Microplastics and cadmium contamination effects on plant physiology and soil biodiversity

By
Attia Rubab Khalid
00000329911

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

Atta-ur-Rahman School of Applied Biosciences (ASAB),
National University of Sciences and Technology (NUST),
Islamabad,
Pakistan 2022

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We hereby recommend that the dissertation prepared under our supervision by:

Student Name: Attia Rubab Khalid **Registration Number: 00000329911**

Titled: "Microplastics and cadmium contamination effects on plant physiology and soil biodiversity" be accepted in partial fulfillment of the requirements for the award of MS Degree in Plant Biotechnology degree with A grade

Examination Committee Members

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

2. Name: **Dr. Fazal Adnan** Signature: ____________

Co-Supervisor's Name: **Dr. Rabia Amir** Signature: ____________

Supervisor's Name: **Dr. Ghulam Haider** Signature: ____________

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

Date: 22-07-2022

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

Date: 22-07-2022

Head of Department

COUNTERSINGED

Date: 22-07-2022

Dean/Principal

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

Thesis Acceptance Certificate

It is certified that final copy of MS thesis written by Attia Rubab Khalid (Registration no.000000329911), of Atta-ur-Rahman School of Applied Biosciences (ASAB) has been vetted by undersigned, found complete in all respects as per NUST Statutes/Regulations/MS policy, is free of plagiarism, errors and mistakes and is accepted as partial fulfillment for the award of MS degree.

Supervisor: ____________________

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

Dr. Ghulam Haider

Associate Professor

Atta-ur-Rahman School of Applied Biosciences,

National University of Sciences & Technology, Islamabad

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

Head of Department: ____________________

Dr. Muhammad Faraz Bhatti

Professor

Atta-ur-Rahman School of Applied Biosciences,

National University of Sciences & Technology, Islamabad

Principal: ____________________

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

Dr. Hussain A. Janjua

Professor and Principal

Atta-ur-Rahman School of Applied Biosciences,

National University of Sciences & Technology, Islamabad

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Attia Rubab Khalid

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Supervisor: Dr. Ghulam Haider

Signature:

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

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I certified that this research work titled "Microplastics and cadmium contamination effects on plant physiology and soil biodiversity" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged/ referred.

Attia Rubab Khalid

Registration # 000000329911

Dedication

I dedicate this work to my supportive family especially my mother who always believed in me and supported me in each step during the study period.

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ABSTRACT

Microplastics (MPs) are tiny plastic particles less than 5 mm in size. The MPs enter the environment due to the breakdown of larger plastic materials. MPs pollution has become the most emerging environmental issue and is almost everywhere in the world. The MPs are divided into two major groups. The primary and secondary MPs. Primary MPs are manufactured in micro sizes, while the breakdown of larger plastic materials forms secondary MPs. Polyvinyl chloride (PVC) is one of the agricultural soil's most abundant and toxic MPs. Agricultural soils are also facing the challenges of heavy metals (loids). For instance, Cadmium (Cd), a heavy metal, is one of the most dangerous pollutants in agricultural soils. Cd is a non-essential, more mobile, and bioavailable heavy metal and thus can be a potential threat to plant growth and soil biota. Biochar (a stable carbon obtained by the pyrolysis of organic wastes under anaerobic conditions) has been investigated and recommended to reduce soil contaminants' bioavailability. In the present study, we investigated the interaction of PVC-MPs (0%, 0.25%, and 0.5%, (w/w)), Cd (600mg/kg), and cotton stalk (*Gossypium hirsutum* L.) biochar (0%, and 0.5% (w/w)), in two controlled experiments, on plant growth, physiology, antioxidants defense, soil microbial community and abundance using 16S rRNA, 18S rRNA and PLFA biomarkers. Results revealed that the PVC-MPs at the low dose of 0.25% increased the dry shoot mass with no significant effect on the dry root mass. There was no significant effect on plant antioxidant activity. However, MP's addition reduced soil urease, dehydrogenase activity, soil organic and microbial biomass carbon, and bacterial and fungal abundance. The Co-application of biochar with PVC-MPs reduced the adverse effects of the MPs and increased

Urease and dehydrogenase activity, soil organic and microbial biomass carbon, and bacterial and fungal abundance. PVC-MP + Cd reduced the plant shoot biomass and did not significantly affect the root dry weight. On the other hand, PVC-MP + Cd reduced the soil enzyme activity and increased the Cd concentration in the plants' roots and shoot, whereas PVC-MP + Cd increased gram-positive bacteria, gram-negative bacteria, total PLFA (phospholipids fatty acids) biomarkers. Adding biochar alleviated the hazardous effects of PVC-MP + Cd contamination as indicated by increased soil enzyme activity and reduced Cd uptake in the shoot, root, plant, and soil. Applying biochar and PVC-MP + Cd causes a further increase in gram-positive bacteria, gram-negative bacteria, and total PLFA. To sum up, it is concluded that applying biochar to high-risk areas of MPs or heavy metals contamination can be a strategy to reduce the environmental impacts of emerging pollutants.

Keywords: Polyvinyl chloride; PLFA biomarker; Plastics; dehydrogenase; Urease

GRAPHICAL ABSTRACT OF OVERALL STUDY

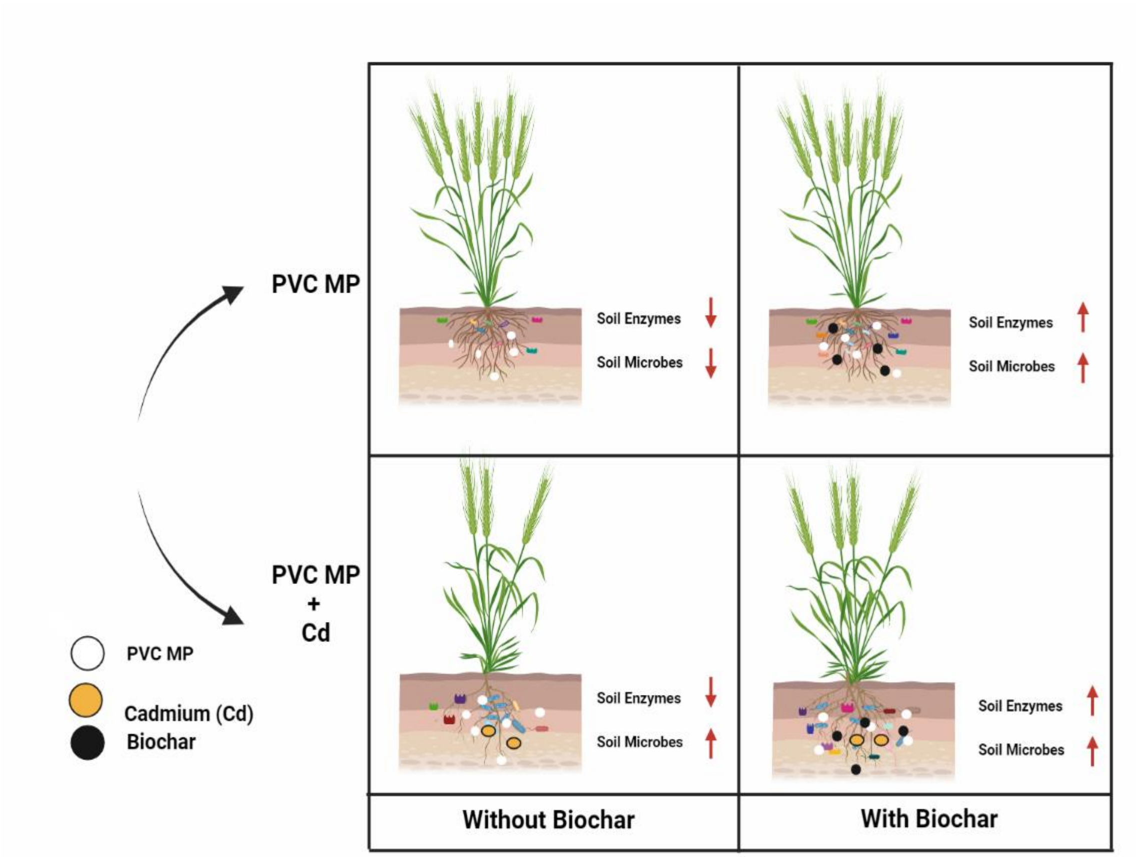

Figure1. Representation of PVC-MP, PVC-MP+Cd and biochar effect on the plant biomass and soil biodiversity

1.INTRODUCTION

1.1. Importance of wheat

Wheat *Triticum aestivum* plays an important role in the economy of the world as it is a major cereal crop and staple food (Hossain et al., 2021). It is an important part of the human diet, 40% of the calories come from wheat, and fulfills 35% of food demand for humans (Kumar & Krishna, 2015). Despite wheat can grow in various environments but optimum soil sites can improve the yield of wheat. It is estimated that a 60% increase is required by 2050 to feed the world population however the production of wheat is not satisfactory as there is only a 1.1% increase in the last decade (Hussain et al., 2022). Various abiotic factors such as MPs and Heavy metals can affect the production of wheat by inhibiting growth, reducing biomass, and changing nutrient assimilation (Wang et al., 2022).

1.2. Plastics and MPs

Plastics are the polymer with harmful chemicals that are derived from Non-renewable crude oil and these chemicals are released into the environment with the disposal of plastics (Lithner et al., 2011). More than 300 million tons of plastics have been produced globally in the last decade but the number of recycled plastics is less than half of the produced plastic and the remaining plastic are becoming part of the landfills, oceans and polluting the water bodies and terrestrial land (Rochman et al., 2013). Plastic in the municipal solid waste is estimated at 79 million tons and in the industrial solid it was estimated at 42 million tons in Asia (Liang et al., 2021) . Plastics are divided into seven different knowns as resin codes, Polyethylene terephthalate PET, high-density polyethylene HDPE, polyvinyl chloride PVC, Low-density polyethylene LDPE, polypropylene PP, polystyrene PS, and other resins are included (Boyle & Örmeci, 2020). Demand of plastics resins in 2019 was PP 19.4%, PE-LD 17.4%, PE-HD 12.4%, PVC 10%, polyurethane PUR 7.9%, PET 7.9%, PS 6.2% and other plastics 18.8% (Plastics Europe - Association of Plastic Manufacturers (Organization), 2020). These plastic resins are the vector for the other contaminant and additives that harm the organisms (Mato et al., 2001). Plastics can be broken down into macro plastics 25 mm to 5 cm (Ng et al., 2018) , mesoplastics 5–25 mm, MPs 100 nm and 500 µm and nano plastics (NPs) >500 µm (Ng et al., 2018). The size of small MPs is 0.2-2 nm and large micro plastics are 2-5 nm (Collignon et al., 2014). Two main problems are associated with the MPs, the extremely small size that is easily available to the biota and the vector for the other pollutant and microorganisms (Rillig, 2012b; Weithmann et al., 2018).

1.3. Sources of MPs

Primary and secondary MPs are a major part of the environment (Cole et al., 2011). Primary MPs are manufactured in microscopic size and are used in the air blasting media (Claessens et al., 2011) , pellets, and cosmetics (Napper et al., 2015). The secondary source of the MPs is that plastic which is derived from plastics when they are a breakdown into smaller particles (Roy et al., 2011). The sunlight exposure and the UV light cause the photo degradation of plastics into smaller plastics, the smallest plastic that was detected in the ocean is 1.6 µm in the size (Andrady, 2011). Biodegradable plastics are thought to be safe for the environment but can cause the MPs as these plastics decompose into the smaller particles that are released into the water bodies (Barnes et al., 2009; Thompson et al., 2004). The MPs are abundant in the sewage water due to the presence of the clothing fibers (Mason et al., 2016), these fibers contain the polyester and the acrylic compound have the same resemblance in composition with those of MPs that were found In the sediments (Browne et al., 2011). These MPs are retained in the form of sludge and used as fertilizer for agricultural land (Nizzetto et al., 2016).

1.4. Effect of MPs on plants physiology

MPs harm plant growth and plant morphological traits (Bosker et al., 2019; de Souza Machado et al., 2019; Qi et al., 2018a). Biodegradable MPs from the mulch have more negative effects than low-density polyethylene on the root, shoot and fruit biomass in the *Triticum aestivum* (Qi et al., 2018). Different sizes of the MPs cause reduction in the root length of the plants and MPs deposit in the pores of the seed coat can slow down the water uptake (Bosker et al., 2019; de Souza Machado et al., 2019). Plant release various antioxidant substances in response to MPs stress, malondialdehyde, and proline content increased in the *Cucumis sativus* cucumber when contaminate with the PS MPs (Li et al., 2020). 100 nm fluorescent PS MPs also increased the toxicity and oxidative damage than 5 mm PS-MPs (Jiang et al., 2019). MPs inhibit the formation of the chlorophyll in *Vicia faba*, the accumulation of MPs led to the blockage of the cell connection that affected the transport of minerals through the water (Jiang et al., 2019).

1.5. Effect of MPs on soil biodiversity

MPs also have a negative effect on the soil microbiota if they change the soil properties (de Souza Machado et al., 2018; Rillig et al., 2019; Wan et al., 2019). Studies reported that

MPs contaminated soil negatively affects the growth of microbes and decreases the soil microbiota (Huang et al., 2019; Wang et al., 2020; Yi et al., 2021). Polyvinyl chloride (10%) shifted the microbial community from gram-positive bacteria to gram-negative bacteria (Zang et al., 2020), however lower concentration of PVC also harmed the bacterial community (Yan et al., 2021), and fungal species (Fan et al., 2022). MPs have a negative impact on soil enzyme activity (Awet et al., 2018; Dong et al., 2021; Yu et al., 2020). MP can reduce the soil organic carbon SOC microbial by inhibiting the growth of microbial community (Yu et al., 2020) and enhancing SOC mineral but due to the stability, it is not available to the microorganism (Rillig, 2018). The study reveals that MPs cause a significant reduction in microbial biomass carbon MBC and nitrogen MBN (Blöcker et al., 2020). These studies indicate that MPs are adversely affecting the soil microorganism, enzyme activity and nutrient cycles need immediate remediation (Zhang et al., 2021).

1.6. Heavy metals effect on plants and soil biodiversity

Heavy metals, sometimes describe as metalloids such as cadmium (Cd), Chromium (Cr), Mercury (Hg) Copper (Cu), Lead (Pb) Nickle (Ni), and Arsenic (As) are part of the soil however, presence of heavy metal in high concentration can alter the soil properties and cause damage the plants (Alengebawy et al., 2021). It became a serious threat to the environment because heavy metals are irreversible and persistent pollutants (Li et al., 2019). Soil is contaminated at five million sites that cover about 500 million ha of land globally with heavy metals exceeding the permissible limits (Liu et al., 2018). Primarily heavy metals can enter agricultural land through the natural process however, natural sources usually are of minor impact (Dixit et al., 2015). Other than natural processes heavy metals can enter through anthropogenic activities (Chen et al., 2015) such as mining, application of pesticides and fertilizers, smelting, and combustion of fossil fuels (Li et al., 2019).

High concentrations of heavy metals in soil damage the properties of soil and soil microorganisms. The presence of heavy metals can inhibit microbial growth, microbial diversity reduces soil nutrients and soil fertility (Terry & Banuelos, 2000). Furthermore, the reduction in microbial biodiversity can also affect plant growth and reduce organic matter decomposition which led to the reduction in soil nutrients (Chibuike, 2014). Heavy metals cause toxic effects on plants such as chlorosis, inhibit growth, reduce biomass, and change nutrient assimilation (Singh et al., 2016). A high concentration of liable heavy metal produces free radicles and reactive oxygen species that are followed by cellular damage (Shivaraj et al., 2020).

1.7. Introduction to biochar

Different strategies are used to reduce the contaminants and the amendment of biochar is one of those strategies that help in the reduction of contaminants. Biochar that is produced under low temperature and limited oxygen can increase the soil organic carbon and contribute to soil carbon sequestration (Wang et al., 2015), provide habitat for the microbial communities (Cai et al., 2021; Ye et al., 2017) and increase the gram-positive bacteria (Zhang et al., 2018), gram-negative bacteria and fungi (Dominchin et al., 2021). Biochar enhances the enzyme activity in the soil (Song et al., 2020; Wang et al., 2015). Numerous studies reported that application of the biochar is useful in the removal of various contaminants (Diao et al., 2021; Yang et al., 2021).

1.8. Objectives

I. Investigation of PVC MP and Cadmium contamination effects on Wheat growth, and dry matter production

II. Investigation of PVC MP and Cadmium contamination effects on Soil microbes and enzymes activity

III. To evaluate, if co-application of biochar with PVC MP and Cadmium can reduce/enhance their effects on:

1. Plant growth, physiology, and biomass production
2. Microbes and soil enzymes activity

2. LITERATURE REVIEW

Plastics are the polymer with harmful chemicals that are derived from Non-renewable crude oil and these chemical are released into the environment with the disposal of plastics (Lithner et al., 2011). Due to the plastics low weight, these are also produced during carbon emission in transport (Miller, 2020). More than 300 million tons of plastics have been produced globally in the last decade but the number of recycled plastics is less than half of the produced plastic and the remaining plastic are becoming part of the landfills, oceans and polluting the water bodies and terrestrial land (Rochman et al., 2013). Plastics are persistent in all environments, from the oceans to the highest mountains (Chiba et al., 2018). Plastic production increased the greenhouse gas emissions, 3.8% of global carbon dioxide was produced due the plastic production in 2015 (Zheng & Suh, 2019). Other gases including methane and ethylene also produced during the degradation of plastics (Royer et al., 2018). Plastic in the municipal solid waste is estimated at 79 million tons and in the industrial solid it was estimated at 42 million tons in Asia (Liang et al., 2021). Plastics are divided into seven different knowns as resin codes, Polyethylene terephthalate PET, high-density polyethylene HDPE, polyvinyl chloride PVC, Low-density polyethylene LDPE, polypropylene PP, polystyrene PS, and other resins are included (Boyle & Örmeci, 2020). Demand of plastics resins in 2019 was PP 19.4%, PE-LD 17.4%, PE-HD 12.4%, PVC 10%, polyurethane PUR 7.9%, PET 7.9%, PS 6.2% and other plastics 18.8% (Plastics Europe - Association of Plastic Manufacturers (Organization), 2020). These plastic resins are the vector for the other contaminant and additives that harm the organisms (Mato et al., 2001).

The retention of a plastic litter in the environment is about thousands of years that becomes more harmful by converting into microscopic size plastics and it is estimated that the normal size plastic is decreasing in the environment however small size plastic is increasing the environment (Barnes et al., 2009). Continuous accumulation, weathering, and fragmentation of plastics led to smaller-sized debris, which has been named MPs (MPs) with size <5 mm (Barnes et al., 2009; de Souza Machado et al., 2019; Rillig, 2012; Thompson et al., 2004). Plastics can be broken down into macro plastics 25 mm to 5cm (Ng et al., 2018), mesoplastics 5–25 mm, MPs 100 nm and 500 mm and nano plastics (NPs) >500 mm (Ng et al., 2018). The size of small MPs is 0.2-2nm and large MPs are 2-5nm (Collignon et al., 2014). Different plastics' origins, morphology, particle size, chemical properties, and persistence could have highly diverse effects under different environmental ecosystems (Wagner et al., 2014). Two main problems are associated with the MPs, the extremely small size that is easily available to the biota and the vector for the other pollutant and microorganisms (Gao et al., 2019; Rillig, 2012).

2.1. Source of MPs

Primary and secondary MPs are a major part of the environment (Cole et al., 2011). Primary MPs are manufactured in microscopic size and are used in the air blasting media (Claessens et al., 2011), pellets, and cosmetics (Napper et al., 2015). The secondary source of the MPs is that plastic which is derived from plastics when they are a breakdown into smaller particles (Roy et al., 2011). The sunlight exposure and the UV light cause the photo degradation of plastic into smaller plastics, the smallest plastic that was detected in the ocean is the 1.6 µm in the size (Andrady, 2011). Biodegradable plastics are thought to be safe for the environment but can cause the MPs as these plastics decompose into the smaller particles that are released into the water bodies (Roy et al., 2011; Thompson et al., 2004).

The MPs are abundant in the sewage water due to the presence of the clothing fibers (Mason et al., 2016), these fibers contain the polyester and the acrylic compound have the same resemblance in composition as those of MPs that were found In the sediments (Browne et al., 2011). These MPs are retained in the form of sludge and used as fertilizer for agricultural land (Nizzetto et al., 2016). It is estimated that 30.7 × 103 particles kg–1 was detected in the dry sludge. In Pakistan, sewage water without any treatment is used for the irrigation of the crops to meet the need of the water shortage and to avoid the other expensive method to drag the underground water (Murtaza et al., 2010). The PE, PS, and PP MPs have been detected in the canal associated with the Ravi river and the same plastics have been detected from the Rawal lake (Irfan et al.,2020; Irfan et al., 2020).

2.2. Source of MPs in soil

MPs enter the terrestrial environment through various channels such as sewage sludge (Nizzetto et al., 2016; Wei et al., 2019), irrigation with wastewater (Zhang et al., 2018), plastic mulching (Huang et al., 2020), Organic fertilizers (Weithmann et al., 2018) and from the atmospheric environment (Dris et al., 2016).

2.2.1. Plastic Mulching as the source of MPs

Plastic mulching is an important practice in the agricultural sector that enhance crop yield by improving the soil temperature, moisture, and pest attack (Liu et al., 2022). Mostly plastic mulch is made of polyethylene, low-density polyethylene, and high-density polyethylene (Shah & Wu, 2020). In China, about 1.4 million tons of polyethylene plastic films were used in 2018 covering about 15% of the agricultural land (Liu et al., 2020).

Despite of beneficial aspects of plastic mulching, disposal practices such as ploughing the mulch in a field or disposing into landfills are not environmentally friendly (Fontenot et al., 2021), However high-cost halts removing or recycling the plastics mulch from agricultural land (Brodhagen et al., 2017) as removal of plastics takes time about 42 hours/h (Velandia et al., 2019) and according to one study in South Georgia vegetable industry it cost about 116.24 dollar/ h (Fontenot et al., 2021). Therefore, the continuous addition of plastic residues in agriculture results in the accumulation of a large number of MPs (Rillig et al., 2017). Plastic mulches were the source of MPs that affect the growth of the plant (Qi et al., 2018). MPs increased in the soil by about 80.3 ± 49.3, 308 ± 138.1, and 1075.6 ± 346.8 pieces/kg soil in fields with 5, 15, and 24 years respectively where the plastic mulch was continuously used (Huang et al., 2020). MPs concentrations were high in mulched soil about 754 ± 477 items kg–1 as compared to the non-mulched soil were about 376 ± 149 items kg–1 and MPs increase with time in mulched soil. (Zhang et al., 2021). Mostly plastic mulch is formed of polyethylene, low-density polyethylene and high-density polyethylene (Shah & Wu, 2020).

2.2.2. Wastewater and Sewage sludge as a source of MPs

Wastewater use for agricultural land is a frequent practice, especially in arid areas where fresh water is not sufficient to provide the fields (Muhammad Ashraf, 2017). Untreated wastewaters contain primary and secondary MPs from industrial and household waste that arrive at wastewater treatment plants (Sol, 2022) . Microbeads are added to personal care and cosmetics released into the wastewater (Cheung et al., 2013). Moreover, the polyester and acrylic fibers from the synthetic clothing are discharged into the wastewater (Napper et al., 2015). 4500 to 94000 microbeads released from sole use of exfoliant and 4000 from the toothpaste (Prata, 2018). Despite the water, the waste plant can remove about 90% of the MPs still a large amount of the MPs discharged into the environment (Masiá et al., 2020).

Wastewater transfer a large number of MPs from its source (Domestic, industrial waste) to soil (Collivignarelli et al., 2021). Wastewater management prevents the MPs from entering the aquatic environment by trapping it in the sludge (Milojevic & Cydzik-kwiatkowska, 2021). About 99% of MPs are retained in sludge after wastewater management (Mahon et al., 2017). Even though the removal of MPs from the wastewater is beneficial for the aquatic environment but harmful to the terrestrial ecosystem (Corradini et al., 2019). MPs present in the wastewater treatment plant accumulates into the sludge that is provided to the agricultural land (Li et al., 2018). This sludge is used as fertilizer to enhance soil fertility however when it employs on agricultural land can contribute to the contamination of soil (Corradini et al., 2019). It is estimated that dry sludge contains 4.20-15.4 * 10 3MPs particles/kg (Mahon et al., 2017).

According to Li et al., (2018) , about 1.56*10 14 MPs particles enter the soil through sludge. In addition, MPs inhibit the sludge hydrolysis and reduce the microorganisms (Zhang et al., 2020).

2.2.3. Organic fertilizer as a source of MPs

Compost is considered a reliable option to enhance soil fertility, carbon sequestration, crop nutrition, crop yield, and organic matter (Imran et al., 2021). Compost can be formed from manure, plant residues, municipal waste, and sewage sludge (Brock et al., 2021). Usage of biosolid fertilizers particularly organic waste can act as a vector in soil (Lambert et al., 2019). Industrial and domestic waste contain plastic that converts into MPs physical and mechanically and unfortunately added in the composting process, used as the organic fertilizers in agriculture (Liu et al., 2018). Previously macroplastic gained attention in compost whereas MPs is higher in number as compared to macroplastic (Gui et al., 2021). According to Vithanage et al. (2021), (about 1.23*10 7 ± 1.93*108 item /ha MPs particles are added into the soil through domestic waste compost. Despite many composting stations pre-treating the waste to reduce the plastic debris, still, a large amount of plastic remains in the compost (Gui et al., 2021).

2.2.4. Atmospheric source of MPs

Less attention was paid to the atmospheric deposing of MPs in the environment that can affect chemically and physically the land surfaces (Cai et al., 2017). Atmospheric deposition is another pathway for the MPs to enter the terrestrial environment (Dris et al., 2016).

