. However, co-application with biochar showed significant increase. And the same trend was observed in the case of MBN i.e., biochar application significantly increased overall MBN compared to control soil without any biochar and same results were observed in combination of PVC-MPs and chromium with biochar. Chromium alone and combination of chromium and PVC-MPs significantly reduced the MBN. However, co-application with biochar showed significant increase.

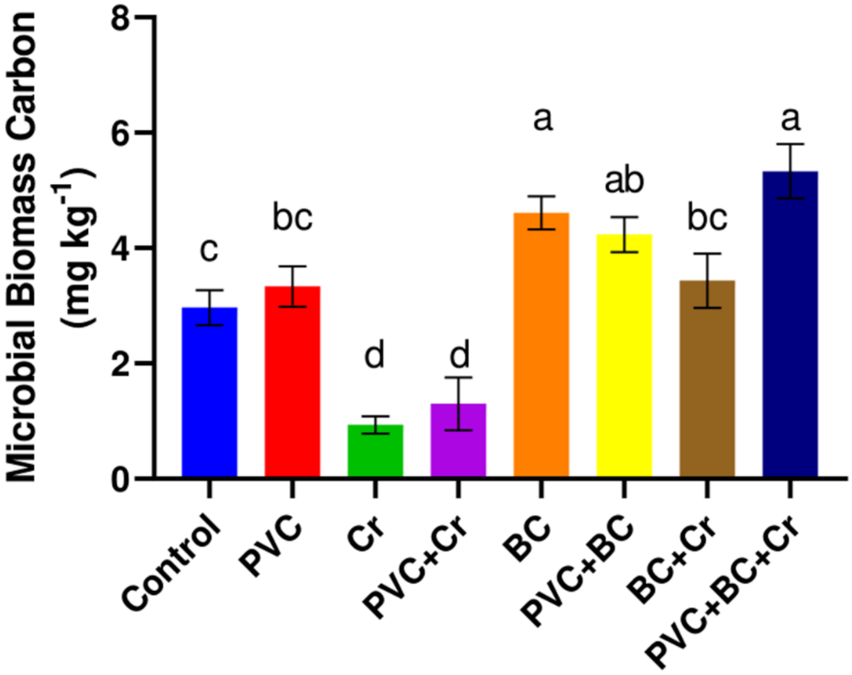

Figure 5. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on microbial biomass carbon. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

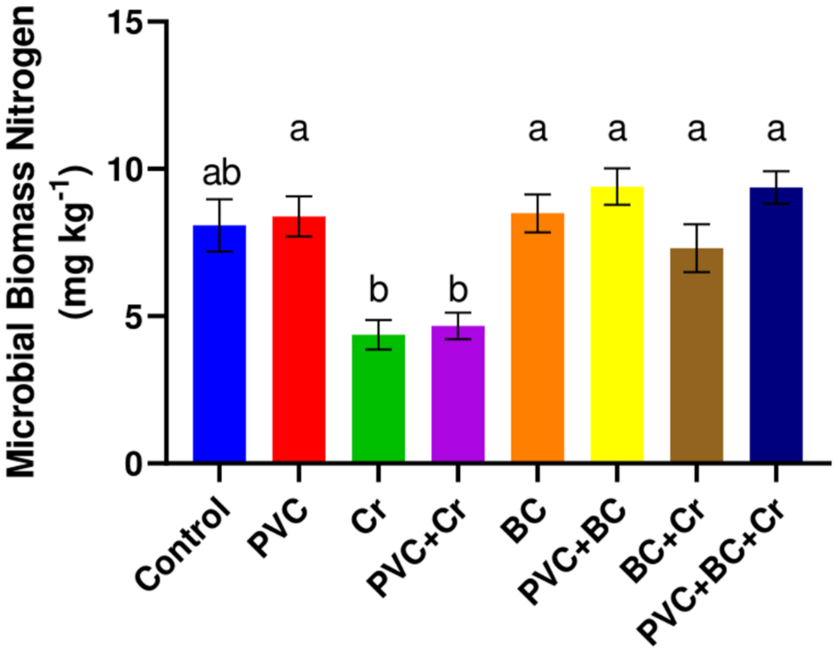

Figure 6. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on microbial biomass nitrogen. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