Table 1. Various sources of MPs in the soil

Source	Site	Shape	Size	Range	Type	Reference
Rural domestic waste compost	China	Fiber, films	0.05 -5 mm	2400±358 items/kg dry weight	Polyester, Polypropylene Polyethylene	(Gui et al., 2021)
Farmland soil (Plastic mulch, biosolid fertilizers	Taiwan	Fiber, Film, Pellet, Foam,	1-3 mm , >5 mm	53.2 items/m2 in 5 cm depth 34.5 item/m2 in 20 cm depth	Polystyrene, Polypropylene , Polyethylene	(Fakour et al., 2021)
Plastic mulch& Municipal organic waste compost	Spain	N/A	>2 mm	2242±984 Mps/kg& 888±500MP/kg	Polyethylene, Polypropylene	(van Schothorst et al., 2021)
Atmosphere	China	Fiber, granules	N/A	212-9020mg/kg	Polycarbonat, Polyurethane, Polypropylene	(Liu et al., 2019)

2.3. Effect of MPs in soil

Soil provides a multitude of ecosystem services to humans such as food, wood raw material for infrastructures, prevention from flood nutrient cycling, carbon sequestration, and biodiversity (Kopittke et al., 2019). However anthropogenic activities such as industries increase the soil contamination that possess harm to the living creatures (Ye et al., 2018) . Soil that is essential for the terrestrial ecosystem confronted the MP contamination that has a strong impact on the soil function and biodiversity (de Souza Machado et al., 2018). Soil structure can be affected by the contamination of the MPs therefore it is necessary to understand how MPs affect the chemical, physical and biological properties of soil (Liang et al., 2019).

2.3.1. Impact of MPs on Biological properties of soil

2.3.1.1. Microbial communities:

Microorganisms such as mycorrhizal fungi and symbiotic bacteria are responsible for the provision of benefits in the agriculture (Jacoby et al., 2017). 80-90% biological activity depend on the enzymatic activity and species of microorganisms (Furtak & Gajda, 2018). Microorganisms provide numerous soil nutrients, enhance soil fertility (Mengqi et al., 2021), and help in recycling the organic waste (Siles et al., 2021). Therefore, management of soil microorganism plays key role in the sustainable agriculture and food safety (Bertola et al., 2021). However anthropogenic activities have negative effect on soil microbial biodiversity that may have adverse impact on the soil health (Yang et al., 2021)

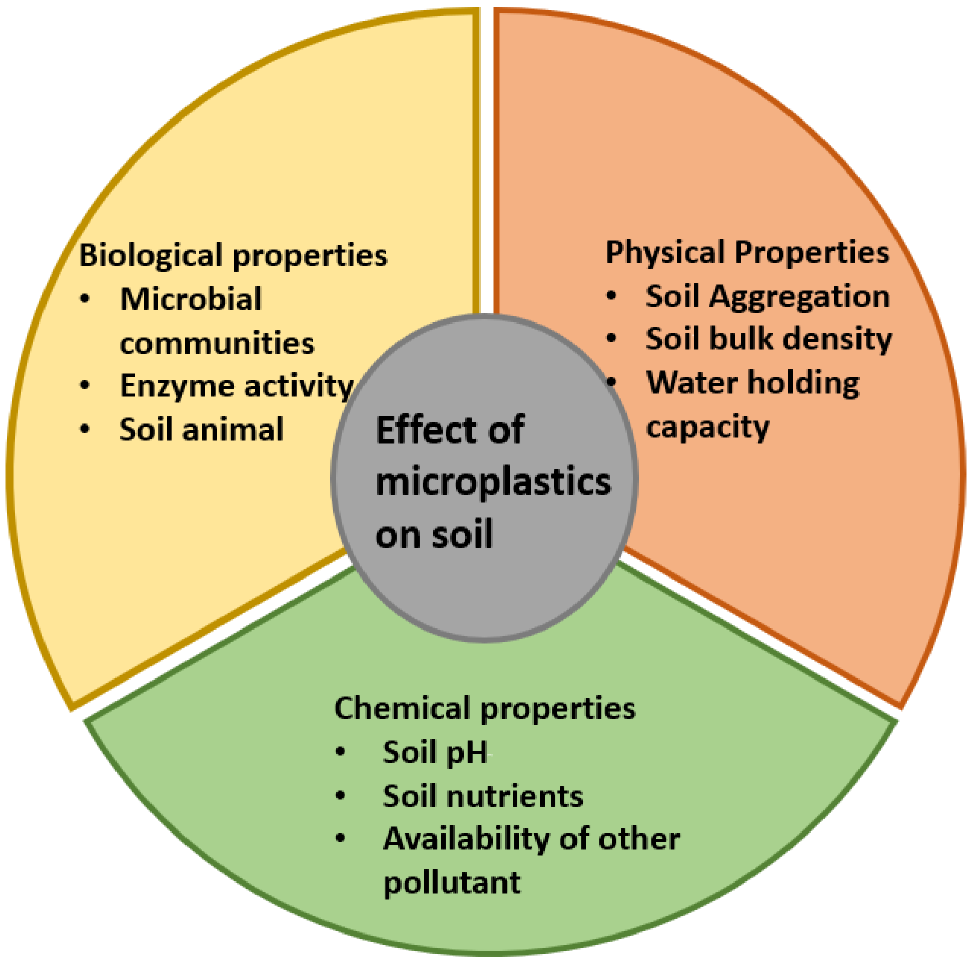

Figure2: Representation of effect of MPs in soil. MPs effect the soil chemical (soil pH, soil nutrients and, availability of other pollutant), physical (Soil aggregation, soil bulk density and, water holding capacity) and biological properties (microbial communities, enzyme activity and, soil animal)

Recent studies showed that MPs interact the microbial communities and affect their biological activities (Sun et al., 2022; Wang et al., 2020; Zhang et al., 2019). MPs provide the hydrophobic surface to heterogeneous bacteria, make these particles more favorable substrate for the microbial communities as compared to the other contaminants (Zhang et al., 2019). MPs with distinct size, shape and polymer had separated effect on the bacterial communities, Polyethylene and polypropylene had stronger effect on the bacterial communities as compared to the polystyrene (Sun et al., 2022). Moreover, soil Physiochemical properties such as soil aggregate alter the microbial communities. Film, fiber, and foam MPs negatively affect the bacterial communities because shape of MPs is linked with the soil physical properties (Sun et al., 2022). Huang et al. (2019) also concluded that MPs abundance and depletion is associated with the soil physical properties like soil bulk density, porosity, evaporation, and soil moisture. MPs enriched the various bacterial phyla (Wang et al., 2020), include *Bacteroidetes* have some pathogen bacterial member that can cause hoof rot diseases in the plants and may threaten for the agricultural ecosystem (Zhang et al., 2019).

MPs act differently on different communities of Fungi, *Basidiomycota*, *Chytridiomycota*, *Ciliophora* and *Rozellomycota* decreased and *Ascomycota* and *Zygomycota* increased with the polyethylene MPs (Ren et al., 2020). *Ascomycota* increased and *Basidiomycota* increased when inoculated with polyethylene MPs (Hou et al., 2021). Moreover, Polyvinyl chloride, Polyethylene, Polystyrene increased *Ascomycota* and decline of *Basidiomycota*, and *Chytridiomycota.*

Table 2. MPs size, shape and types effect on the bacterial communities

Microbes	MP Size, shape	Type	Effect on microbial communities	Reference
Bacteria	150 µm diameter Foam, Film Fiber	Polystyrene, Polyethylene, polypropylene	Polystyrene had weaker effect on bacteria as compared to the other two, *Patescibateri*a increased	(Sun et al., 2022)
Bacteria	<100 µm, Particles	Polyethylene	increase the abundance of *Actinobacteria, Chloroflexi* and *Firmicutes* and decrease the abundance of *Gemmatimonadetes* and *Bacteroidetes*	(Hou et al., 2021)
Bacteria	<5 µm	Polyethylene	*Abundance Actinobacteria, Bacteroidetes, Proteobacteria, Cyanobacteria* and *Deinococcus-Thermus.*	(Zhang et al., 2019b)
Bacteria	2 mm × 2 mm × 0.01 mm, Fragments	Low density polyethylene	*Abundance Acidobacteria, Armatimonadetes, Bacteroidetes, Gemmatimonadetes, and Proteobacteria*	(Wang et al., 2022)
Bacteria	2 mm × 2 mm × 0.01 mm films	Low density polyethylene	*Acidobacteria, Bacteriodietes, Gemmatimonadetes, Nitrospirae,* and *Proteobacteria* were significantly enriched *Actinobacteria* and some *Nitrospirae* were depleted	(Huang et al., 2019)
Bacteria	100 µm diameter, particle	Polyvinyl chloride, Polyethylene, Polystyrene	abundance of Proteobacteria, Actinobacteria and decline of Acidobacteria	(Fan et al., 2022b)

Table 3. MPs size, shape and types effect on the fungal communities

Fungi	MP Size, shape	Type	Effect on microbial communities	Reference
Fungi	100 µm diameter, particle	Polyvinyl chloride, Polyethylene, Polystyrene	Abundance of Ascomycota and decline of Basidiomycota, and Chytridiomycota	(Fan et al., 2022b)
Fungi	<13 µm and <150 µm	Polyethylene	*Basidiomycota*, *Chytridiomycota*, *Ciliophora* and *Rozellomycota* decreased and *Ascomycota* and *Zygomycota* increased with the polyethylene MPs	(Ren et al., 2020)
Fungi	<100 µm, Particles	Polyethylene	*Ascomycota* increased and *Basidiomycota*	(Hou et al., 2021)

2.3.1.2. Enzymatic activity

Soil enzyme production is essential for energy flow and nutrient cycling (Cui et al., 2021). Soil enzymes play a significant role in soil health, they are associated with soil physical properties, catabolism of soil organic compounds, microbial activities, and the indicator of soil health (Farooq et al., 2021) .

MPs can increase the soil enzyme activity depending on the different type of MP and their concentration (Fan et al., 2022). FDAse activities were increased with the addition of polyamide beads, polyacrylic fibers, polyethylene fragments, polyester fiber (de Souza Machado et al., 2018), and polypropylene MPs (Liu et al., 2017). The enzyme activity of urease and catalase was increased with the addition of polyethylene MPs however slight increase occurred in the invertase enzyme (Huang et al., 2019). Catalase enzyme increases in polyvinyl chloride treatment by about 38%, alkaline phosphatase in polyethylene by about 20.8%, and 63.2% of Urease with the addition of polystyrene MP (Fan et al., 2022). However, polyethylene MPs reduce the activity of Catalase, Phenol oxidase, Urease, manganese peroxidase, laccase, and β-glucosidase (Yu et al., 2020).

Recent studies showed that MPs can change the soil properties such as soil aggregates which may be associated with the soil enzyme activity (Zhang et al., 2018b). Furthermore Yu et al, (2020), also concluded that different enzymes act differently in soil aggregates.

Table 4. MPs size, shape and types of effect on the soil enzymatic activity

Enzyme	**MP size shape**	**MP type**	**Effect**	**Reference**
FDAse	<180μm	Polypropylene	28% of PP MPs increased Enzymatic Activity	(Liu et al., 2017)
β-glucosidase β-celluliosidase N-acetyl-b-glycosaminidase Phosphatase	0.03 mm diameter 0.008mm diameter	Polyester fiber Polyacrylic	Enzyme Activity decreased in polyester fiber	(Liang et al., 2021)
Urease, Catalase, invertase	2 mm × 2 mm × 0.01 mm, Films	Low density polyethylene	Enzyme activity increased in first two, and did not significantly increased	(Huang et al., 2019)

2.3.1.3. MP effect on Plants

MPs affect plant growth and plant physiology. In the *Vicia faba* plant, a significant reduction in growth was observed after 48h exposure to MPs. Plant biomass and catalase enzyme decreased by MP however peroxidase and superoxide dismutase enzyme were increased. MP induces genotoxicity and oxidative damage to *V. faba*. Toxicity was increased with the decrease in the size of MP. Particles inhibit the nutrient imbibition that causes blockage in the cell wall pores (Jiang et al., 2019). Some studies suggest that translocation and accumulation of MP beads in the wheat plants, 0.2 µm microbeads can easily translocate to stem and leaves through the apoplastic pathway (Li et al., 2019). MPs and NPs can easily penetrate the plant's tissues through the apoplastic pathway and proceed toward the different parts of plants (Li, et al., 2020). The smaller MPs of size 0.2 micrometer translocates efficiently in the wheat, Carrot, and lettuce plants as compared to the larger MPs (Li, et al., 2020) . PSMP of sizes 500 and 700 nm was accumulated in the aerial part of the plants like the stem, leaf tissue, calyx tissue, and fruit of the cucumber plant (Li, et al., 2020). The accumulation of large MPs of 1 µm was accumulated in the carrot stems there were no traces in the leaf (Dong et al., 2021).

The component of the MPs can reduce the root tissue density (de Souza Machado et al., 2019). Biodegradable MPs from the mulch have a more negative effect than low-density polyethylene on the root, shoot and fruit biomass in the *Triticum aestivum* (Qi et al., 2018). Different sizes of the MPs on *Lepdium sativum* cause a reduction in the root length of the plants and MP's deposit in the pores of the seed coat of *L. sativum* can slow down the water uptake (Bosker et al., 2019). The component of the MPs reduced the root tissue density in *Allium fistulosum* (de Souza Machado et al., 2019). *The* root diameter of the cucumber plant decreased with the inoculation of the 300 -500 nm polystyrene plastics (Li, et al., 2020). The biomass of root and leaf in *Daucus carota* carrot decreases with increasing concentration of the polystyrene MPs and Arsenic (Dong et al., 2021b).

The chlorophyll plays a major role in the photosynthesis in absorbing the scattered sunlight and radiation ,however in the *Lolium perene* rye grass MP's affect the chlorophyll a / Chl b ratio (Boots et al., 2019). *T*he MPs inhibit the formation of the chlorophyll in *Vicia faba* the accumulation of MPs can lead to the blockage of the cell connection that affects the transport of mineral through the water and 100 nm fluorescent polystyrene MPs also increase the toxicity and oxidative damage than 5 mm PS-MPs (Jiang et al., 2019). The activity of Rubisco decreases the contamination of the arsenic and PS MPs (Dong et al., 2021). Plant release various antioxidant substances in response to any antibiotic stress (Zhang et al., 2015). Malondialdehyde and proline content increase in the cucumber *Cucumis sativus* when contaminant with the polystyrene MPs (Li, et al., 2020). In another study in *Zea mays* maize, the coexistence of heavy metals and MPs conclude that these affect the function of the plant and affect the symbiotic relationship of the plants with the arbuscular mycorrhiza (Wang et al., 2020). In community study of plants, the inoculation of the micro fibers increases the efficiency of the allopathic species *Hieracium* and decreases the growth of the *Festuca* Specie, increase in the growth of the *Hieracium* may lead to the reduction in the growth of the *Festuca* species under the drought condition due the allelopathy nature of *Hieracium* (Lozano & Rillig, 2020) as the allelopathic species can reduce the growth and performance about 25 % . MP's particles found in the carrot and apple may be due to the high vascularization and complex root system but it needs more research to find facts (Conti et al., 2020).

Table 5. MPs type and size and their effect on plant physiology

Plant specie	MP types	MP size	MP Effect	Reference
Viccia Faba	Polystyrene	5µm . 100nm	Genotoxicity, oxidativ stress	(Jiang et al., 2019)
Triticum aestivum	P.S microbeads	0.2 ,2 , 7µm	Uptake by wheat plant	(Li et al., 2019)
Allium cepa	P.S nano particles	50 nm	Genotoxic, cytotoxic, oxidative stress	(Giorgetti et al., 2020)

2.3.2. Impact of on Physical properties of soil

The main factor that affects the soil properties is the MPS shape that is found in multiple varieties such as fragments, fiber sphere columns, and foam (Wang et al., 2022) .

The most abundant MPs regarding shape are the fibers and dye to their linear shape they can affect the soil aggregation (Zhang & Liu, 2018). Soil aggregates stability is the main soil physical property that aids in the prevention of erosion, provides aeration, soil fertility and soil aeration (Liang et al., 2021).Water stable soil aggregates are essential for measuring the soil structures and assessment of the behavior of MPs is necessary that these contaminants interact with the soil aggregates (Zhang & Liu, 2018). MPs Fiber act as binding agents and take part in the soil aggregation (Zheng et al., 2016). According to Zhang and Liu. (2018), 72% of MPs contribute into the soil aggregation 16% with micro aggregate, 56% with macro aggregates and the rest 28 % are in a desperate state.

MPs can negatively affect soil aggregation (de Souza Machado et al., 2019a; Lehmann et al., 2019; Lozano & Rillig, 2020). Respective to the shape the MP fiber decreased soil aggregation by about 29%, films by 20%, and 27 % whereas, polymer LPDE foams decreased the 35% polypropylene by 32% and polycarbonate by about 31% (Lozano et al., 2021), Meta-analysis study revealed that MPs hurts the soil aggregation (Li et al., 2022). Moreover, another study revealed that MPs with organic matter like wheat straw and *Plantago* had a negative effect on soil aggregation (Liang et al., 2021).

Table 6. MPs size, shape and type and their effect on plant physical properties

Shape	**Size**	**Type**	**Effect**	**Reference**
Fiber, films, foams, and particles	>5 mm	Polyamide Polyester, polypropylene, polyethylene terephthalate, polyethylene, polyurethane	Negatively affected the new aggregates formation	(Lehmann et al., 2021)
Fiber, Films, foams, fragments	>5 mm	Polypropylene Low density Polyethylene, Polyethylene terephthalate, Polyurethane, Polystyrene,	Decreased soil aggregation	(Lozano et al., 2021)
Bead	15-20 µm diameter	Polyamide		
Fiber	Length 5000 µm, diameter 8 µm	Polyester (PES)		
Fragments,	2 mm sphere, Dimension > 800 µm	Polyethylene high density,		

	2 mm sphere, dimension 647-754 µm	Polypropylene	Soil structure affected by all MPs	(de Souza Machado et al., 2018)
	547-55 µm dimension,	Polystyrene,		
	2 mm cylinder 22-258 µm 2mm cylinder	Polyethylene terephthalate		
Fiber	4.56 ± 0.94, 4.20 ± 1.37, 4.05 ± 0.1.14 mm length	Polyester 1, Polyester Polyacrylic	Decrease water soil aggregation with the addition of organic mater (Wheat straw and *Plantago*)	(Liang et al., 2021)
Microfiber	0.37 to 3.14 mm length 0.026 ± 0.005 mm diameter	Poly acrylic	No significant effect	(Liang et al., 2019b)

2.3.3. Impact of MPs on the soil Chemical properties

Numerous studies indicated that MPs could affect the soil's chemical properties like soil pH (Qi et al., 2020), soil organic matter (Liu et al., 2021), and soil nutrients (Li et al., 2021). In most cases, MPs increased the soil pH. Lozano et al. (2021) found an increase in pH when inoculating the soil with 0.4% w/w MPs. However, some studies revealed low pH or no significant effect on the pH. High-density polyethylene MPs reduced the pH however the PLA MPs did not show any effect on the pH (Boots et al., 2019). Other factors are also responsible for the alteration of the pH, for example during the photodegradation of the MPs, MPs release additives chemical that alters the pH of the soil (Bandow et al., 2017). Moreover, the MPs change the microbial communities that affect the pH of the soil (Kim & Rillig, 2022). However, more effort and research are required to explore the effect of MPs on the pH of soil

Soil organic matter is substantial for soil health. There is a mixed result about MPs effect on the soil organic matter. Soil organic matter in the presence of MP is more affected in alkaline soil than acidic and increased with the increase of MP concentration (Liu et al., 2021). Soil organic matter increased in the presence of MP because the MPs itself is the carbon source and released carbon in the soil (Lee & Hur, 2020). Plastic decomposing bacteria present in soil promote the degradation of MPs in soil and promote the carbon content (Zhang et al., 2019). However, some studies suggested low SOM in MP inoculated soil (Dong et al., 2021) or a significant effect (Ren et al., 2020).

Soil nutrients in the presence of MPs can be increased, decreased, or have no effect. For example, in the rice field PVC-MPs decreased the available nitrogen and phosphorus by about 13% and 30% respectively (Yan et al., 2021) . PLFA MPs increase the NO3-N and NO2-N under low carbon conditions (Chen et al., 2020). Moreover, in another study, the phosphorus content increased in the plasticized PVC and decreased in the absence of non-plasticizer plastic (Yan et al., 2021). Furthermore, the high dibutyl phthalate content in the PVC was also found to greatly affect the nitrogen attributes of soil Zhu et al. (2022) suggests that plasticizers can play a substantial role in the availability of nutrients. Other factors can influence the nutrient availability as MP can affect the AMF community that play role in the P availability

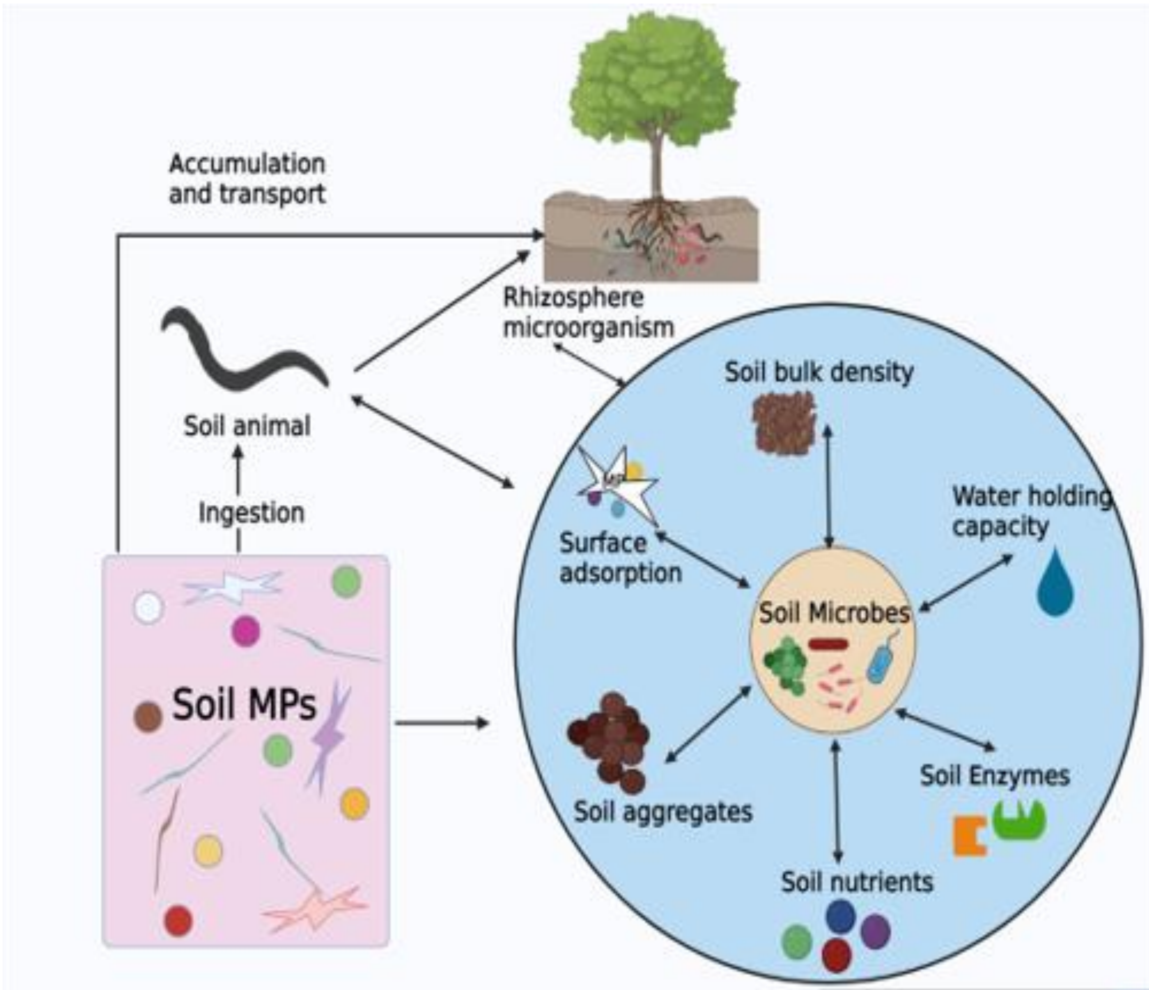

Figure 3. MPs effect on soil biological (Soil microbes, soil enzymes, soil animals and plants), chemical (Surface adsorption and soil nutrients), and physical properties (Water holding capacity, soil bulk density and soil aggregates

2.4. Interaction of MP with Heavy metals

Heavy metals are sometimes known as metalloids such as chromium (Cr), cadmium (Cd), Nickle (Ni), Arsenic (As), Copper (Cu) and Mercury (Hg) are part of the soil but when these metals cross permissible limit these can affect the soil properties and plant growth (Alengebawy et al., 2021). Heavy metal is an irreversible and persistent pollutant, therefore, these are the serious threat to the environment (Li et al., 2019) . Heavy metals can enter agricultural land through anthropogenic activities (Chen et al., 2015) *such* as mining, application of pesticides and fertilizers, smelting, and combustion of fossil fuels (Li et al., 2019). Soil is contaminated at 5 million sites that cover about 500 million ha of land globally with heavy metals exceeding the permissible limits (Liu et al., 2018).

Limited studies have been done on the interaction of MP and heavy metals. (Pinto-Poblete et al., 2022) revealed the Cd concentration in root and soil increased

when inoculated with the MP. The heavy metal distribution also depends on the soil aggregates (Deng et al., 2018), and MPs contamination affects soil aggregation. The ability of MPs to adsorb the heavy metal depends upon the size of MP and small size MPs have a more adsorbing capacity as compared to the larger particles (Gao et al., 2019). Presence of the MP in soil increase the desorption capacity and decreases the desorption capacity which leads to enhancement of the bioavailability of metals (Li et al., 2021). Li et al. (2021) suggested that the adsorption capacity of cadmium on MPs also depend on the functional group of the MP and pH therefore MPs act as the carrier for the heavy metals.