4.3. Biochar, chromium and PVC-MPs effect microbial communities and soil bacteria

PVC-MP did not show any effect on the abundance of the gram-negative bacteria in PVC-MP soil as compared to the control treatment. The same trend was observed in the case of chromium contaminated soil. However, biochar application with 0.5% PVC-MPs and chromium contaminated soil increased the abundance of gram- negative bacteria. PVC-MP did not affect the abundance of the gram-positive bacteria in PVC-MP soil as compared to the control treatment, but the effect was non-significant. The same trend was observed in the case of chromium contaminated soil. However, biochar application with 0.5% PVC-MPs and chromium increased the abundance of gram-positive bacteria respectively. PVC-MP addition increased AMF community as compared to control, but the effect was non-significant. Chromium sole and combination of chromium and

PVC MPs drastically reduced AMF community as compared to control. While the addition of biochar to chromium and combination of chromium and PVC MPs significantly increased the AMF community. total PLFA was also increased with the addition of biochar as compared to PVC sole, chromium sole and combination of chromium and PVC MPs.

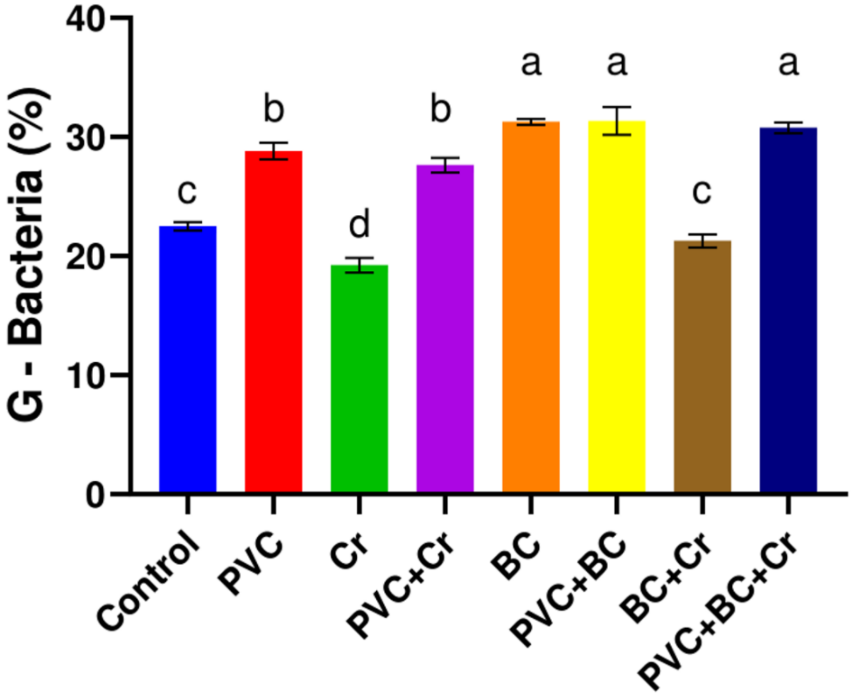

Figure 7. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on gram negative bacteria. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at P < 0.05.