Cadmium and MPs decreased the root and shot biomass and affect the soil enzyme activity (Poblete et al., 2022). Zhu et al. (2021) revealed that MP+ arsenic combined less affected the bacterial community. However, there is scarce information about the interaction of heavy metals and MPs. More research is needed to explore the interaction between MP and Heavy metals.

2.5. Interaction of MP with Biochar

Biochar produces under low temperature and limited oxygen can increase the soil's organic carbon and contribute to soil carbon sequestration (Wang et al., 2015). Biochar provides a habitat for the microbial communities (Cai et al., 2021; Song et al., 2020; Ye et al., 2017) and increases the gram-positive bacteria (Zhang et al., 2018) , gram-negative bacteria, and fungi (Dominchin et al., 2021) . Biochar enhances the enzyme activity in the soil (Song et al., 2020; Wang et al., 2015). Assorted studies reported that the application of biochar is useful in the removal of various contaminates (Diao et al., 2021; Yang et al., 2021).

Few studies explore the interaction between biochar and MPs. Li et al. (2022) and Wang et al. (2020) investigate the co-application of biochar and MPs for the remediation of the MPs. A study revealed that biochar can be stuck, trapped, and entangled in the MPs sphere (Wang et al., 2020) . Li et al. (2022) investigated that metal-loaded PVC reduced the bioavailable metal in biochar. Moreover, biochar increased the enzyme activity and bacterial community (Palansooriya et al., 2022; Dissanayake, 2022).

3. MATERIAL AND METHOD

3.1. Soil, Biochar and MPs properties

3.1.1. Soil Properties

The soil used in the experiment was collected from the agricultural research field of MNS University of Agriculture Multan, located at 30.1598° N, 71.4502° E, with an elevation of 178 m above the sea level in the suburbs of city Multan, south of Punjab. The crops in the agricultural field are mostly irrigated from canal water in summer and in winter tube well water from the ground is used to irrigate the fields. Tube well irrigation is quite expensive as it required a continuous supply of electricity, therefore, farmers use sewage water to irrigate crops thinking that sewage water contains the essential nutrients for suitable crop growth. Wheat (*Triticum aestivum* L.), Cotton, Maize (*Zea maize* L.), and sometimes mustard or vegetable crops as well were cultivated in the fields. 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 a shovel. All the samples were mixed thoroughly for further physiochemical analysis. The sample were then air dried, cleaned from stem and roots residues of crops, and sieved through 2mm mesh. The sample was stored at a temperature of 20±2ºC in the glasshouse.

The soil was weakly structured silt loam with moderately calcareous brown river alluvium and had fluventic haplocambic, hyperthermic, cambic, and orchric subsurface horizons (Miani Soil Series). Soil have electrical conductivity (EC, 5.8 mScm-1) ºC, pH (0.01 M CaCl2) of 7.8 and a low amount of organic matter (0.71 %), total nitrogen (1.9 %), phosphorous (CAL-P, 8.4 mg kg-1), potassium (KAL-K, 155 mg kg-1)

3.2.1. Biochar properties

Biochar was produced from the cotton using Kon Tiki flame Curtin Pyrolysis Kiln uses for small-scale production of biochar worldwide. Biochar produces in a kiln at a specific temperature of 650-750ºC. The produced biochar was analyzed from Eurofins Umwelt Germany for detailed physical and chemical properties. The particle size and surface morphology of biochar were determined by using Scanning Electron Microscope (SEM, JEOL JSM-6490A, Analytical Scanning Electron Microscope) at 20 kV, X20,000, and X50,000.

Table 7. 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

3.3.1. MPs properties

Polyvinyl chloride (PVC-MP) was used in this study as it is an emerging contaminant wastewater that uses 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 particles shape was round and spherical and varied sizes ranging between <0.5 µm – 10 µm.

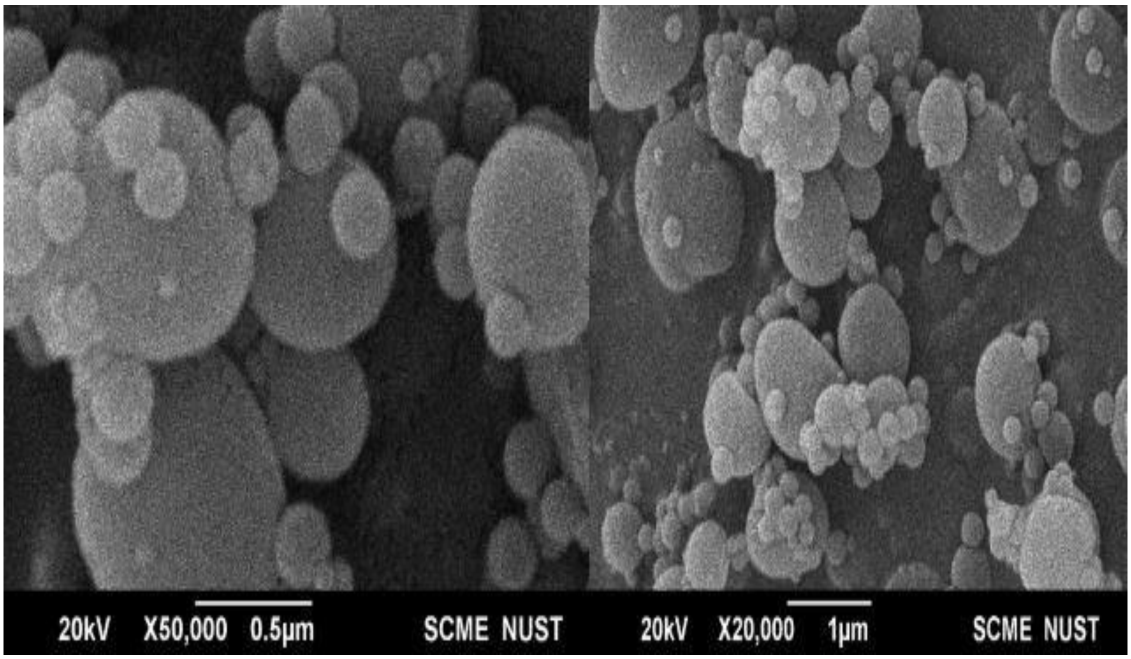

Figure 4. Size of MPs determined under the SEM

3.2. Experimental Design:

Wheat was chosen for the study because of its economic importance in Pakistan. Two experiments were done under controlled glasshouse conditions at the plant Biotechnology Department, at the National University of Science and Technology. In first study experimental treatments consist of PVC-MPs (i) control, ii) 0.25 % and iii) 0.5 %, (w/w)) and cotton stalk biochar (i) control, ii) 0.5 % (w/w). In Second study experimental treatment comprised of (i) control, ii) Cadmium 600mg, iii) 0.25 % and iv) 0.5 %, (w/w)) and cotton stalk biochar ((i) control, ii) 0.5 % (w/w)) The PVC-MP concentration was based on the previous studies of Li et al. (2020) and Dong et al. (2021). Biochar concentration was selected from the study by Haider et al. (2015) as the study concluded that biochar at low concentration provides a better crop response.

All the treatments had 6 replicates (n=6, N=18) and two additional repeats. One set of experiments was used for growth treatments and physiology and other sets for the chemical analysis and biodiversity-related molecular analysis. Rhizoboxes for the plants were created from the transparent polyethylene boxes having length = 15.5cm, diameter= 10.5cm and height = 3.5cm. MPs and Biochar were mixed in the soil for about 15 minutes in each treatment. Rhizoboxes were filled with 500g of soil each and incubated for a week. In first experiment treatment received 0.25% (1.25g) and 0.5% (2.5g) PVC-MPs while 0.5% (2.5g) biochar kg-1 of dry soil. Another experiment treatment receives the same concentration of PVC-MP and Biochar along with Cd 600mg kg-1 in soil. Each Rhizobox was watered at 60% of the total water holding capacity. water holding capacity was measured according to the method described by Kammann et al. (2011).

The wheat seeds of the cultivar 'Super' were obtained from National Agriculture Research Centre 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 rhizoboxes. Plants were grown in the glass house under controlled conditions: the 23-24ºC temperature at daytime and 16-17 ºC at night, 14/10 day/night photoperiod, and 65% relative humidity. After 7 days seedlings were thinned to three plants in each rhizobox. The rhizoboxes position changed after every three days for uniform environmental conditions. Boxes were irrigated regularly according to field capacity. Plants were grown for 22 days from 1st November 2021 to 23rd November 2021 after germination and then harvested.

3.3. Dry Biomass

After harvesting, the steel sieve was used to remove the soil particle from the roots. Forceps were used to remove all the debris from roots. Roots and shoots were separated and dried. The dry weight of the shoot and root were measured by digital balance.

3.4. Antioxidant activity:

0.5g of leaf from each sample was ground in phosphate buffer and centrifuged at 13000rpm. The supernatant was separated into a small tube. For superoxide dismutase (SOD), a reaction solution was formed that contain NBT, Riboflavin, methionine, EDTA, and 0.025ml enzyme. The optical density(OD) value was measured at 560 nm on a 3500 UV-VIS spectrophotometer (Agilent technologies). Peroxidase was measured in the reaction solution of hydrogen peroxide, guaicol, and 0.1ml enzyme. OD value measured at 470nm.

3.5. Measurement of soil enzyme activities

The soil dehydrogenase activity has been used for overall microbial activity (Casida, 1977) , and it plays a crucial role in the soil carbon cycle. The dehydrogenase activity was measured by spectrophotometer according to (Kassem Alef, 1995) . The soil (5g) was taken in a 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 optical density of the soil, the 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 (Kassem Alef, 1995)(Alef, 1995).

To determine the urease activity moist soil collected from the experimental pots was sieved from a 2mm sieve and saved in the polyethylene 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. After the filtration process ammonium content was measured at the optical density of 690 nm (Kandeler & Gerber, 1988).

3.5. Determination of microbial abundance

Bacterial and fungal abundance were estimated through cultural-independent technique. 0.5-gram soil was from the experimental pots was taken for DNA extraction. DNA extracted by using the Power soil DNA extraction kit (MoBIO). Nanodrop was used for the estimation of DNA concentration (Bustin et al., 2009) (Bustin et al.,2009). Total bacterial and fungal abundance with 16S rRNA and ITS rRNA respectively were assessed by real time PCR. Each reaction for PCR contains (25 µL), SYBER Green Master Mix (12.5 µL), DNA (10 ng), each primer (0.2 µmol) and BSA (0.2 mg/mL). Plasmid of 16S rRNA (bacterial) and ITS rRNA (fungal) target region was utilized for the construction of standard curve (Fierer et al., 2005) (Fierer et al., 2005).

3.6. Phospholipid Fatty Acid (PLFA) 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 separate 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 trans esterify 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 the following pattern: Arbuscular mycorrhizal fungi (AMF): 16:1ω5c; 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 (Frostegard et al., 1993; Olsson, 2006; Zelles, 1999; Zelles et al., 1992) (Frostegard et al., 1993; Olsson, 2006; Zelles, 1999; Zelles et al., 1992).

3.7. Soil Organic Carbon and total nitrogen:

The soil organic carbon was measured by using the standard wet chemistry technique of soil sample extraction 'rapid dichromate oxidation of organic matter (Nelson, D., Sommers, 1982). Briefly, concentrated sulphuric acid (H_2SO_4) and 1 N $K_2Cr_2O_7$ (potassium dichromate) were added to a 0.5 g soil for wet digestion. After digestion, the solution was allowed to cool down before halting the reaction. 0.5 N ferrous ammonium sulfate solution was used for titration. For the determination of total nitrogen, soil samples were digested with H2SO4, and followed by distillation by adding sodium hydroxide (NaOH). Nitrogen was analyzed according to the method described by (van Schouwenberg, 1973).

3.9. Microbial Biomass Carbon and Microbial Biomass nitrogen:

5 g of soil was fumigated at 25ºC with CHCl3 (ethanol-free chloroform) for 24 hours, and the samples were extracted using 0.5M K2SO4. The same steps were done for the nonfumigated soil. The MBC was calculated after titration according to (Wu et al., 1990) (Wu et al., 1990).

$$MBC = (Cabonfumigated - Carbonunfumigated) \text{ x } 2.64$$

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.

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

3.10. Cd concentration measurement in plant:

Cd concentration was measured in shoot, root, plant and soil through atomic absorption spectroscopy after completing the acid digestion by the combination of sulfuric acid and nitric acid of the shoot, root, plant and soil.

3.11. Statistical analysis:

Two-way ANOVA was performed for both for investigation interaction of PVC-MPs + Cd and biochar. Difference between the treatment were compared through the Tukey's test at $p < 0.05$. All the graphs were generated using statistic 8.1 software.

4. RESULTS

4.1. EXPERIMENT-1: EFFECT OF PVC-MP ON PLANT AND SOIL IN BIOCHAR AMENDED SOIL

4.1.1. PVC-MPs and biochar effects on plant growth, dry matter production and antioxidant activity

The PVC-MPs and cotton stalk biochar were used to investigate their effects on wheat growth, dry matter production and plant antioxidant activity (Figure5 - 8). Biochar addition and low dose (0.25%) of PVC-MPs significantly increased shoot dry matter production compared to control treatment and the use of high dose (0.5%) of PVC-MPs (Figure5). The combination of PVC-MPs and biochar showed that the biochar and PVC-MPs addition produced a relatively greater shoot dry weight than the control or high dose (0.5%) of PVC-MPs. However, there was no dose dependent response of PVC-MPs addition on plant dry matter production. The PVC-MPs and biochar addition showed no significant effects on root dry matter production (Figure6). However, overall biochar plus PVC-MPs treatments showed trend of positive biochar effects on wheat growth and dry matter production. There was no significant effect of PVC-MPs addition at any dose. Furthermore, the biochar addition as well showed no significant effect on plant antioxidant activities like superoxide dismutase (SOD) and peroxidase (POD) Figure 7 and 8, respectively.

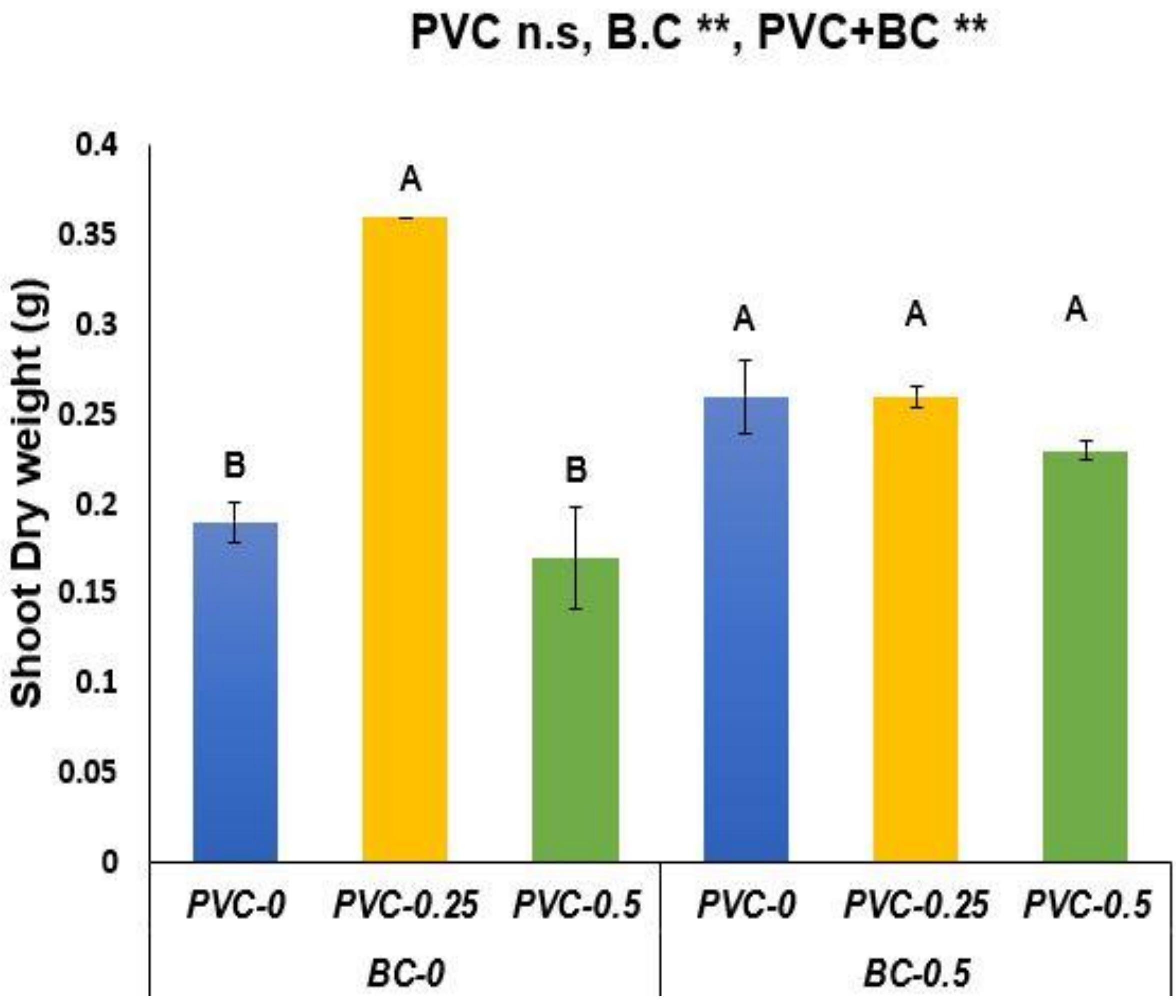

Figure 5. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on plant shoot dry weight. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

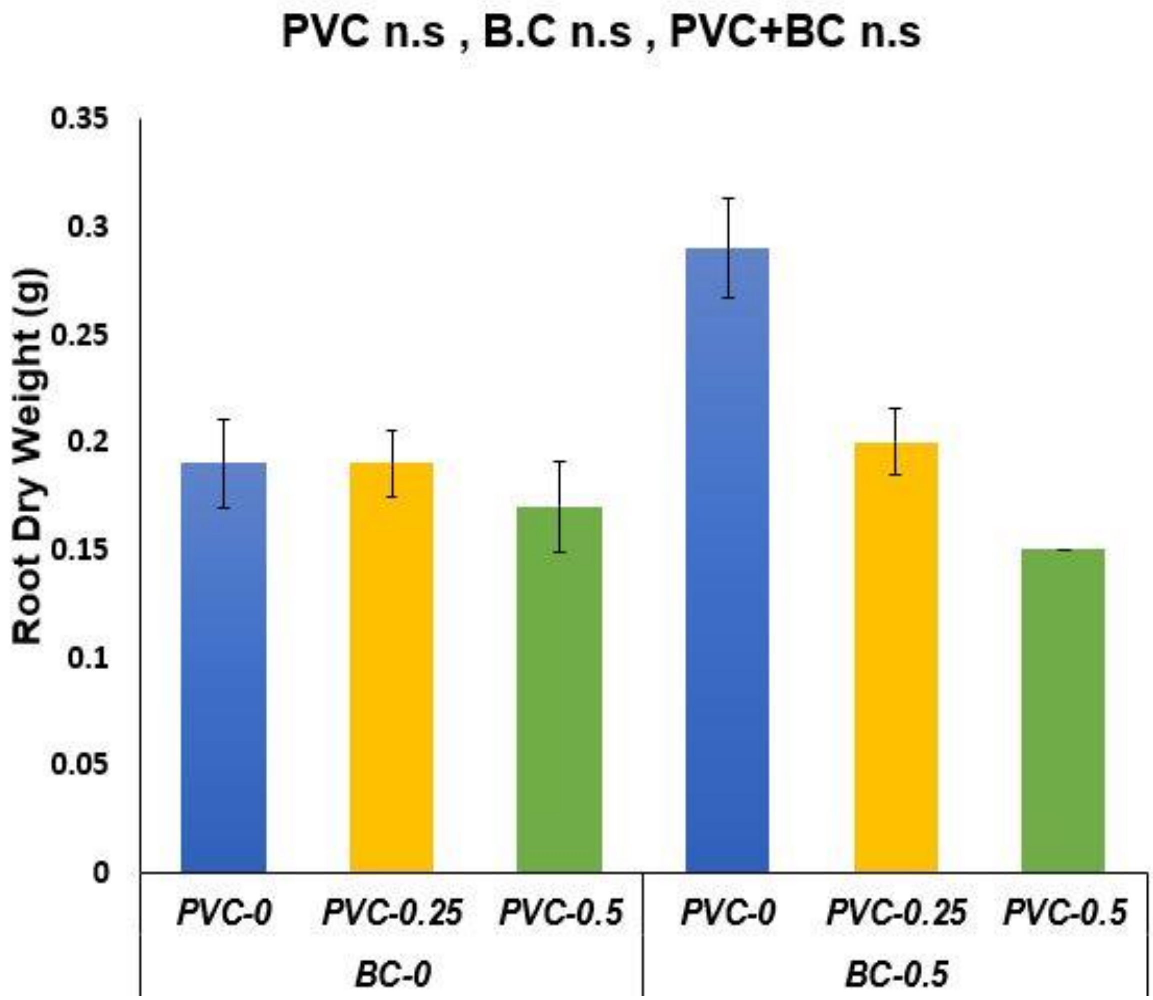

Figure 6. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on plant root dry weight. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %

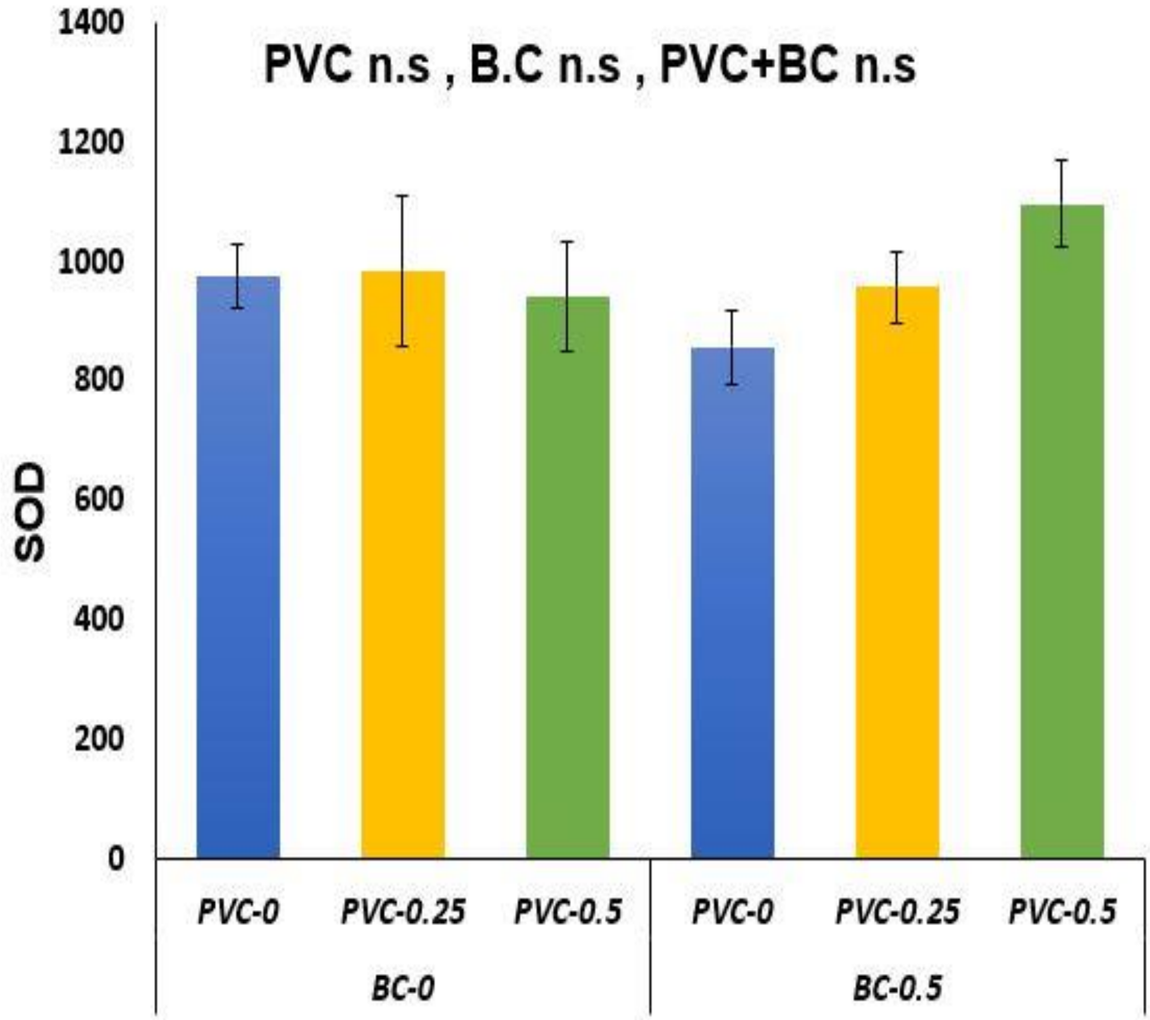

Figure 7. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on plant SOD. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

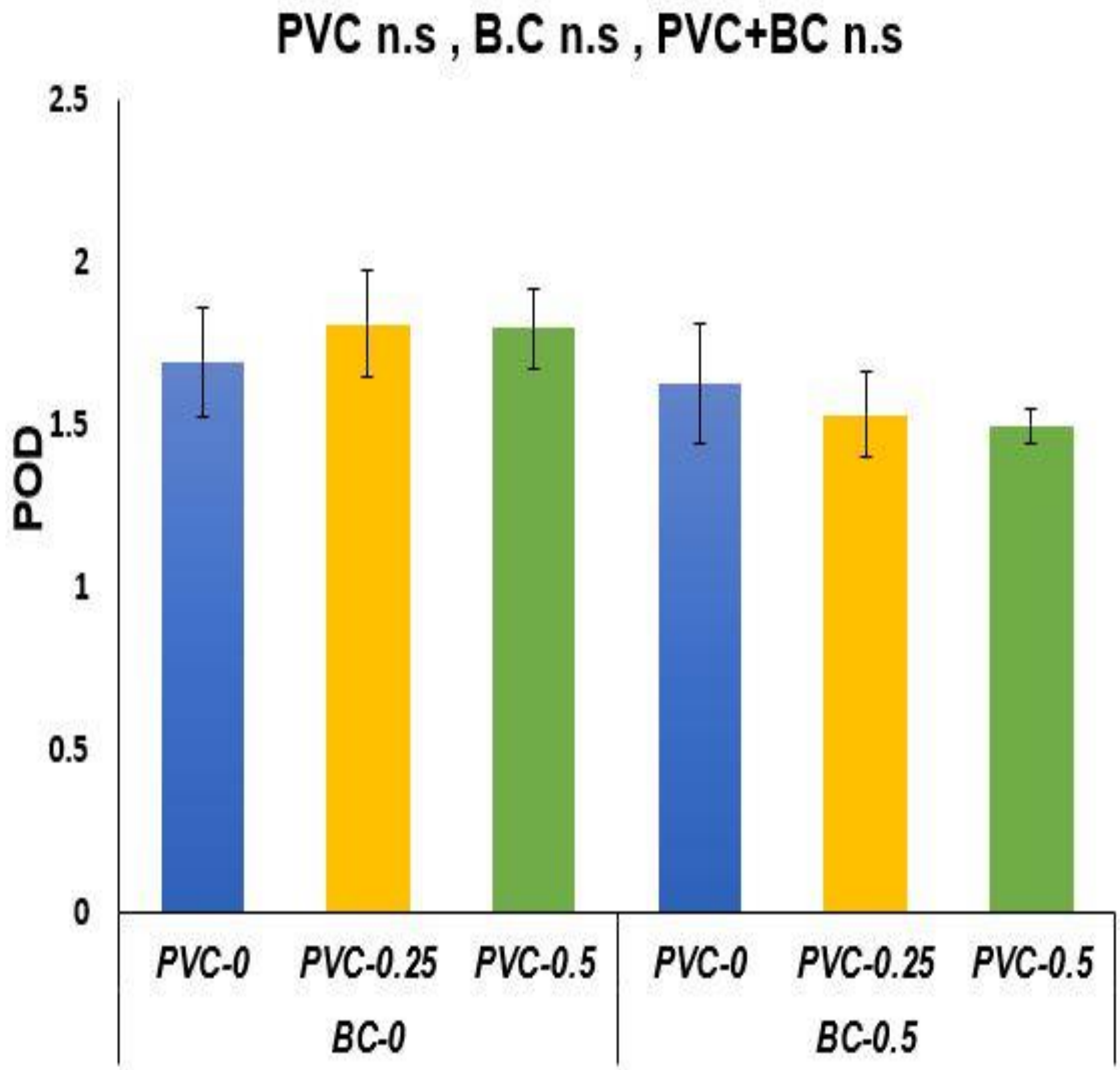

Figure 8. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on plant POD. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

4.1.2. PVC-MP and Biochar effect on soil organic carbon, total nitrogen, microbial biomass carbon and nitrogen

The PVC-MPs and cotton stalk biochar were used to investigate their effects on soil organic carbon and nitrogen (Figure 9, 10). The addition of PVC-MPs significantly affected total soil nitrogen and organic carbon as compared to controls. PVC-MPs soil has no significant effect on the total nitrogen in both the PVC-MPs concentration. However, soil with PVC-MPs and biochar application increased total nitrogen by +33.96% and +40.77% as compared to PVC-MPs residues treatments without biochar (PVC 0.25%, and 0.5%, respectively) (Figure10). Both the concentration if PVC-MP 0.25% and 0.5% reduced the soil organic carbon. At the same time, the co-application of biochar with PVC-MPs reduced the negative effects of MPs on soil organic carbon. Co-application of PVC-MP and Biochar increased the soil organic carbon about +37.8% and +41% (PVC 0.25%, and 0.5%, respectively) (Figure 9).

Generally, biochar application (alone or with 0.25% and 0.5% PVC-MPs) significantly increased overall MBN compared to control soil without any biochar or increasing PVC-MPs addition While as compared to respective control treatments, PVC-MPs addition (0.25% and 0.5%) reduced MBN when applied alone (-31.76% and -41.64%, respectively) However, it was visible that biochar addition combined with PVC-MPs showed a positive effect and increased the MBN about +32.8% and +41.3% respectively (Figure 12). PVC-MPs 0.25% and 0.5% without biochar reduced the MBC about -12.03% and -16.9%. However, co-application with biochar showed no significant effect but slightly increased the MBC by about +9.54% and +13.6% (Figure 11)

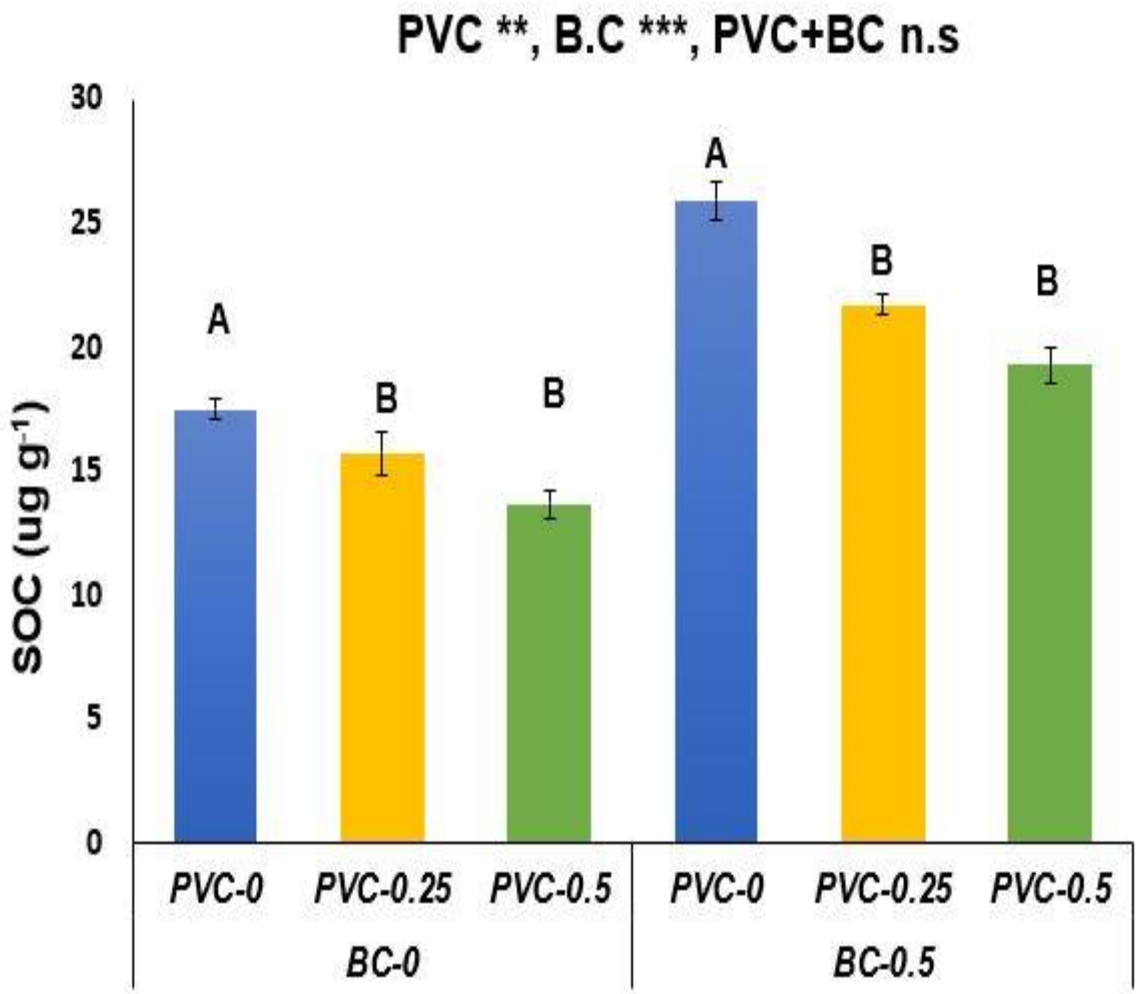

Figure 9. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on SOC. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

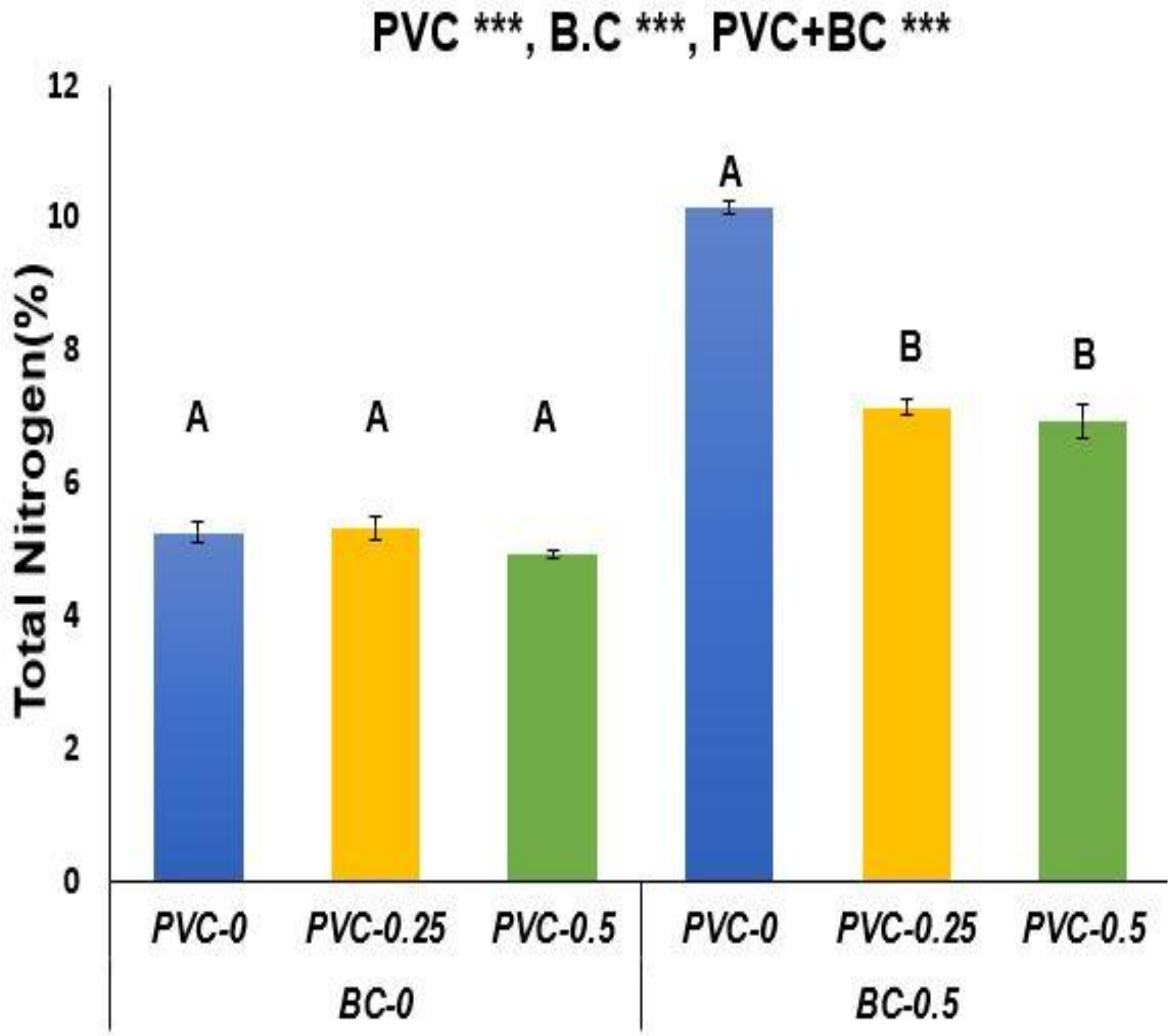

Figure 10. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on total nitrogen. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

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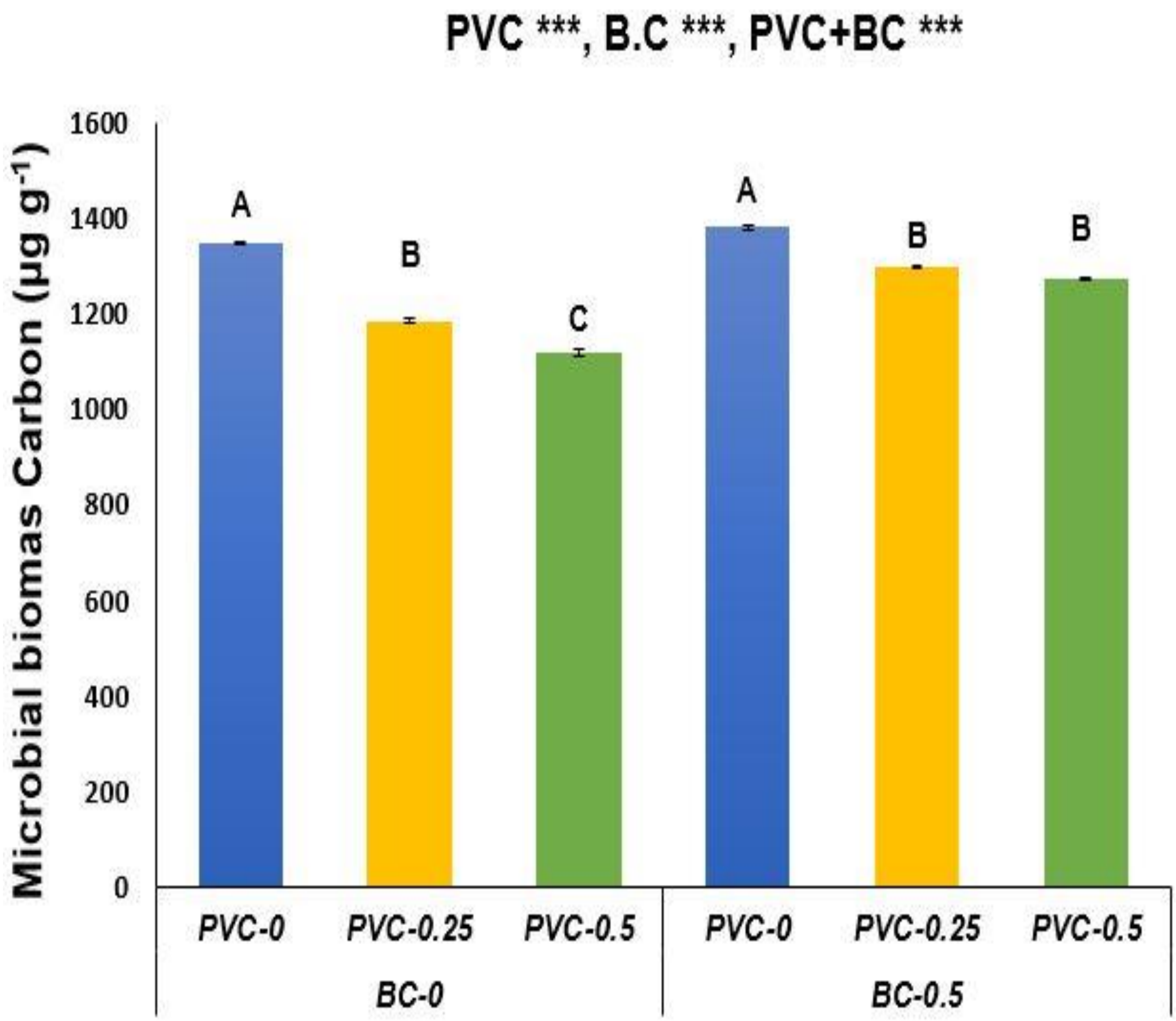

Figure 11. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on microbial biomass carbon. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

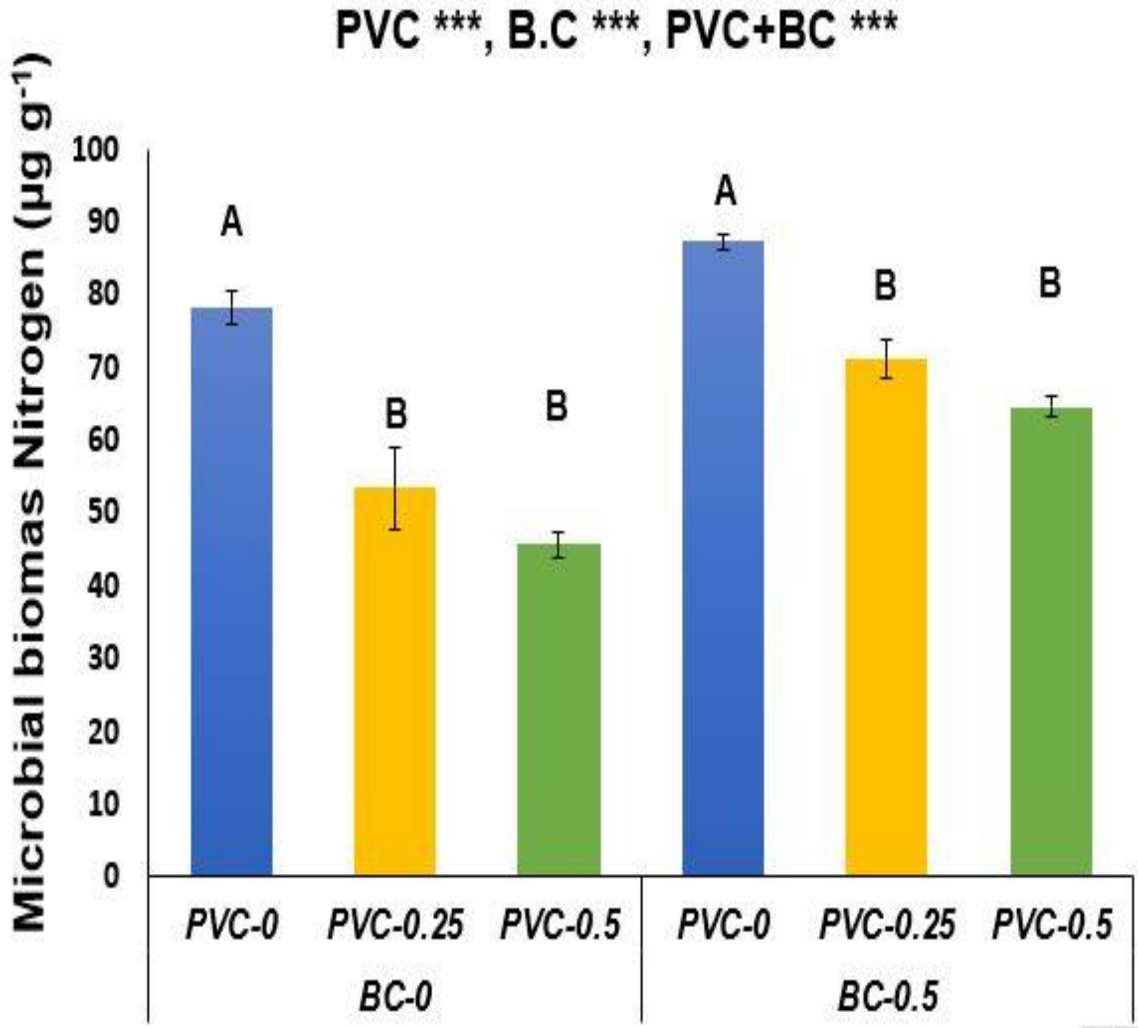

Figure 12. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on microbial biomass nitrogen. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

4.1.3. 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 and dehydrogenase. The urease activity was significantly reduced by -11.93% and -18.75% due to PVC-MPs addition at 0.25% and 0.5% addition as compared to control (no PVC-MPs addition). However, when biochar was applied (0.5%) in combination with 0.25% and 0.5% PVC-MPs, it showed no negative effects; rather, it increased urease activity compared to only biochar addition in control soil (Figure 13).

Dehydrogenase activity reduced in PVC-MPs by about -5.9% and -9.11% (0.25% and 0.5% respectively) highest concentration cause more reduction than the low concentration of PVC. However, biochar application did not affect significantly to reduce the effect of PVC-MPs but cause slight increase in the dehydrogenase activity as compared to the soil where PVC-MPs inoculated alone (Figure 14).

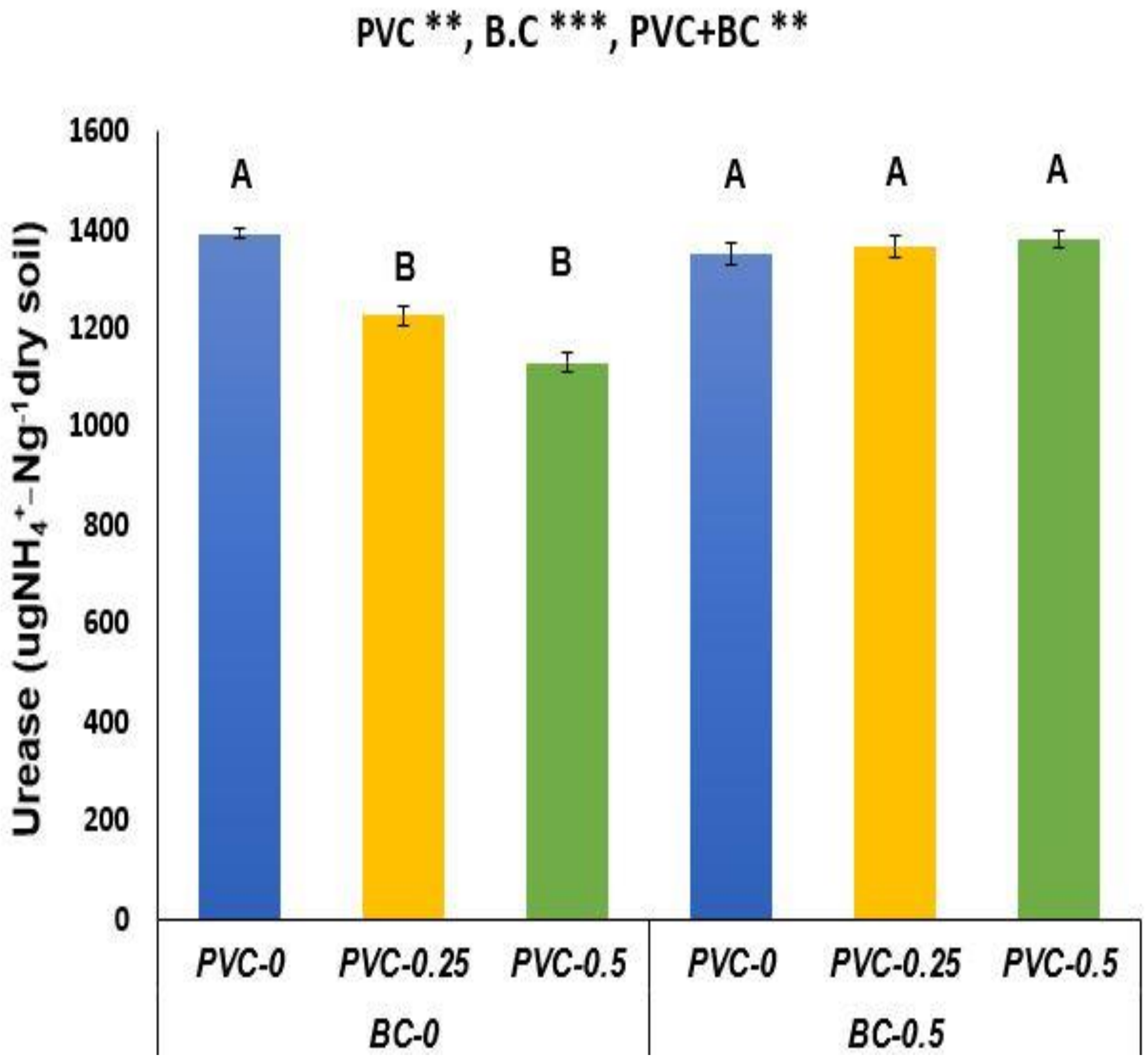

Figure 13. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on urease. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