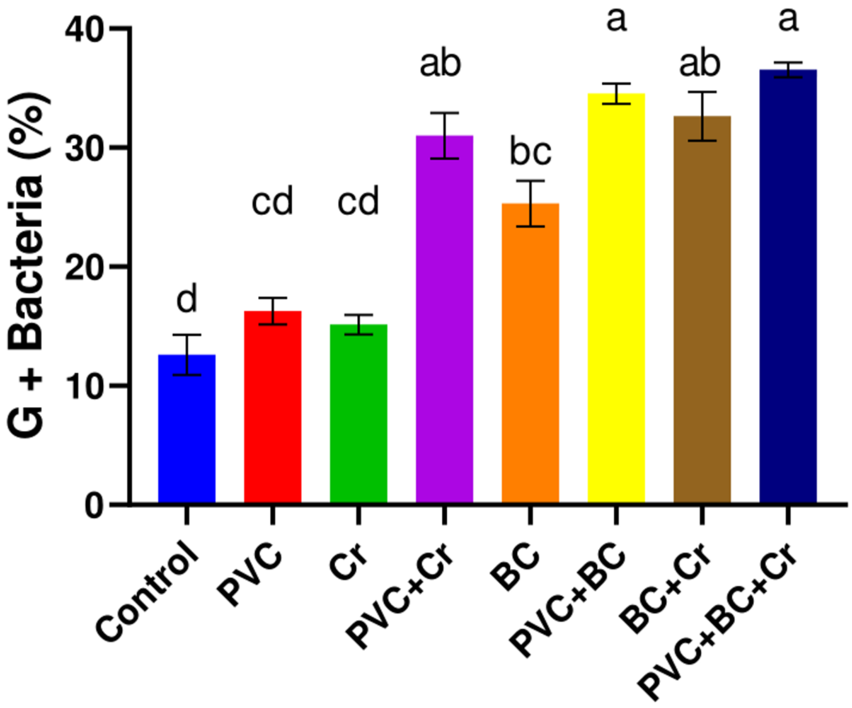

Figure 8. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on gram positive bacteria. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at P < 0.05.

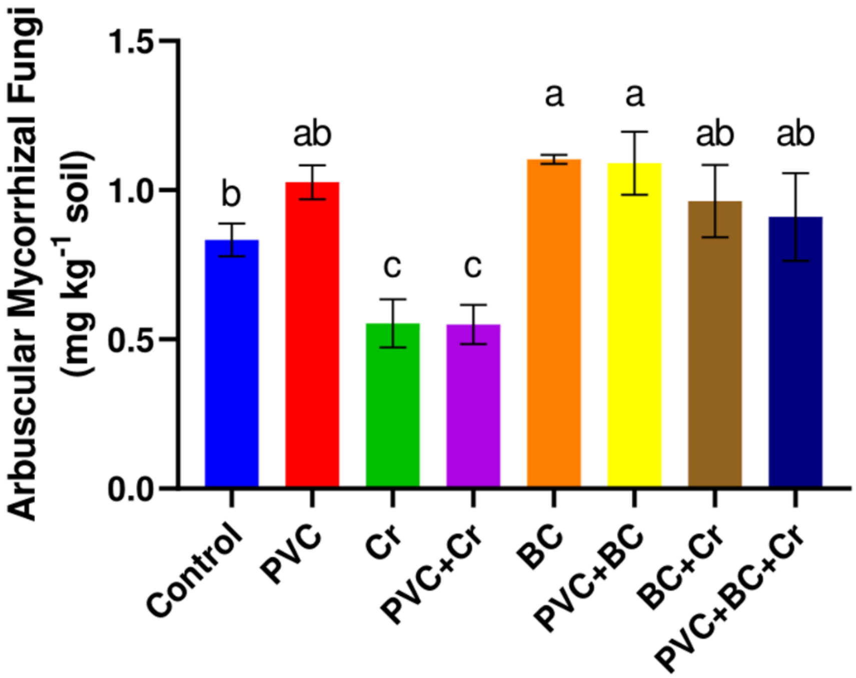

Figure 9. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on AMF community. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at $P < 0.05$.