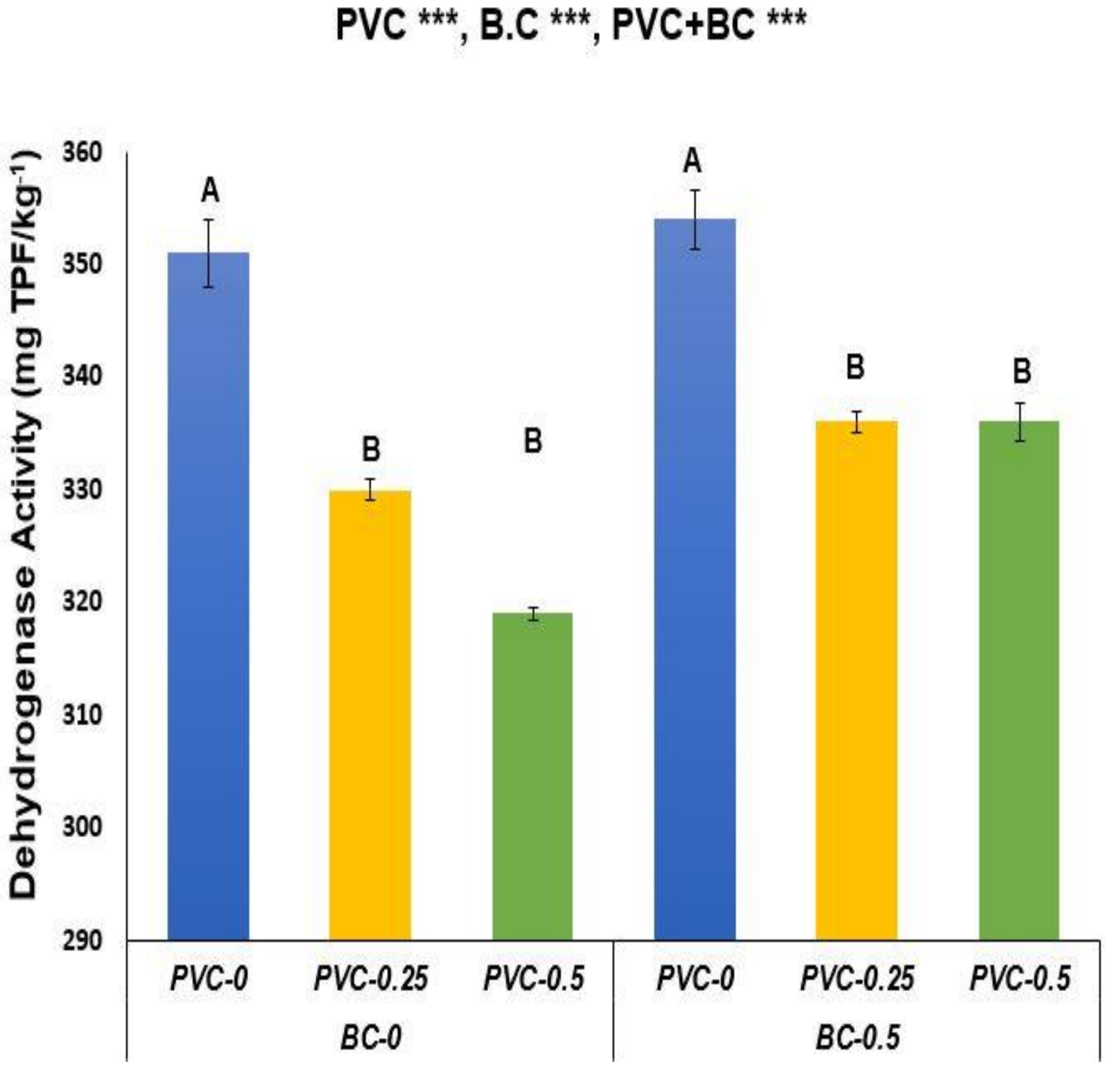

Figure 14. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on dehydrogenase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

4.1.4. Biochar and PVC-MPs effect microbial communities, soil bacterial and fungal gene abundance:

PVC-MP did not affect the abundance of the gram-positive bacteria in PVC-MP soil as compared to the control treatment. However, biochar application with PVC-MPs 0.25% and 0.5% increased the abundance of gram-positive bacteria by +6.76% and +5.86% respectively. Gram-negative bacteria are reduced in the presence of PVC-MPs however biochar application increased the gram-negative bacterial abundance. PVC-MPs (0.25% and 0.5%) decreased the gram-negative -10.09% and -12.97%. Co-application of PVC-MP, and Biochar increased the gram-negative bacteria by about +8.96% and +1.12%. Pseudomonas community drastically reduced -23.31% and -41.24% with inoculation of PVC-MP at 0.25% and 0.5% respectively. Biochar addition and PVC-MP treatment reduced the effect of MPs toxic and increased pseudomonas by about +28.5% and +17.6% in 0.25% and 0.5% PVC-MPs respectively. The PVC-MPs addition (0.25%, and 0.5%) reduced ($p < 0.001$) AMF community up to -8.39% and -20.16%, respectively over control treatment While the biochar addition (biochar alone, biochar+0.25%, and biochar+0.5% PVC-MPs) significantly enhanced AMF community up to +9.82%, and +7.01%, respectively, over control soil with no MPs or biochar addition (Figure 15-18)

The 16S rRNA gene abundance was reduced by -12.03% and -16.9 due to 0.25% and 0.5% PVC-MPs addition in soil without and with biochar, respectively but a significant improvement was observed with biochar plus increasing PVC-MPs addition (+9.54% and +13.68%, respectively). Total fungal gene abundance was significantly enriched in biochar added PVC-MPs treatments over no biochar with PVC residues. The PVC-MPs residues drastically reduced (-31.6% and -41.67%, with MPs 0.25% and 0.5%, respectively) total fungal gene abundance compared to the control treatment. Interestingly, biochar addition with 0.25 % and 0.5 % PVC-MPs reduced their toxic effects on fungal gene abundance and increased fungal abundance by about +32.8% and +41.3% (Figure 19, 20).

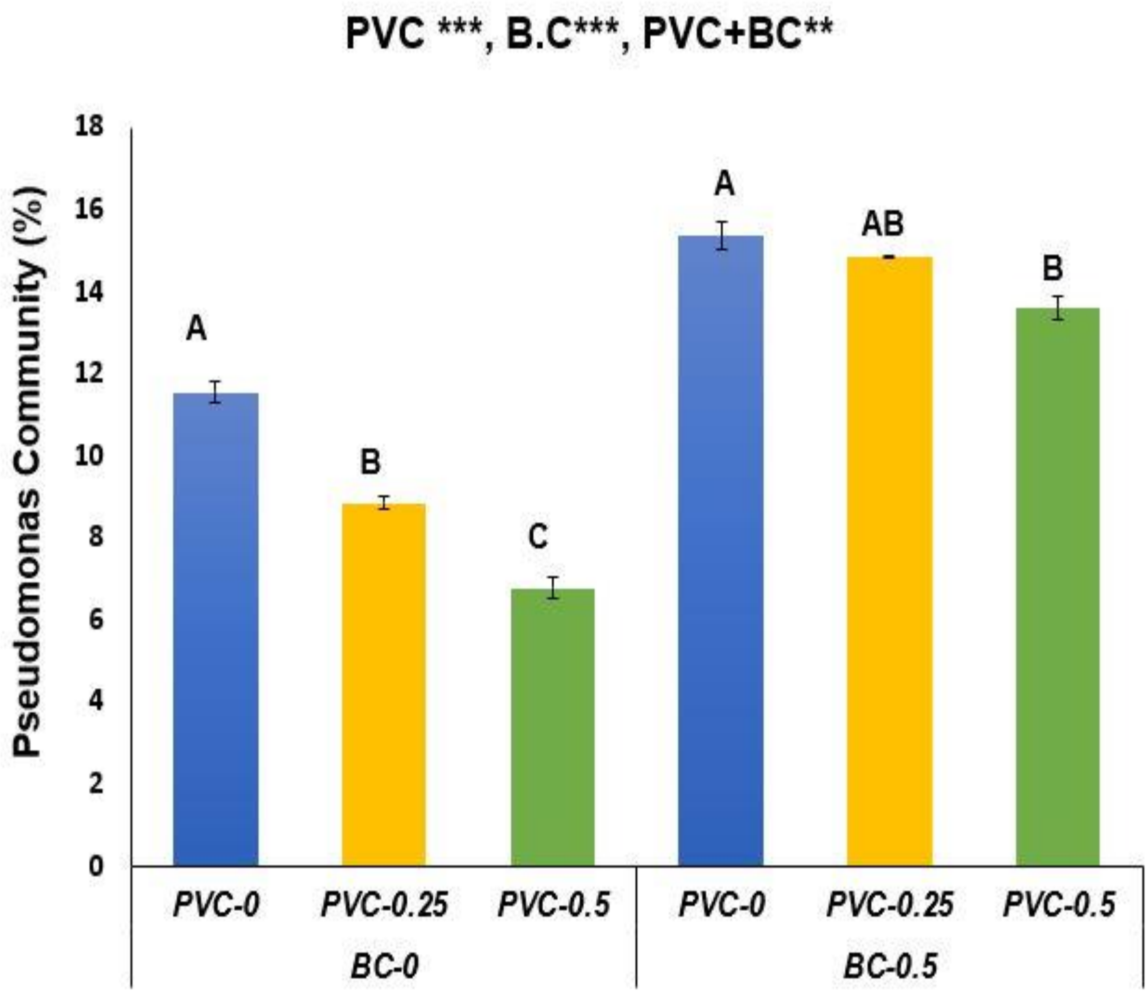

Figure 15. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on pseudomonas community. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

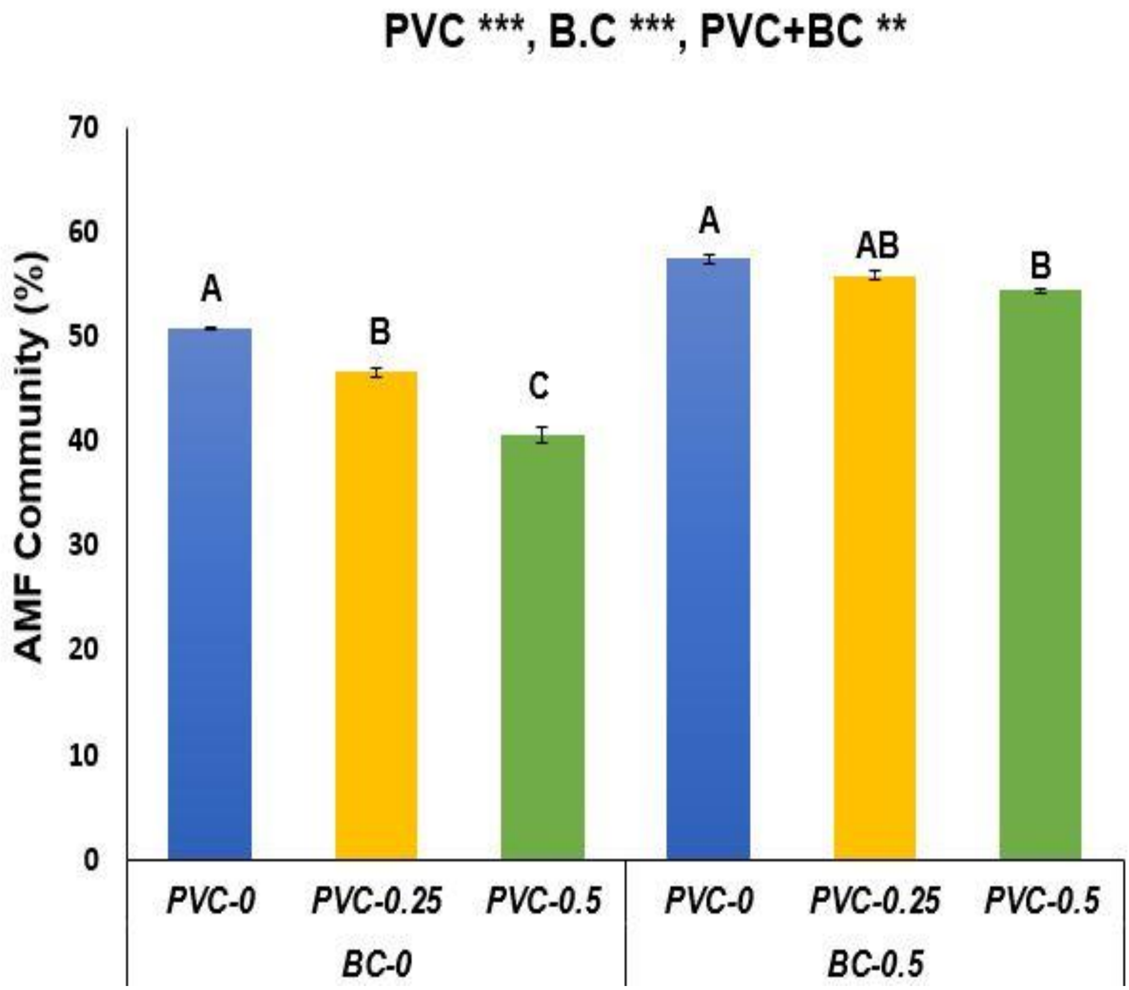

Figure 16. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on AMF community. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

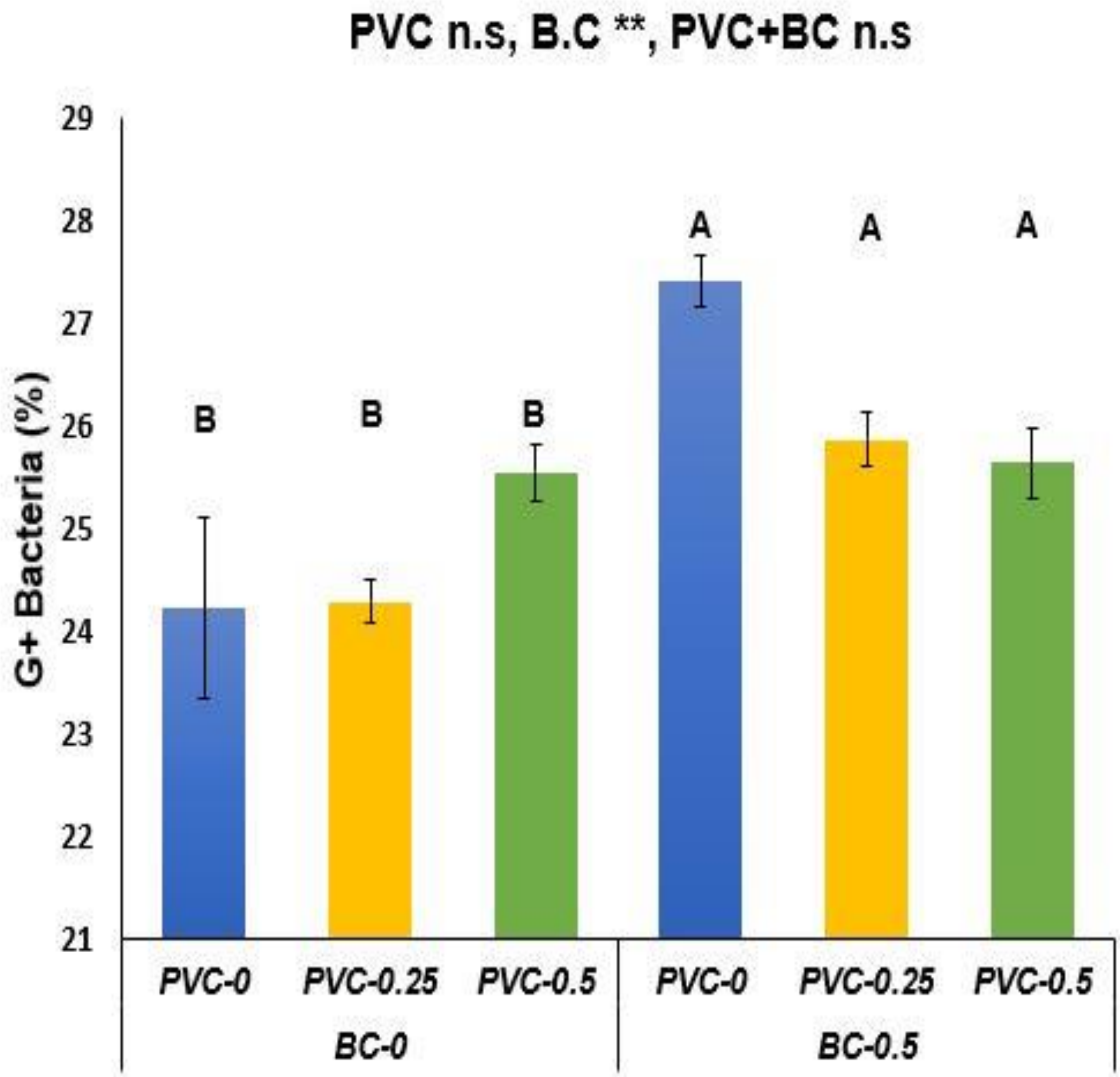

Figure 17. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on G+ve bacteria. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

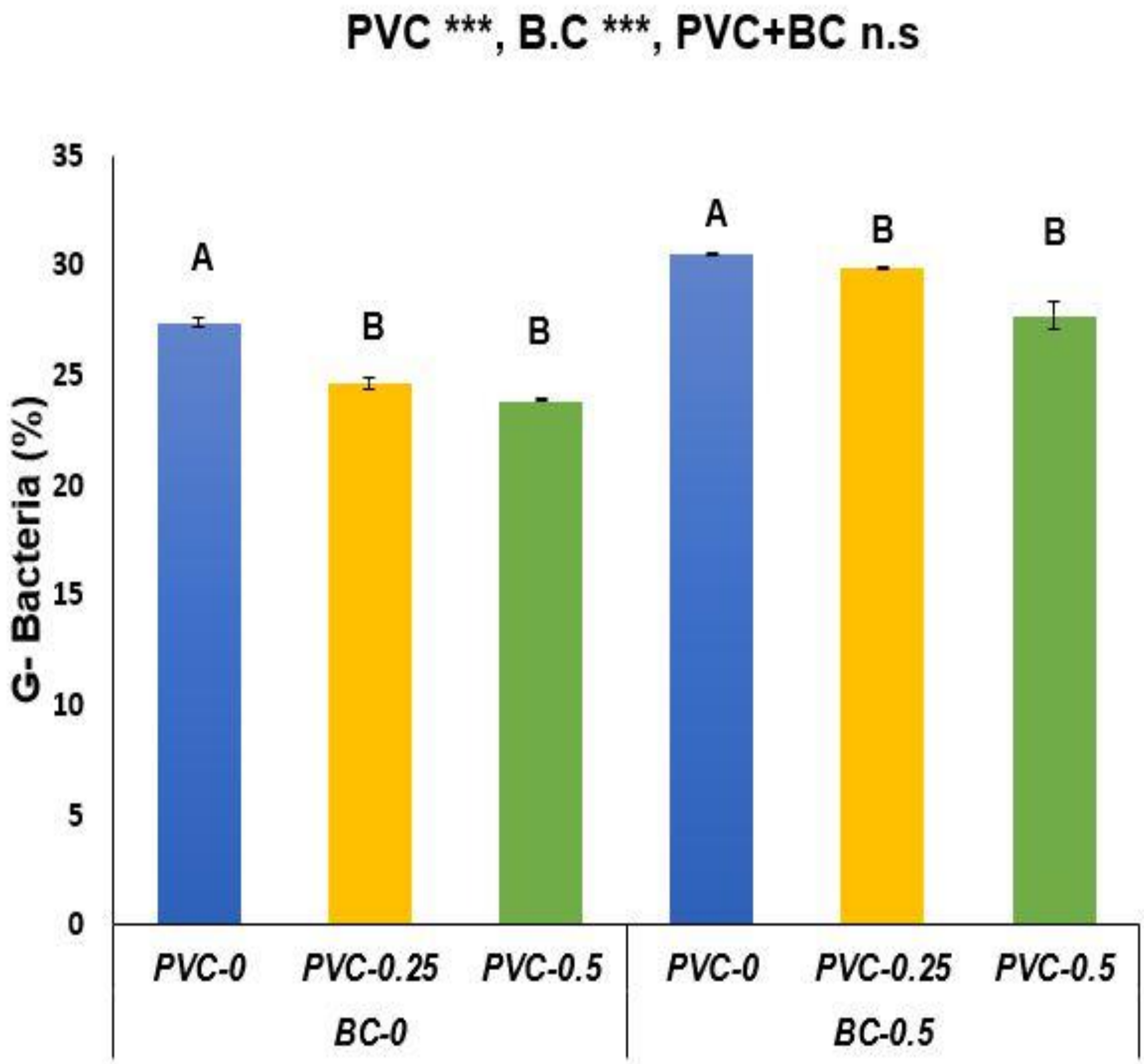

Figure 18. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on G-ve Bacteria. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

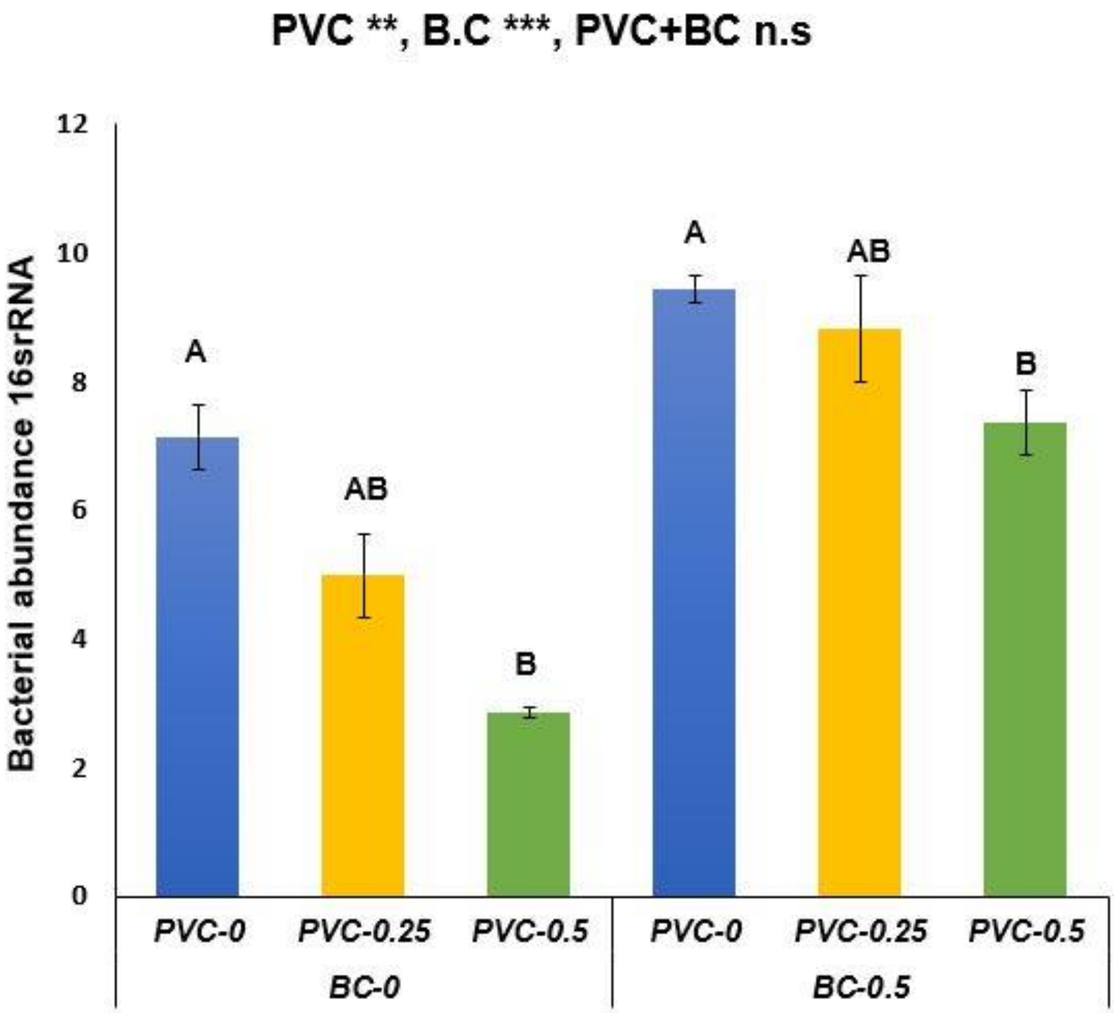

Figure 19. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on bacterial abundance. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

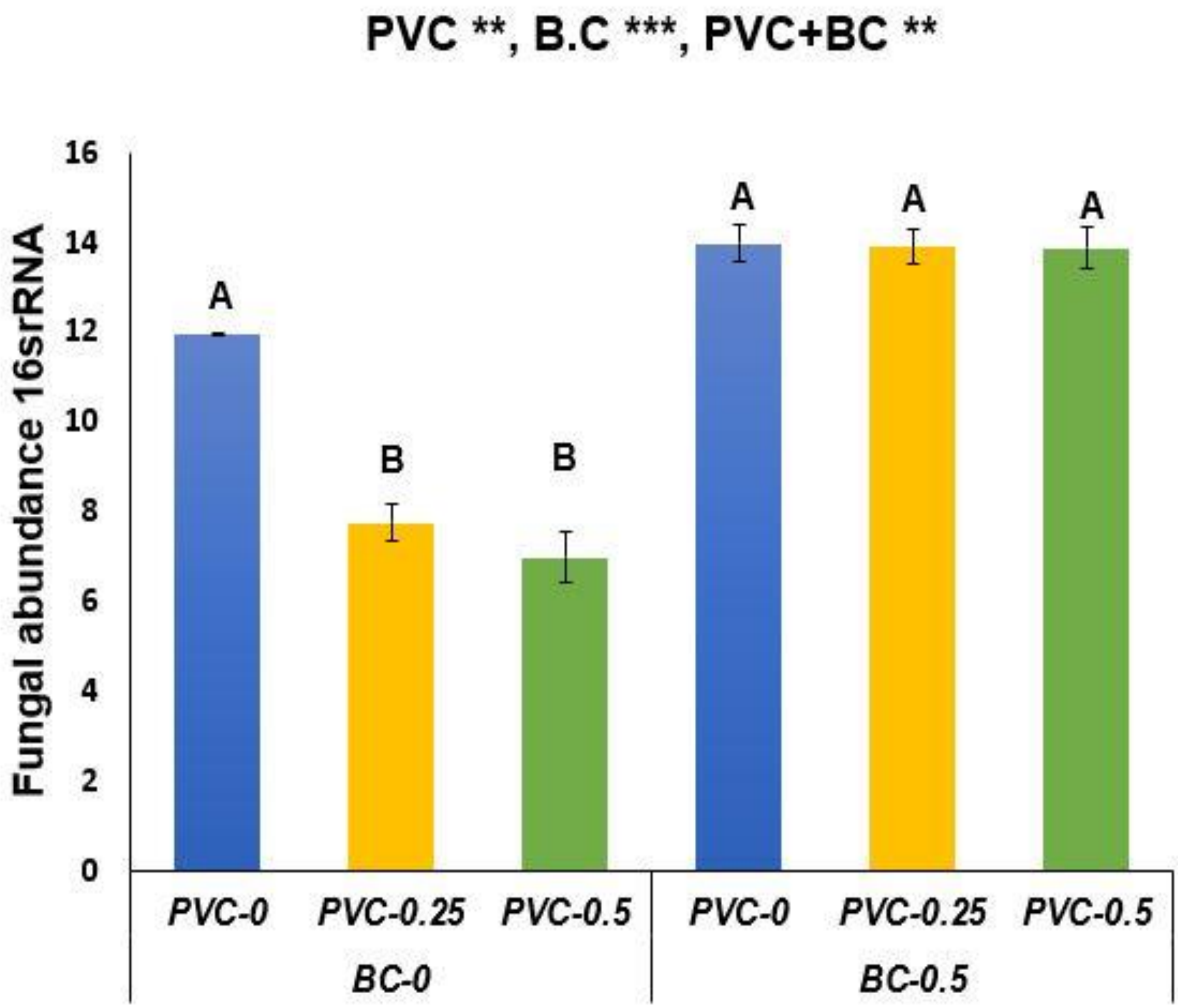

Figure 20. Effect of PVC-MP (control = 0, PVC-0.25%, PVC-0.5%, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on fungal abundance. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

4.2. Experiment:2 Effect of contaminants (PVC-MP + Cd) on plant and soil in biochar augmented soil

4.2.1: PVC-MP + Cd and biochar effect on the shoot and root dry biomass

The PVC-MPs + Cd and cotton stalk biochar were used to investigate their effects on wheat growth, shoot dry weight and root dry weight (Figure 21, 22) PVC-MP+Cd significantly affects the shoot dry weight as compared to control. 0.25% PVC-MP+ Cd reduced the biomass by about -68.4% and 0.5% PVC-MPs + Cd reduced the shoot biomass by about -47.3 %. Biochar alone increased the shoot biomass as compared to the Cd and PVC-MP s+ Cd contaminant soil. Co-application with biochar reduced the effect of both contaminants and increase shoot dry mass by about 116.6 % and 50 % in 0.25% and 0.5% PVC-MPs concentration (Figure21). PVC-MPs + Cd did not affect significantly the root biomass of the plant. However, biochar addition in the PVC-MP+Cd soil reduced the negative effect of these contaminants and increased the root biomass by about + 333.3% and +22.2% in 0.25% and 0.5% PVC-MPs concentration. Biochar alone increased the root biomass as compared to Cd and PVC-MPs + Cd contaminant soil (Figure 22)

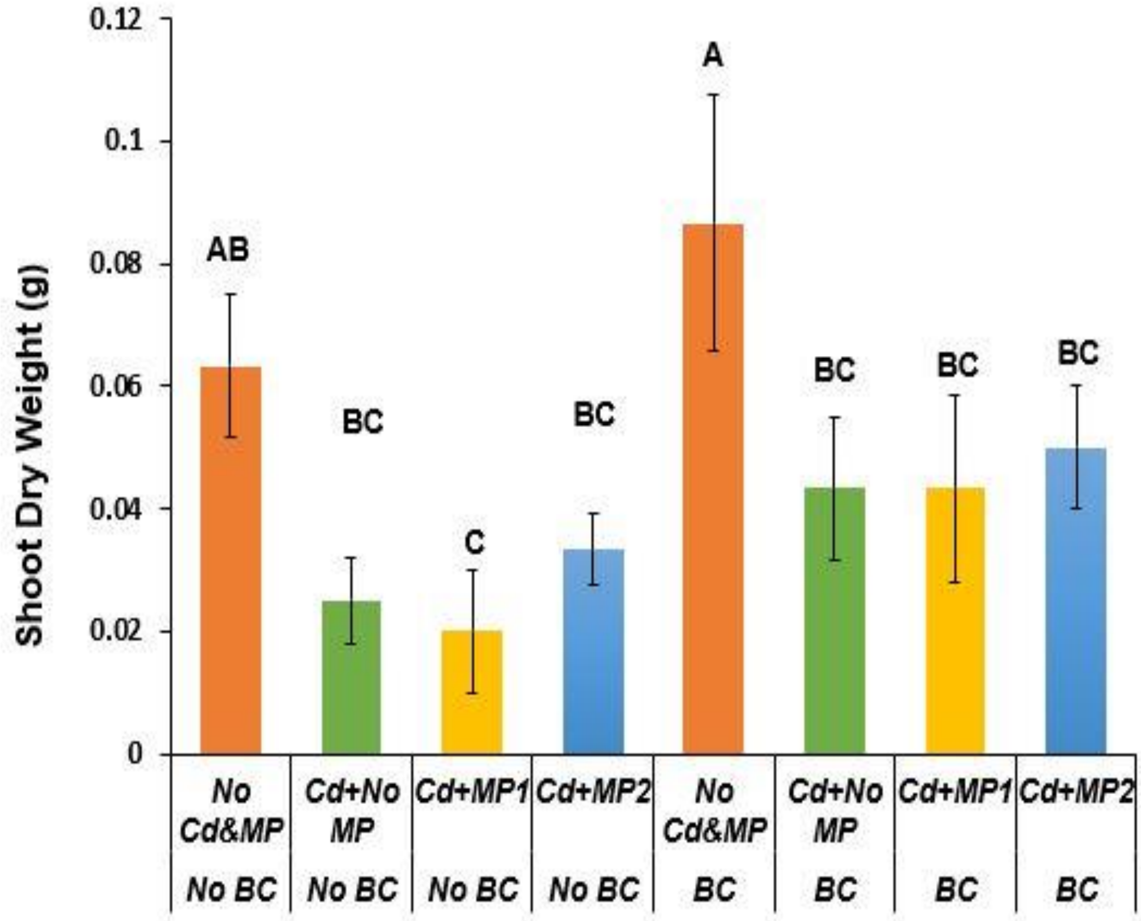

Figure 21. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on shoot dry weight. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

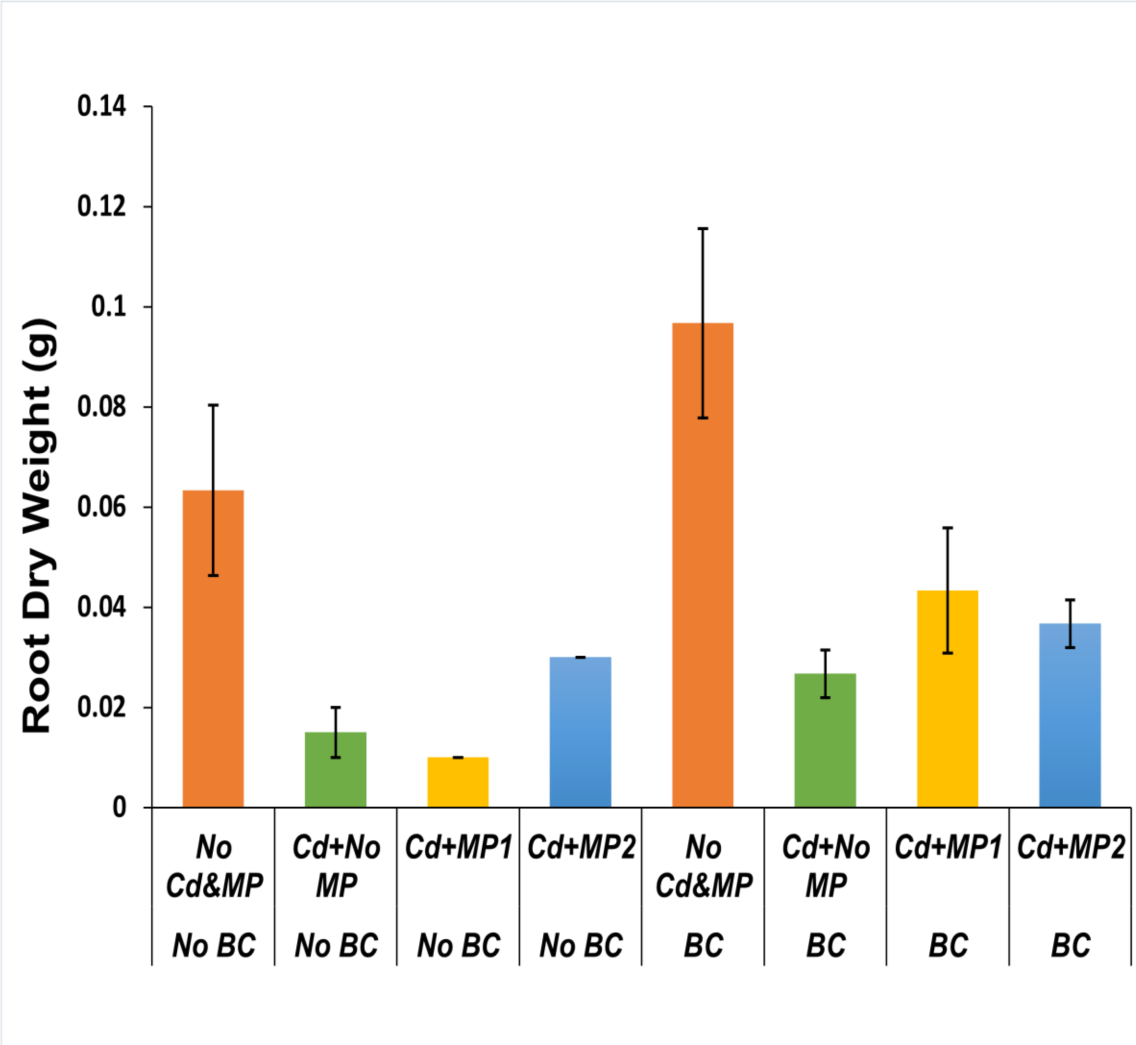

Figure 22. Effect of PVC-MPs + Cd PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on root dry weight. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

4.2.2. PVC-MP + Cd and biochar effect on soil enzyme:

The PVC-MPs + Cd and cotton stalk biochar were used to investigate their effects on soil enzyme activity, urease dehydrogenase, acid phosphatase and β glucosidase (Figure 23-26) The addition of the PVC-MP + Cd and biochar significantly affected the soil enzyme activity, Urease, dehydrogenase and β-glucosidase activity decreased in Cd + PVC MP by as compared to control however acid phosphatase activity increased in the PVC-MP + Cd contaminated soil. Biochar addition significantly increases the urease activity as compared to the no biochar treatment by about +11 % and +15.4% (0.25% and 0.5% PVC-MPs respectively) (Figure 23). B- glucosidase activity was reduced in PVC-MP+ Cd contaminated soil however biochar relieved the effect of both contaminants (Figure 24). There was no significant effect in dehydrogenase activity with or without biochar. However, control and only biochar treatment showed enhanced dehydrogenase activity (Figure 25). Acid phosphatase showed the significant result as compared to control. Cd + PVC-MP 0.5% showed the highest activity as compared to control. Treatment without biochar showed more activity as compared to the biochar treatments. PVC-MP + Cd showed different trend in the acid phosphatase activity as compared to the other tested soil enzymes (Figure 26). These results suggested that PVC + Cd reduced the soil enzyme activity except acid phosphatase however biochar reduced the effect of PVC-MP + Cd.

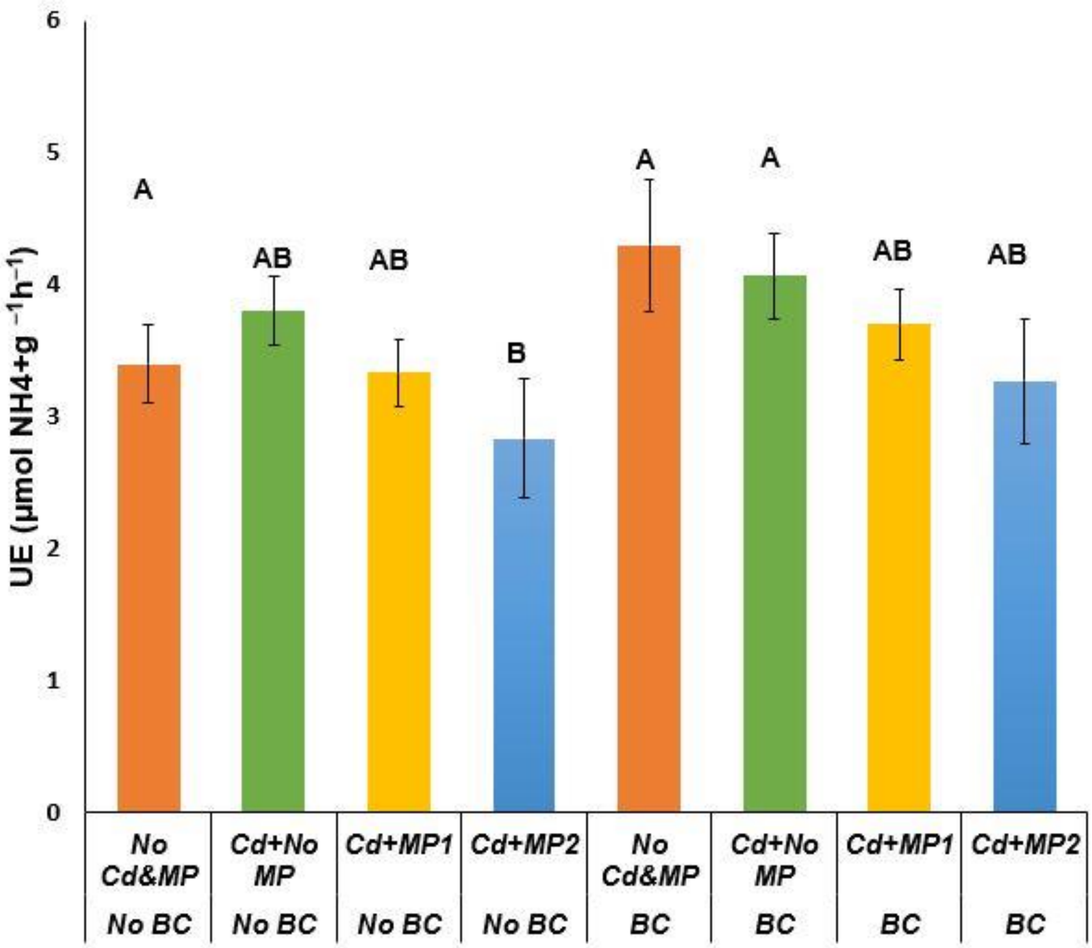

Figure 23. Effect of PVC-MPs + Cd PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on urease. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

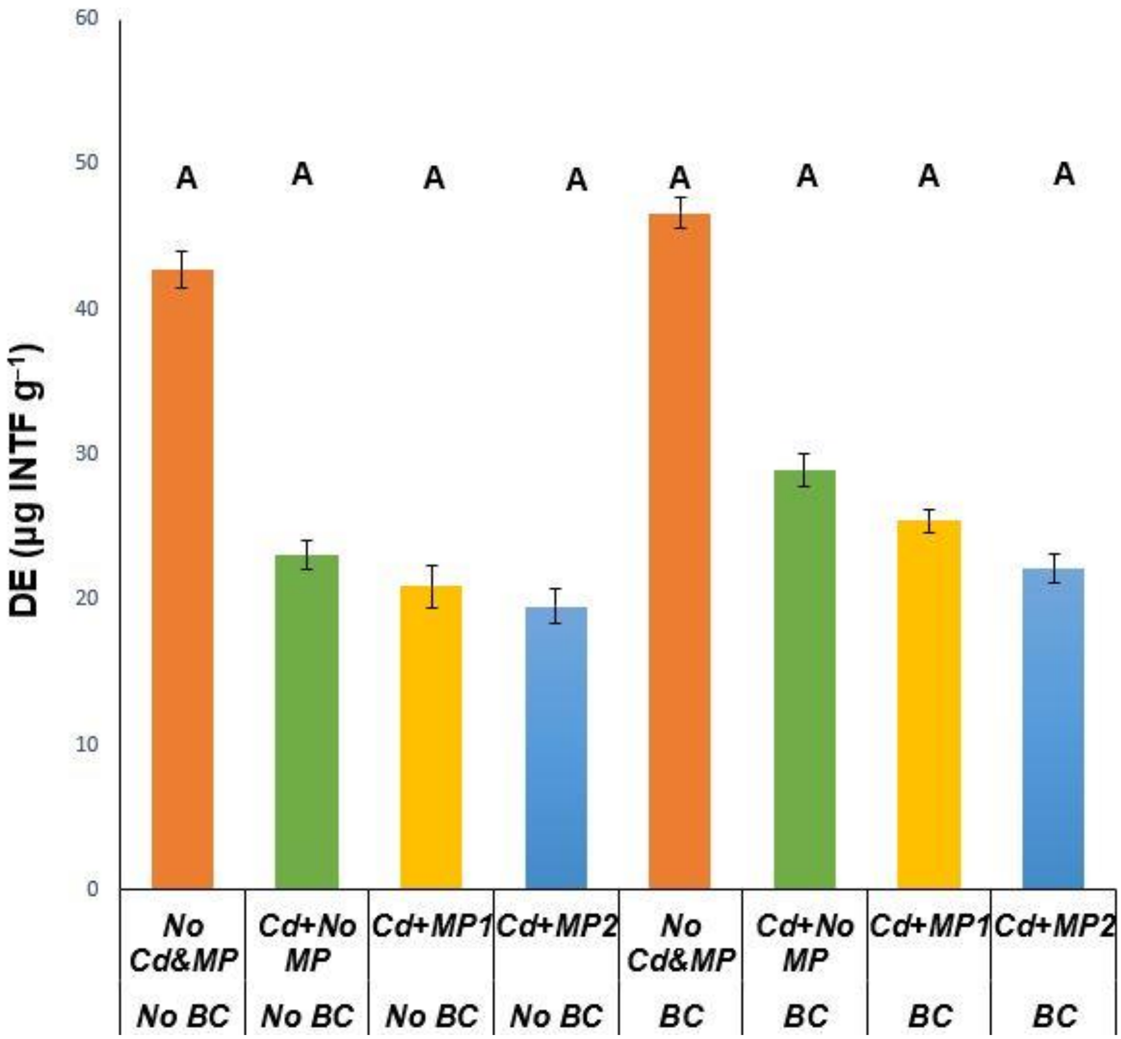

Figure 24. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on dehydrogenase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

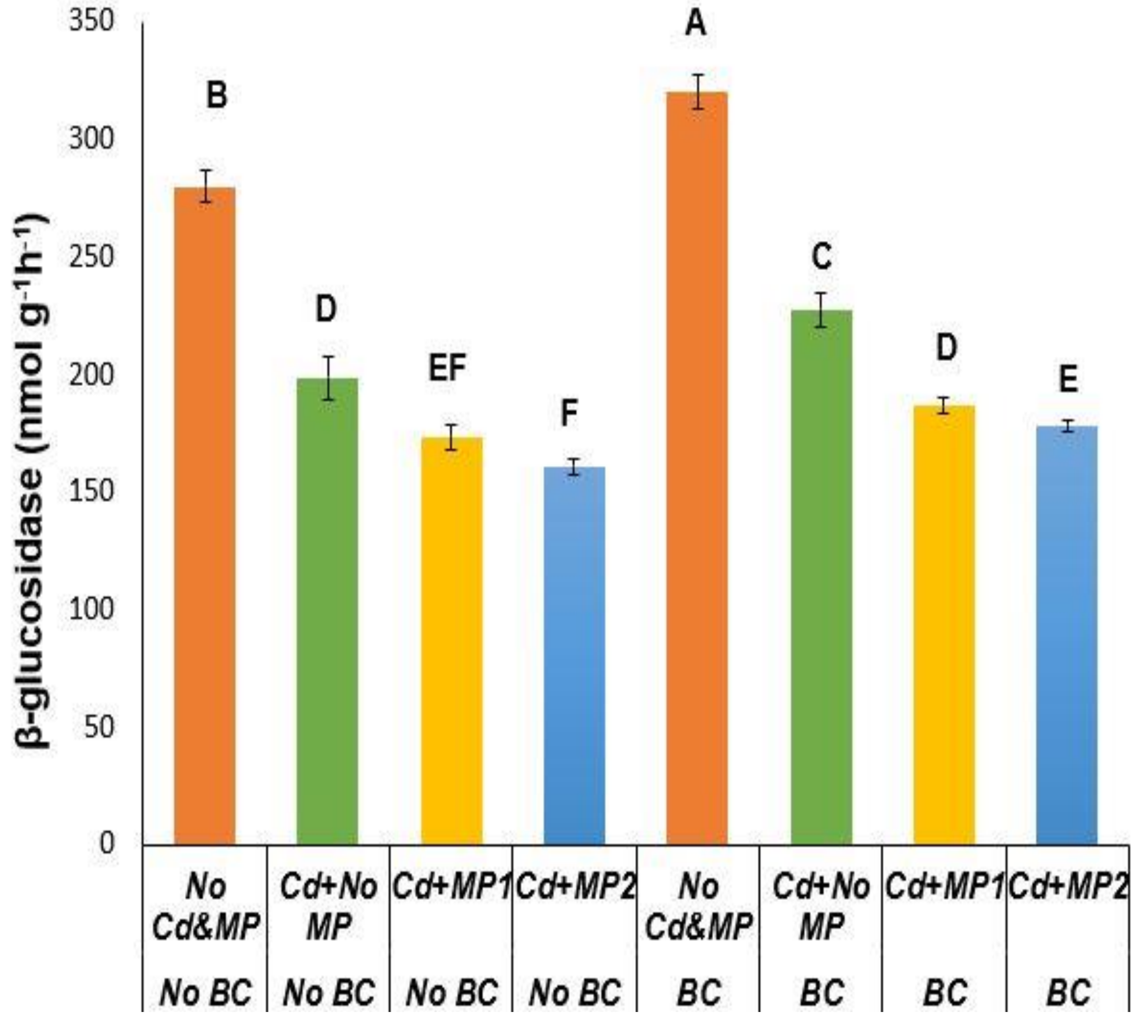

Figure 25. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on β-glucosidase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

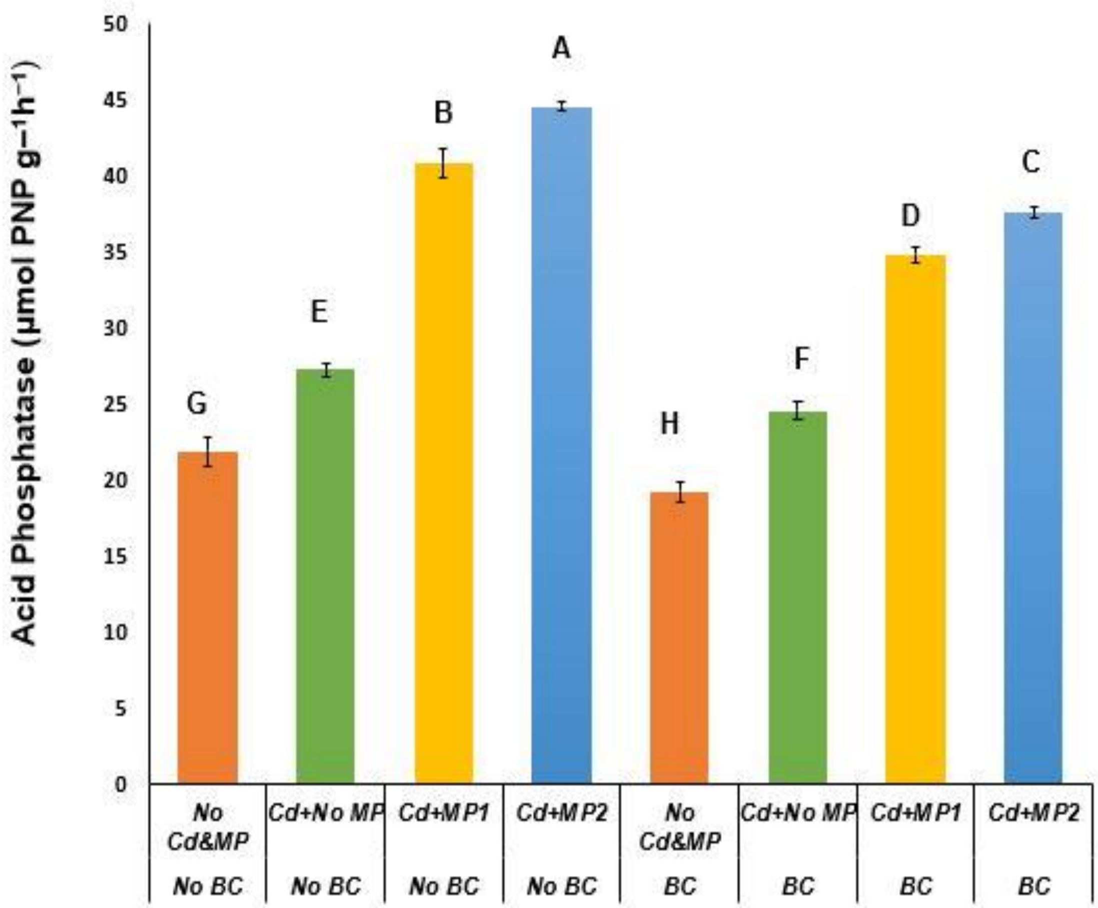

Figure 26. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on acid phosphatase. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

4.2.3. PVC-MP + Cd biochar effect on MBC and MBN:

The PVC-MPs + Cd and cotton stalk biochar were used to investigate their effects on soil microbial biomass nitrogen and soil microbial biomass carbon (Figure 27, 28) MBN significantly decreased in PVC-MPs + Cd contaminated soil as compared to control about -53.7% and -29% in 0.25% and 0.5% concentration of PVC-MP + Cd. Biochar application in Cd and PVC-MPs + Cd soil reduce the negative effect of the Cd and PVC- MP and increased the MBN. High concentration of 0.5% PVC-MPs + Cd reduced the MBN more than the low concentration (Figure 27).

In case of Microbial biomass carbon, the result showed opposite trend as compared to the microbial biomass nitrogen. Cd alone reduced the MBC as compared to the control. However, PVC-MPs + Cd contaminated soil elevated the MBC about +128.2% and +164.1%. In soil with Cd and PVC-MPs + Cd, biochar cause further increase in the Microbial biomass carbon suggested that biochar is required to reduce the hazardous effect of Cd and MPs (Figure 28). Results suggested that PVC + Cd reduced the MBN but increased the MBC and biochar co application with the PVC + Cd reduced the effect of contaminants.