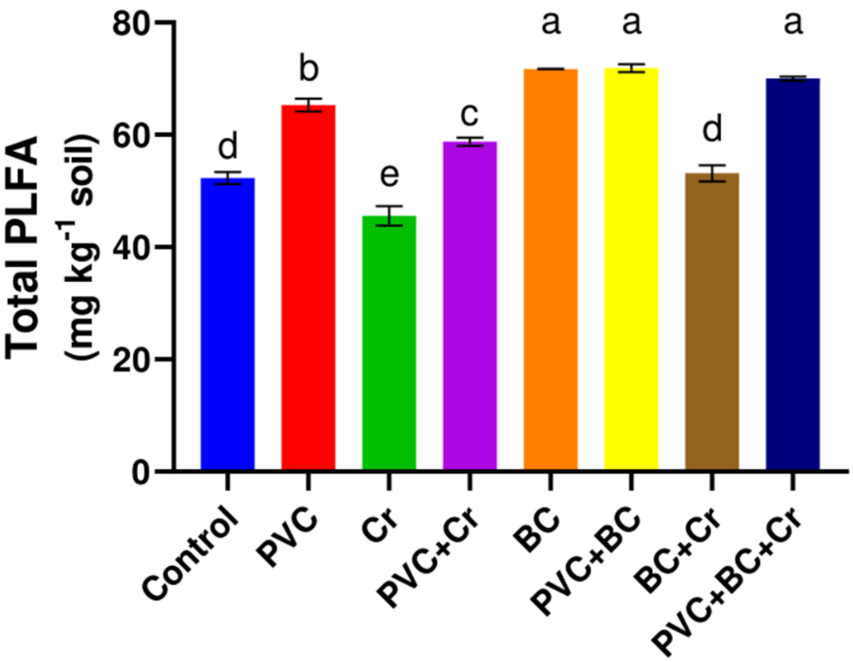

Figure 10. Effect of PVC-MP (control = 0%,0.5%, on dry weight basis (w/w)) and biochar (control = 0%,0.5%, on dry weight basis (w/w)) on total PLFA. The vertical bars are based on the treatment means, while the error bars are based on standard deviation. The bars sharing similar letters are not significantly different from each other at P < 0.05.

CHAPTER 5

DISCUSSION

Discussion

Plastics infiltrate the soil through both point sources (such as sewage sludge and industrial plastic manufacturing) and diffuse contamination (such as runoffs from urban and rural regions), both of which are the result of poor waste management (Edo et al., 2020). Current studies have demonstrated the toxic effect of PVC-MPs and Cr on soil enzymes, community structure, and metal uptake in the soil-plant system (Brown et al., 2023; Fu et al., 2022; Zeb et al., 2022). When plants are watered with purified wastewater, the crack-entry mechanism of uptake is seen as crucial (Li et al., 2020). Enzymes are active substances found in soil that play an important role in biogeochemical processes and nutrient cycling (Daughtridge et al., 2021). Indicators of soil quality, soil enzymes are very susceptible to both natural and human-caused changes (Aponte et al., 2021). Therefore, the assessment of soil enzyme activity is widely used to evaluate the physiochemical characteristics, disturbances, succession, fertility, microbial community structure, and biological activities of the soil (Xu et al., 2021). Here we found a reduced urease enzyme activity due to PVC-MPs addition (Fig.1). There are mixed results of reduced (Han et al., 2022) and increased (Yanan Fan et al., 2022; Fei et al., 2020) urease activity due to different MPs pollution in various experimental settings. In this study, adding biochar increased urease activity even in the presence of PVC-MPs, suggesting that PVC-MPs and Cr inhibited soil microorganisms regulating urease activity whereas biochar sustains microbial activity. The same trend was observed for dehydrogenase activity (Fig.1). MPs, Cr, and biochar applications may show different responses to urease and dehydrogenase activities under different crops. Furthermore, these activities are very sensitive to soil moisture as well. Biochar addition increases soil water holding capacity; therefore, higher urease and dehydrogenase activities in biochar amended treatments in the present study might be due to the more excellent moisture retention in soil due to biochar. Phosphorus in soils is converted and recycled with the help of acid phosphatase enzymes (Behera et al., 2017; Xie et al., 2017). As a bio-indicator of soil phosphorus and heavy metal toxicity, phosphatase activity is becoming increasingly important (Yang et al., 2018). In our study, acid phosphatase activity was considerably enhanced (-15.1044%, -3.49464% and 4.6048%) by the addition of biochar to the PVC-MPs, Cr, and PVC-MPs+Cr treatments. However, the absence of biochar reduced the acid phosphatase activity for PVC-MPs, Cr and PVC-MPs +Cr treatments. As the soil pH rises after applying bamboo biochar, acid phosphatase activity drops (Cao et al., 2021). Similarly, (Zhou et al., 2013) found that biochar application resulted in a rise in soil pH, leading to a drop in acid phosphatase activity and an increase in alkaline phosphatases activity. In addition, acid phosphatase activity in soil can be suppressed if too much nitrogen, phosphorus, or potassium is present (Lopes et al., 2021). Beta-glucosidase activity showed a similar pattern. Decreased enzyme activity might reduce plant accessible nutrients, which could reduce agricultural output or crop quality in the future if microplastic fibers and Cr continue to accumulate (Paz-Ferreiro et al., 2014).