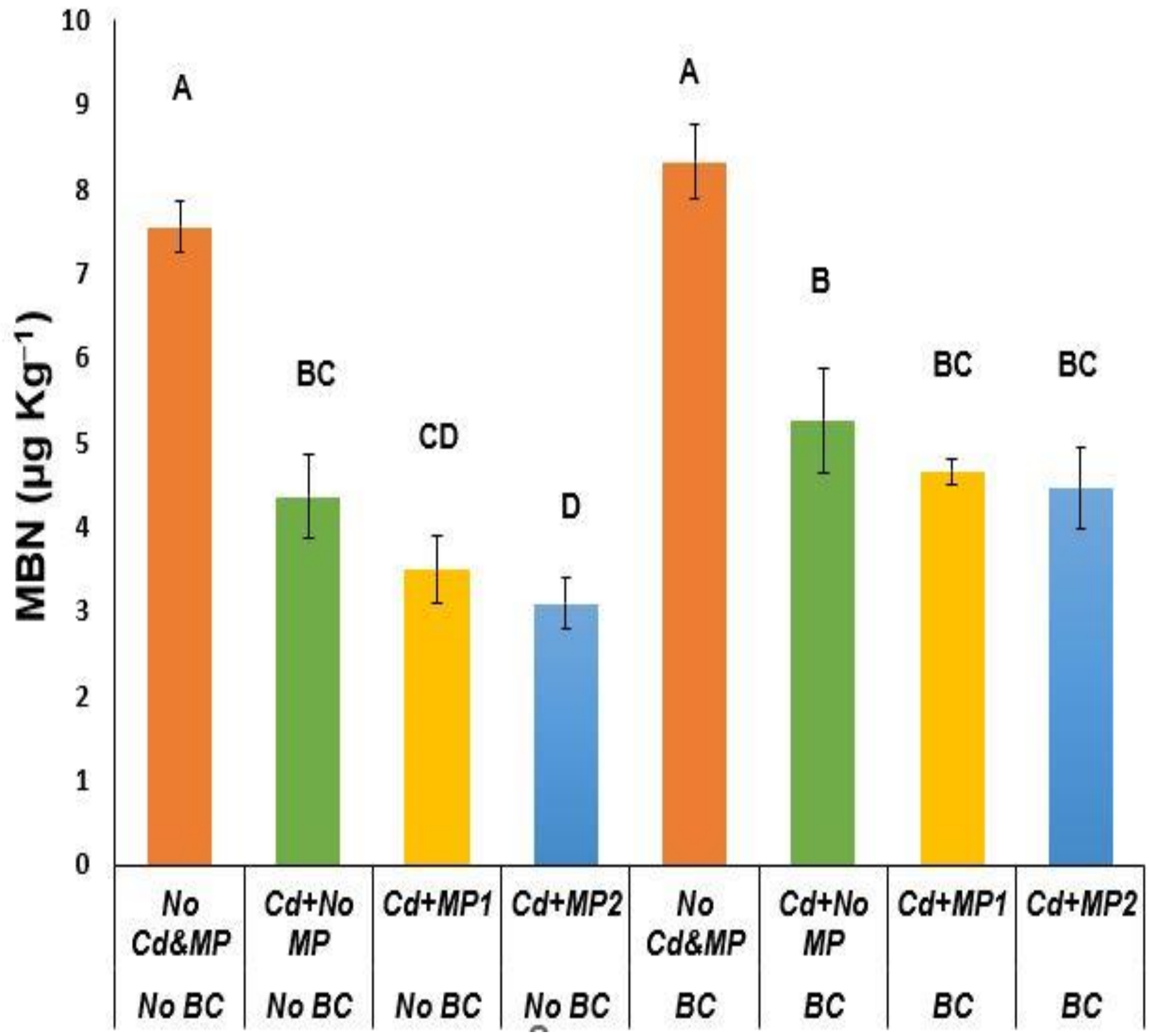

Figure 27. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on MBN. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

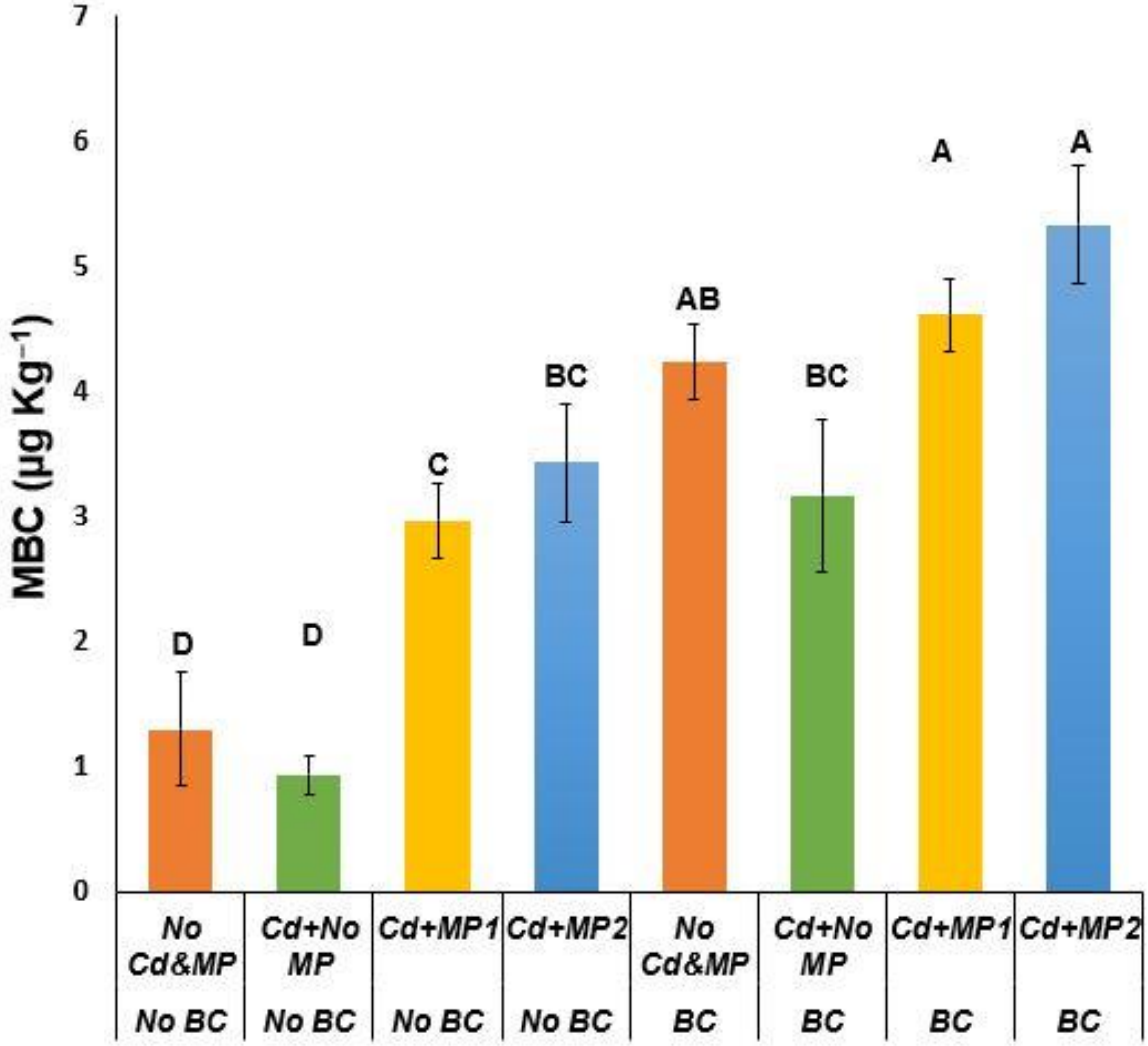

Figure 28. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg , on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on MBC. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

4.2.4. PVC-MP + Cd effect on the Microbial community

The PVC-MPs + Cd and cotton stalk biochar were used to investigate their effects on Total PLFA, gram –ve bacteria and gram +ve bacteria (Figure 29-31) Total PLFA was significantly reduced in the Cd-contaminated soil as compared to control however significant increases were observed in the soil where Cd+PVC-MP were inoculated without biochar. The highest increase was reported in the biochar treatment alone and biochar +Cd+PVC MP. Biochar reduces the effect of Cd in the Cd + Biochar treatment (Figure 31). The same trend was observed in the G-ve bacteria Cd alone reduce the G-ve bacteria however it increased in Cd+PVC MP by about +26.8% and +32.9% (0.25% and 0.5% PVC-MPs +Cd respectively). Biochar reduced the effect of Cd as compared to the Cd alone treatment. Biochar+Cd+PVC MP showed an elevated number of G- bacteria suggesting that biochar is an essential component to reduce the effect of Cd (Figure 30). G+ve bacteria significantly increased in the Cd and Cd+PVC MP contaminated soil as compared to control soil. However, in presence of biochar increases were observed in all the treatments (Figure 29). The results showed microbial community increased in the presence of the PVC + Cd contaminated soil and Co application of biochar and PVC + Cd further increase the microbial community.

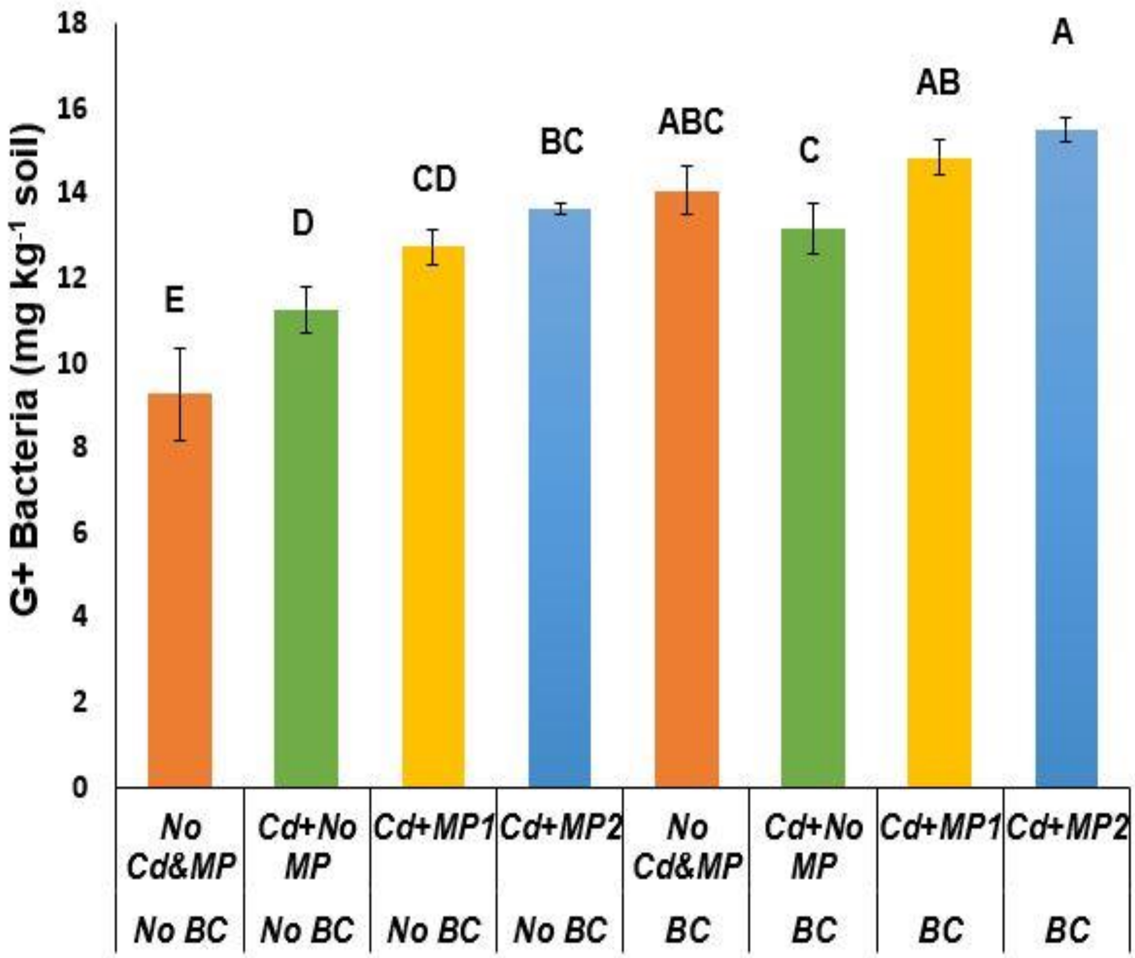

Figure 29. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg , on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on G+ve. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

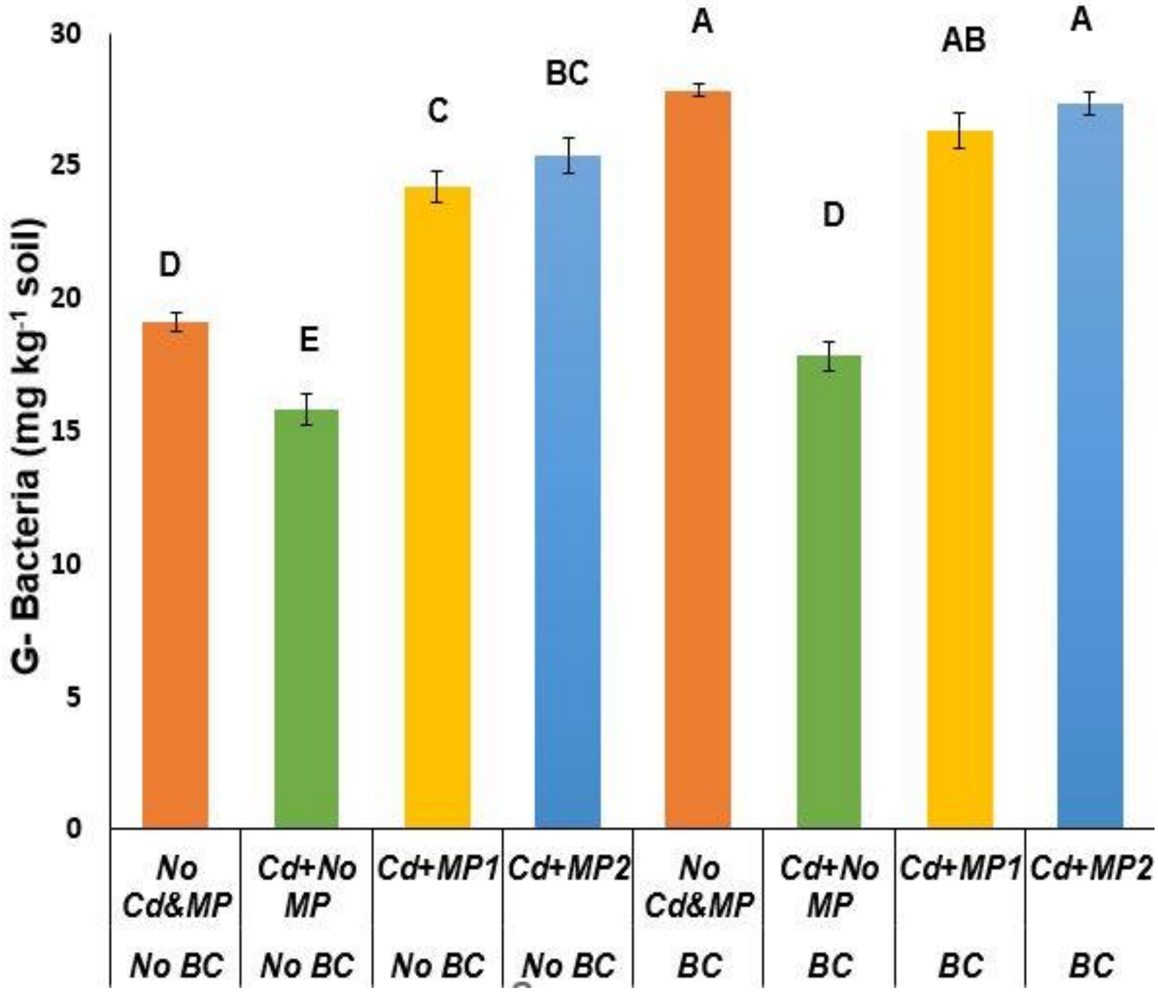

Figure 30. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg , on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on G-ve bacteria. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at $P < 0.05$ %.

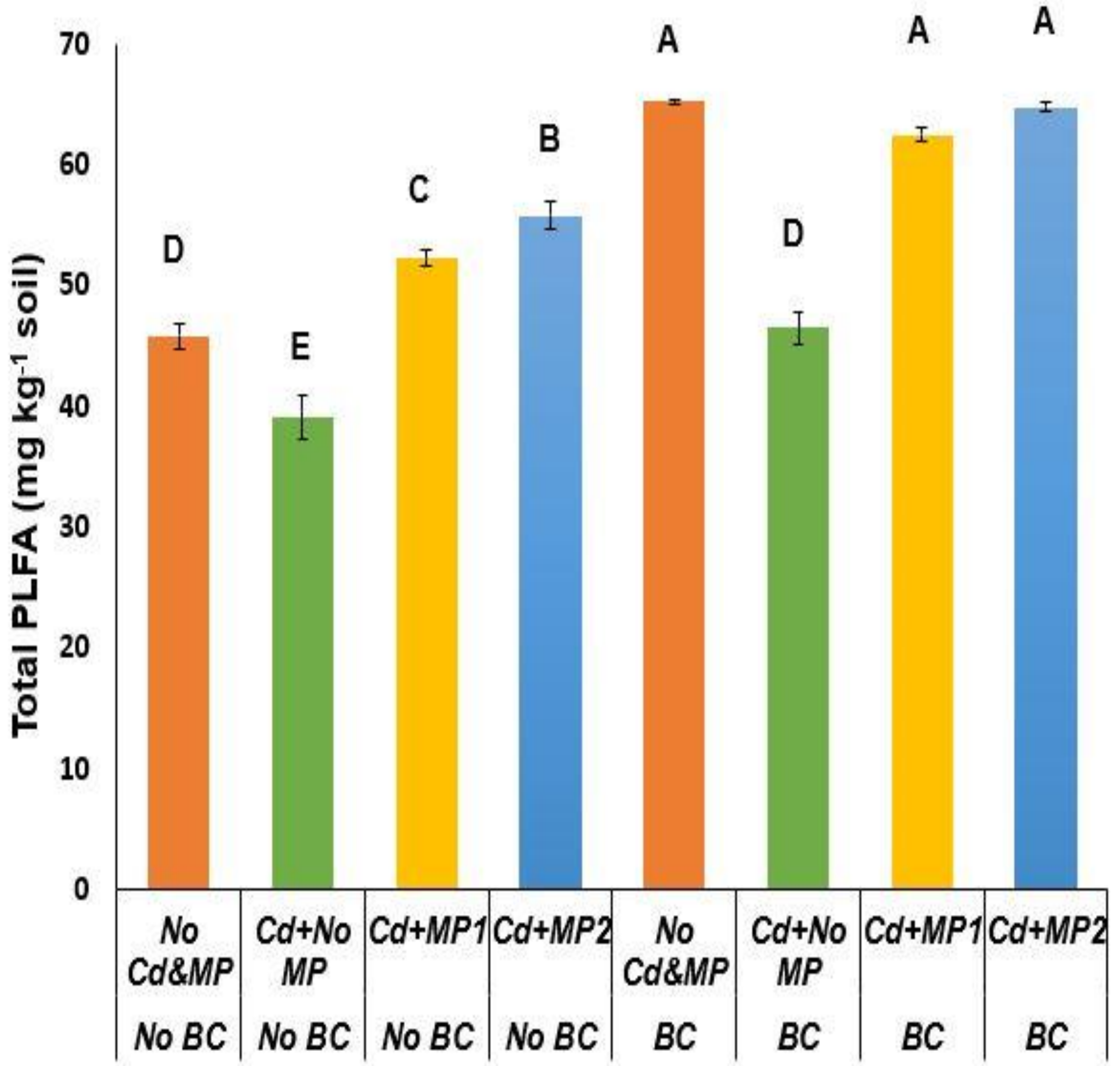

Figure 31. Effect PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on Total PLFA. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

4.2.5. PVC-MP + Cd effect on root, shoot, plant, and soil Cd concentration

The PVC-MPs + Cd and cotton stalk biochar were used to investigate their effects on Cadmium concentration in the root, shoot, plant, and soil (Figure 32-35). PVC-MP+Cd 0.25% and 0.5% increased Cadmium concentration in the root, shoot, plant, and soil however biochar stuck the cadmium, reduce the concentration in the co-application of biochar and PVC-MP+Cd contaminated soil and PVC-MP+Cd 0.25% and 0.5% increased the cadmium concentration in root about +73.8% and +390.3% respectively. However, biochar co-application with the PVC-MP+Cd reduced the concentration in the root by about -59.6% and -50.3% (Figure 32). Cd concentration increased in the shoot in contaminated soil is about +83.7% and +100.8% however biochar decreased the Cd concentration by about -97.4% and -94.8% (Figure 33). The same trend was observed in soil and plant where PVC-MP+Cd increased the Cd concentration and biochar relieved the effect of MPs and reduce the concentration of Cd -50% and -48.5% in soil and-47.9 % and -46.7% in plants (Figure34,35). These results suggested that PVC-MP+Cd increased the bioavailability in the root, shoot, plant, and soil however co-application of biochar with PVC-MPs + Cd reduce the bioavailability in the root, shoot plant, and soil

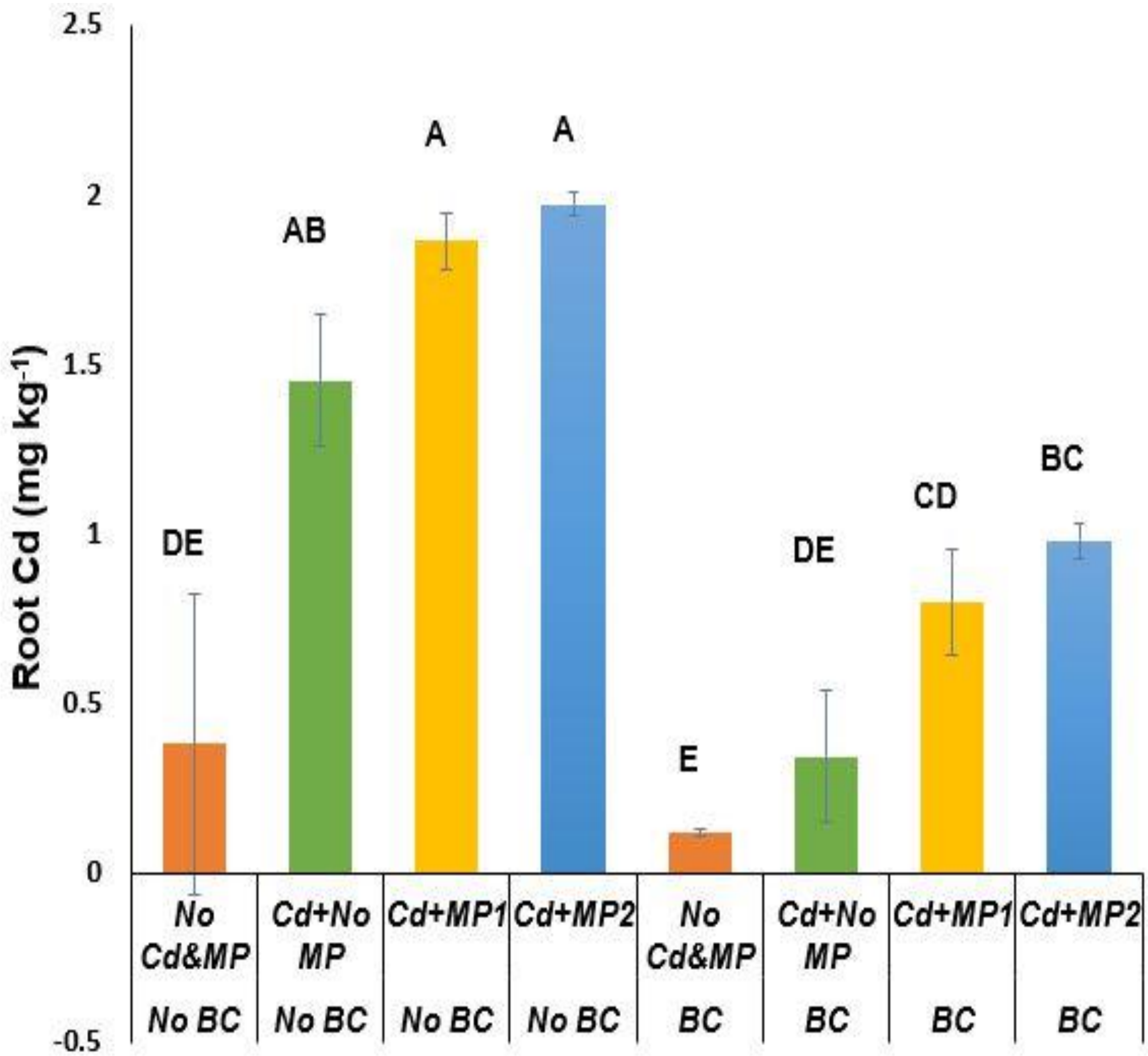

Figure 32. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on Root Cd. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