The soil microbial characteristics are considered important indicators of soil quality changes and health (Schloter et al., 2018). Generally, soil pollutants, for instance, heavy metals or microplastics can reduce microbial biomass and activity (Yifei Fan et al., 2022) . There are primarily three ways in which MPs might influence the soil microbial community in the soil's microenvironment. Altering the soil's physical and chemical composition might affect the soil microorganisms' habitat (Lozano et al., 2021; Ren et al., 2021). The second is that MPs can act as a medium for microbial growth (Rogers et al., 2020); and the third is that MPs can change the rhizosphere soil metabolites (such as root exudates, microbial metabolites) to promote the formation of new dominant microbial communities (Y. Dong et al., 2021; Xie & Shou, 2021). In the present study, we found that PVC-MPs and Cr significantly decreased microbial community structure (gram-negative bacteria, gram-positive bacteria, total PLFA, AMF community, general bacteria, general fungi, total bacteria, and total fungi) compared to control (Fig.2). At the same time, the addition of biochar to PVC-MPs and Cr treatments (sole or combined) significantly relieved the toxic effect of PVC-MPs and Cr on microbial community structure (Fig.2 and 3). These results suggest that biochar addition to PVC-MPs and Cr-contaminated soil significantly alters soil C cycling. The presence of MPs in the soil also influences soil porosity, pH, electrical conductivity, nutrient transformation, water-holding capacity, and soil microbial and enzyme activities (de Souza Machado et al., 2019) . In addition, MPs may alter soil structure (such as soil aggregation and bulk density), which in turn may alter the makeup of the soil microbiome and have further effects on plant development (Rillig et al., 2019), although there is little knowledge about the direction of changes and functional consequences. A few studies on MPs in soil have reported alteration of the soil microbial community (Li et al., 2014; Tanunchai et al., 2021; Zhang et al., 2016), while others reported no significant change (Bandopadhyay et al., 2020).

The PVC-MPs+Cr treatment had the highest root and shoot Cr content compared to the other treatments in the current investigation (Fig. 4). This data demonstrates that MPs increase both the bioavailability and bioaccumulation of Cd in soil. The size of the MPs has a significant impact on the Cd input to the soil and the rate of Cd release (Li et al., 2021). In addition, MPs increase the bioavailability of metals to soil biota by lowering their adsorption capacity and increasing their desorption capacity in the soil-plant system (Li et al., 2021). The retention capacity of heavy metals depends on the type and size of plastic particles (Turner & Holmes, 2015), evidencing that strong hydrophobicity and high specific surface area are responsible for the accumulation of heavy metals in MPs . It has been shown that the accumulation of heavy metals in plants is highly related to the concentrations of bioavailable heavy metals (He et al., 2019). MPs can also increase the bioavailability of Cd in the soil by altering soil properties and occupying sorption sites, which keeps labile MPs mobilized and prevents stabilization (de Souza Machado et al., 2019), thus promoting the mobilization, release, and subsequent accumulation of Cd in the plant (Luo et al., 2016).

CHAPTER 6

CONCLUSION

Conclusion

Here we investigated the PVC-MPs and Cd contamination effects on mash bean plant. Our results clearly show that PVC-MPs and Cr negatively affect soil enzymatic activities and can cause significant shift in the structure and functioning of the soil microbial community. PVC-MPs generally have a minor effect on soil enzymatic activities and microbial community structure, however, Cr alone and in combination with PVC-MPs significantly reduce these traits. Furthermore, PVC-MPs enhanced soil microbial biomass carbon and nitrogen (+15.69% and +9.48%) and altered the structure of microbial community (indicated by PLFA) in the presence of biochar. However, addition of biochar to PVC-MPs and Cr contaminated soil considerably reduced the toxic effect of PVC-MPs and Cr in a soil-plant system. The results presented here suggest that PVC-MPs and Cr can have significant impact on key pools and fluxes in the agroecosystem. In addition, more study is needed to obtain empirical evidence on the potential effects of MP and Cr pollution on soil ecosystem functions and higher plants, especially when employing different farming practices, by examining shifts in soil properties, microbial communities, and plant growth performance under PVC-MPs and pollution.

In this work, we explore pure analytical grade PVC MP to gain knowledge and get familiar with it. However, commercial plastics, which contain various additives (plasticizers) to provide forms, colors, and strength to plastic materials, should also be considered in future investigations. Plastic polymers with other heavy metals may have synergistic effects on soil physicochemical characteristics and nutrient absorption, which should be investigated in future research. Examining how MPs of varying sizes and densities could perform is also crucial. Learn more about how MPs affect the development and physiological traits of many plant species in the future.

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