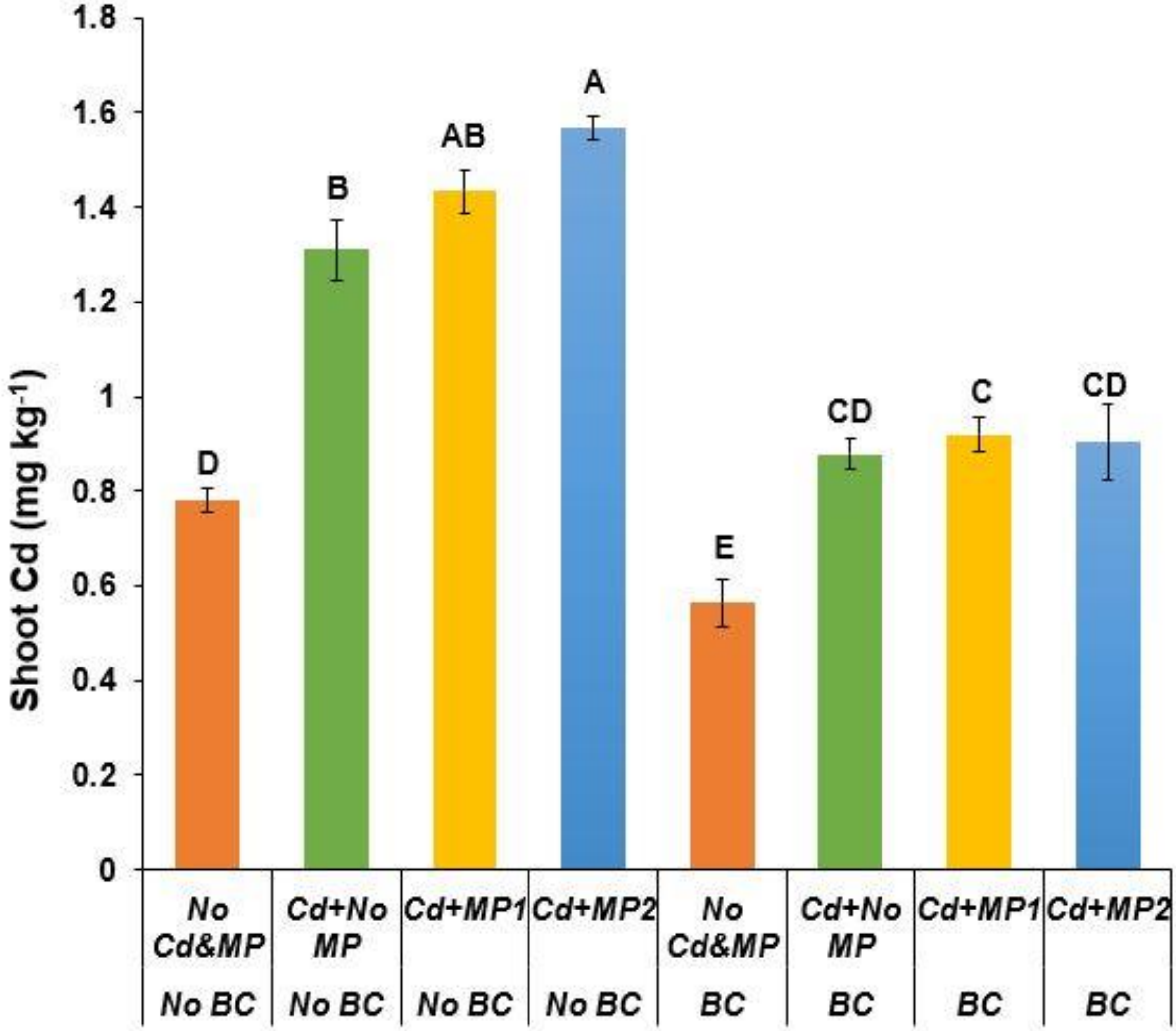

Figure 33. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on shoot Cd. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

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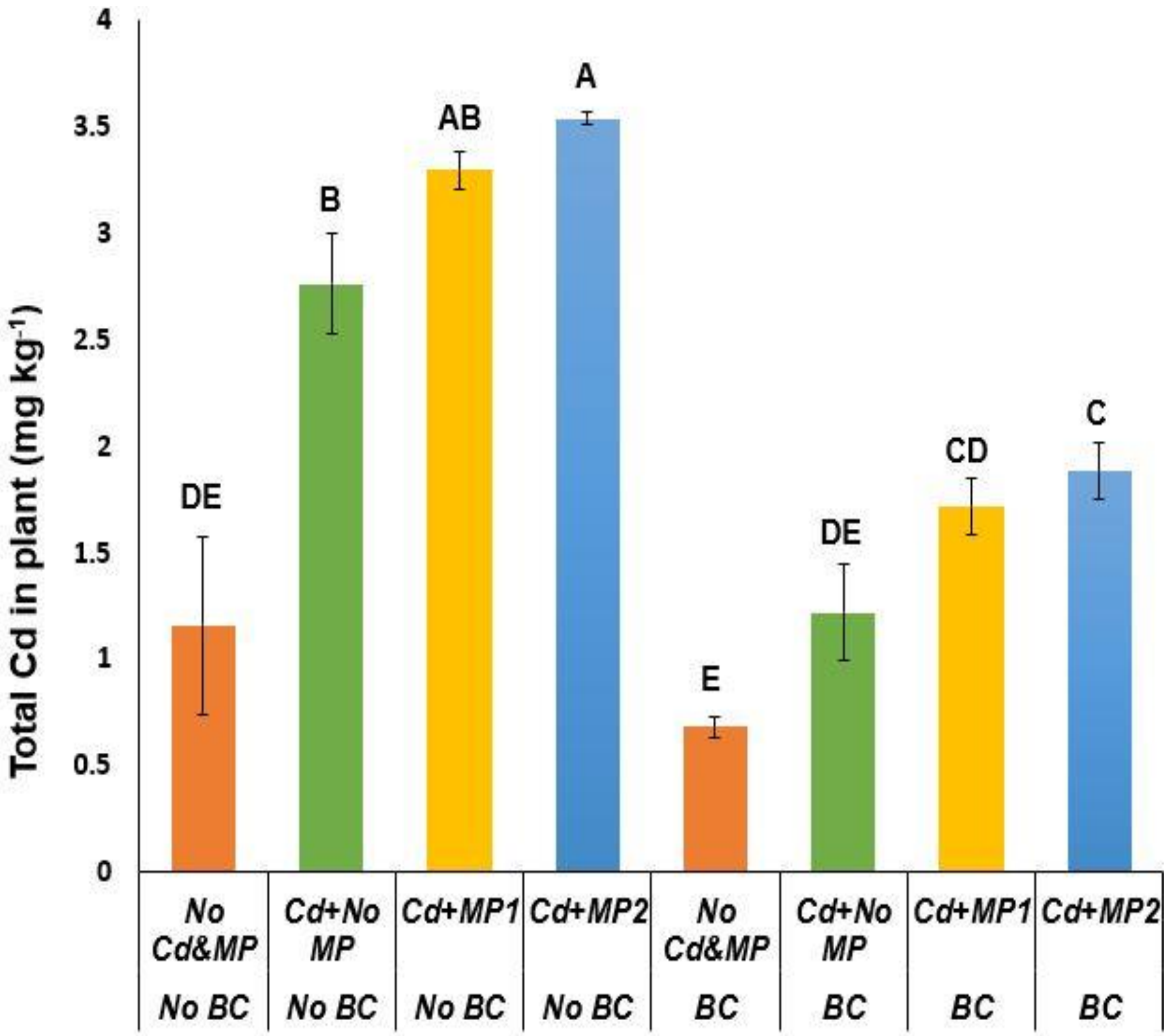

Figure 34. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on total Cd in plant. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (n = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

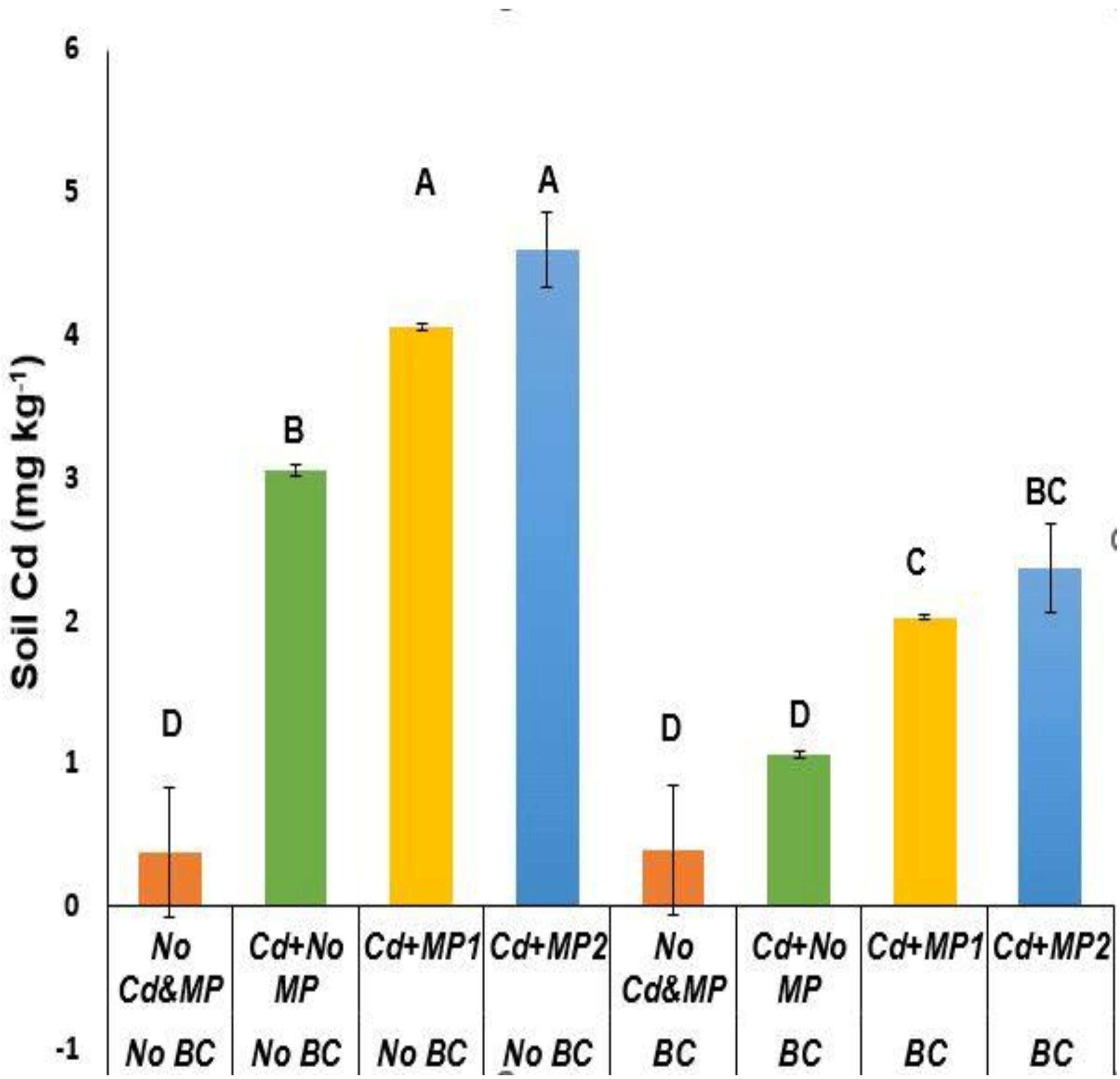

Figure 35. Effect of PVC-MPs + Cd (control = 0, Cd = 600mg/kg, PVC-0.25% + 600mg/kg, PVC-0.5% + 600mg/kg, on dry weight basis (w/w)) and biochar (control = 0, BC = 0.5%, on dry weight basis (w/w)) on soil Cd. The vertical bars are based on the treatment means, while the error bars are based on standard deviation (*n* = 3). The bars sharing similar letters are not significantly different from each other at P < 0.05 %.

5. DISCUSSION

5.1. Experiment no 1: Effect of PVC-MP on Plant and Soil in Biochar Amended Soil

5.1.1. PVC-MPs and biochar effects on the wheat shoot and root dry biomass

The results of dry wheat biomass suggested that co-application of the Biochar with PVC-MPs alleviated the adverse effects of PVC-MPs in the soil system. Our result with PVC-MPs 0.5% showed no meaningful difference compared to the control. However, Zang et al. (2020) and Ren et al, (2021) found contradictory results to our study, but they used different MPs sources like Polyethylene and polystyrene, respectively. Zang et al. (2020) found a reduction in the wheat and root biomass following Polyethylene-MPs addition, while Ren et al, (2021) used Polystyrene beads which caused a reduction in the Chinese cabbage by affecting its photosynthesis and soil microbial activity. MPs can affect plant growth due to direct plastic contamination and plasticizers and cause changes in soil properties, microbial communities, and activity (Gu et al., 2017; R. Qi et al., 2020; Rillig et al., 2019). However, in the present study, PVC-MPs with a low dose of 0,25% caused an increase in the dry shoot mass compared to the control group. Increased growth was also observed in carrot (*Daucus carota)* and spring onion (*Allium fistulosum)* plants (de Souza Machado et al., 2019a; Lozano & Rillig, 2020).

However, our study revealed that PVC-MPs did not affect the root dry biomass. Similar results were observed by Qi et al. (2018), where PVC-MPs did not show any effect on the total wheat biomass production. The reduction in plant biomass can be explained by other factors like alternation in soil chemical and biological properties (Boots et al., 2019;

Leifheit et al., 2021). The Biochar co-application with the PVC-MPs significantly increased shoot biomass compared to control soil or the treatment with a high rate (0.5%, w/w) PVC-MPs addition. Applying Biochar in MPs contaminated soils has been observed to positively affect the soil microbial community in optimum water conditions (Palansooriya et al., 2022) . Thus, biochar may help to relieve adverse abiotic stress by modulating the soil properties like the water holding capacity.

5.1.2. Effect of PVC-MPs and Biochar on total nitrogen, soil organic carbon, microbial biomass carbon, and nitrogen

PVC-MPs affected the total nitrogen, soil organic carbon, microbial biomass carbon, and microbial biomass nitrogen. However, co-application with Biochar with PVC-MP had a limiting effect on total soil nitrogen. Biochar induced nitrogen limitation in soil (Haider et al., 2015) , whereas Gao et al. (2016) found that biochar increased the soil NO3--N and NH4+-N by +33% and +53%, respectively. The reduction in total nitrogen is due to the toxicity of the MPs. Other studies also suggest that MPs contamination reduces the nitrogen content in soil (Liu et al., 2017; Wang et al., 2016; Zhu et al., 2021). Nitrogen content decreased by about 63% and 91% in the MPs-contaminated soil (Zhu et al., 2022). Propylene MP and Plastic mulch residues decreased the nitrogen, phosphorus, and organic carbon content (Liu et al., 2017; Wang et al., 2016).

Few previous studies have observed that plastic residues with larger particle sizes (20 mm × 20 mm debris) in the soil can reduce soil microbial carbon and nitrogen (Wang et al., 2016). MP can reduce the soil organic carbon SOC microbial by inhibiting the growth of the microbial community (Yu et al., 2021). Another study reveals that MPs cause a significant reduction in microbial biomass carbon MBC and nitrogen MBN (Blöcker et al., 2020). Our findings agree with Urbina et al. (2020), who found that bioaccumulation of MPs in the rhizosphere can significantly decline nitrogen content, transpiration, and plant growth. However, biochar addition with PVC-MPs remarkably enhanced these traits compared to PVC-MPs treatment. Notably, the adverse effects of PVC-MPs were still significantly apparent between only biochar addition versus biochar+ PVC-MPs addition. It suggests that biochar's addition to MPs contaminated soils can promote the above traits but cannot completely undo the hazardous effects of PVC-MPs.

5.1.3. Effect of PVC-MPs and biochar on soil enzymes activity

The present study revealed that urease activity and dehydrogenase activity were affected by PVC-MPs residues. Soil enzyme plays a crucial role in the nutrient cycle (Cui et al., 2021). MPs can affect soil physiochemical properties, therefore, can affect the enzyme activities of the soil (de Souza Machado et al., 2019; Du & Wang, 2021; Yu et al., 2020). Recent studies showed that MPs could change the soil properties, such as soil aggregates which may be associated with the soil enzyme activity (Zhang et al., 2018). Furthermore, Yu et al. (2020) also concluded that different enzymes act differently on soil aggregation. The urease activity was increased with the addition of polyethylene MPs; however slight increase occurred in the invertase enzyme (Fan et al., 2022; Huang et al., 2019). In another study, three types of MPs, microsphere polypropylene, membranous Polyethylene, and fibrous polypropylene contamination increased the urease activity in the first two weeks, and then it decreased by 31% to 41% (Yi et al., 2021) . These results indicate that PVC-MP contamination also decreased the dehydrogenase activity however, biochar did not significantly increase the enzymatic activity. Our result contradicts Yi et al. (2021), where dehydrogenase activity increased in all three MPs (microsphere polypropylene, membranous Polyethylene, and fibrous polypropylene.

Biochar addition in PVC-MPs contaminated soil increased the urease activity because the microbial activity decreased in the MP contaminated soil was regulated by the biochar addition and increased the soil urease activity. In another study, biochar increased soil enzyme activity (Dissanayake, 2022) . Feng et al. (2022) observed a reduced urease activity in biochar + polyethylene inoculated soil in rice crops but reduced in polyacrylonitrile + biochar. The Urease activity also depends on soil moisture content. A decline in soil moisture of about 10-67% causes a 21% decline in urease enzyme activity (Sardans & Peñuelas, 2005). Biochar addition increases the soil moisture and water holding capacity; therefore, urease activity increases in the biochar amended PVC-MPs soil.

5.1.4. Effect of PVC-MPs and biochar on microbial communities

Microorganisms such as mycorrhizal fungi and symbiotic bacteria are responsible for providing ecosystem services in agriculture (Jacoby et al., 2017). Microorganisms provide numerous soil nutrients, enhance soil fertility (Mengqi et al., 2021), and help in recycling organic waste (Siles et al., 2021). However, anthropogenic activities harm soil microbial diversity that may harm soil health (Yang et al., 2021). Different MPs composition has been reported that affect soil microbiota(Huang et al., 2019). This study showed that microbial communities declined in the presence of the PVC-MPs, suggesting a harmful PVC-MP effect on bacterial and fungal communities.

MPs with distinct sizes and shapes affect bacterial communities differently (Sun et al., 2022). Microbial activity depends upon the soil's physiochemical properties that alter the microbial communities. Microbial abundance or depletion is affected by physical properties like evaporation, soil moisture, porosity, and soil bulk density (Huang et al., 2019). MPs affect all these properties and microbial communities (de Souza Machado et al., 2018). MPs act differently on different communities of Fungi, *Basidiomycota*, *Chytridiomycota*, *Ciliophora*,and *Rozellomycota* decreased, and *Ascomycota* and *Zygomycota* increased with the polyethylene MPs (Ren et al., 2020) *(Ren et al.,2020)*. *Ascomycota* and *Basidiomycota* increased when inoculated with polyethylene MPs (Hou et al., 2021).

Biochar application in PVC-MP soil reduced the contamination of MPs and increased both bacterial and fungal communities. In a recent study, biochar application with polyethylene MPS alleviated the negative effect of MP and stable the microbial communities. Palansooriya et al. (2022) maintained the 60% water holding capacity and suggested that in well-watered conditions, biochar is stable to the microbial communities compared to drought conditions.

5.2. Experiment no 2: Effect of contaminants (PVC-MP+Cd) on plant and soil in biochar augmented soil

5.2.1. Biochar effect on the soil enzyme activity

Soil enzyme activities are an indicator for evaluating heavy metal contamination (Cui et al., 2021). Heavy metals can act as a prosthetic group and enhance enzyme activity by alternating their surface charge (Dong et al., 2021). However, heavy metals can also compete for the enzyme activities, denature the protein, and inhibit the enzyme activity (Zhang et al., 2018). In this study, enzyme activity showed different trends depending upon

MP concentration. This study showed a reduction in the urease activity in Cd spiked soil. It has been observed that urease is extremely sensitive to Cd and can be used as an indicator of cadmium-polluted soil (Zheng et al., 2019). After two weeks, urease activity was reduced with MPs inoculation (Yi et al., 2021). However, adding biochar enhanced the urease activity in heavy metal contaminated soil. The increase in urease activity in biochar amended soil is due to labile carbon and other nutrients enhancing microbial activity (Ali et al., 2019). Poblete et al. (2022) found no significant effect of Cd+MP contamination on urease activity. There was no significant effect on dehydrogenase activity; however, the increase of enzyme activity can be seen in the control and biochar alone treatment. The Cd contamination reduces the acid phosphatase activity; however, in this study, the Cd and Cd+PVC-MP enhanced acid phosphatase activity. Our study showed a similar result to (Poblete et al., 2022), which found increased acid phosphatase activity in cadmium and MP-contaminated soil. The highest increase was observed in the Cd+PVC-MP because acid phosphatase activity depends on the soil moisture (Sardans & Peñuelas, 2005), and MPs increases the water holding capacity of the soil (de Souza Machado et al., 2018). MPs increases the diazotrophs abundance that is associated with the production of the acid phosphatase (Fei et al., 2020)

5.2.2. Biochar and Cd+PVC-MPs effect on microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)

Cadmium contamination can decrease metabolic activity and microbial biomass (Kandeler et al., 2000). Moreover, MPs have also been reported to affect microbial biomass (Huang et al., 2019). In this study, cadmium contamination reduced the microbial biomass carbon. Previous studies showed a similar response that MBC is negatively correlated with cadmium (Tang et al., 2022). The soil microbial biomass reduces because microorganisms require more energy in the heavy metal stress, which reduces their reduction (Nwuche & Ugoji, 2008). The MBC was increased in this study, suggesting that carbon sources enhance microbial growth and biomass (Zhou et al., 2021). Biochar application increases the microbial biomass carbon and biomass nitrogen in contaminated soil because biochar can enhance soil moisture and improve soil nutrients and microbial activity(Bashir et al., 2018). Lin et al. (2020) showed no significant effect of MP on the MBC and MBN. However, we found different results of Cd+MP contamination on MBC and MBN in the present study. Biochar reduced the effect of Cd and MP and increased the microbial biomass carbon and microbial biomass nitrogen as compared to

5.2.3. Effects of PVC-MP+Cd and biochar on the microbial community

Soil having a high concentration of Cd contamination decreases the PLFA for gram-negative bacteria; however, for gram-positive bacteria, the indicator PLFA show increase with an increasing level of Cd contamination (Shentu et al., 2008) . Gram-positive bacteria increased because, at first addition, Cd inhibits the microbes and then favors the resistant microorganism over time (Kandeler et al., 2000). Previous studies suggest that MPs contamination enhanced the biodegradable plastic degrading bacteria (Sun et al., 2022). The diversity of actinobacteria increased with MPs when 14% PVC was added (Fan et al., 2022). It is speculated that the increase in bacterial communities in PVC-MPs containing treatments might be due to the increase in the plastic degrading group of bacteria and the metal-resistant bacteria, which might use MPs as the source of Carbon (Rillig et al., 2021). Our results align with (Zhu et al., 2021), who found fewer inhibitory effects of MP+arsenic on the bacterial community.

5.2.4. Effects of biochar on plant and soil Cd concentration in a PVC-MP+Cd contaminated soil

MP contamination increased the Cd concentration in plants, roots, shoots, and the soil. Our results align with Poblete et al. (2022), where the Cd concentration in roots and soil was increased when inoculated with the MP. The presence of the MP in the soil increases the adsorption and decreases the desorption capacity, which leads to the enhancement of the bioavailability of metals (Liu et al., 2021). The ability of MPs to adsorb the heavy metal depends upon the size of MPs, and small size MPs have more adsorbing capacity as compared to the larger particles (Gao et al., 2019). Liu et al. (2021) suggested that the adsorption capacity of cadmium on MPs also depend on the functional group of the MP and the medium pH; therefore, MPs act as the carrier for the heavy metals. The heavy metal distribution also depends on the soil aggregates (Deng et al., 2018), and MPs contamination has been reported to affect soil aggregation.

Biochar addition relieved the adverse effects of MP on the Cd availability in the study.Alaboudi et al. (2019)also suggested that biochar reduces the bioavailability of Cd by 50, 45, 66, and 77% in control, 200, 400, and 600 mg/kg^{-1} Cd concentration.

CONCLUSION

Here we investigated the PVC-MPs and Cd contamination effects on wheat growth, physiology, antioxidants, soil enzyme and microbial community, and abundance in the presence or absence of cotton stalk biochar. The PVC-MP contamination showed no adverse effect on plant growth, as indicated by overall dry matter production. Instead, at any application rate, a low dose of MPs addition or biochar plus MPs showed a significant increase in total biomass production. Furthermore, there was no significant effect of PVC-MPs contamination on plant antioxidant activity. The PVC-MP contamination can significantly reduce MBC, MBN, SOC, TN, gram-positive bacteria, gram-negative, Pseudomonas, and AMF. However, the co-application of Biochar in MPs contaminated soils can alleviate the toxic effects of MPs by sustaining microbial and enzyme activity. PVC-MP+Cd synergies the toxic effects on plant growth, dry matter production, soil enzymes, and microbial activity. However, PVC-MP+Cd increased gram-positive bacteria, gram-negative bacteria, and total MBC. Biochar reduces the harmful effects of PVC-MP+Cd but not as much as it can help in the presence of only one pollutant, e.g., PVC-MPs. Co-application of Biochar and PVC-MP+Cd facilitates more increase in gram-positive bacteria, gram-negative bacteria, and total MBC. The study suggested that PVC-MP and PVC-MP+Cd are more hazardous to soil biota than plants; however, biochar alleviated the harmful effect of the contaminants.

Here we have investigated the pristine analytical grade PVC MP to develop a basic understanding and background knowledge. However, future studies should also consider using commercial plastics which contain different additives (plasticizers) to give shapes, color, and strength to plastic materials and are actual pollutants of our environments. Future studies should consider the combined effect of plastic polymers with different heavy metals on soil physicochemical properties and nutrient uptake. It would also be essential to investigate the effect of different shapes and concentrations of MPs. In the future, explore more about the effect of MPs on physiological characteristics and growth of the different types of plants.

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Abstract

Microplastics (MPs) are tiny plastic particles less than 5 mm in size. The MPs enter the environment due to the breakdown of larger plastic materials. MPs pollution has become the most emerging environmental issue and is almost everywhere in the world. The MPs are divided into two major groups. The primary and secondary MPs. Primary MPs are manufactured in micro sizes, while the breakdown of larger plastic materials forms secondary MPs. Polyvinyl chloride (PVC) is one of the agricultural soil's most abundant and toxic MPs. Agricultural soils are also facing the challenges of heavy metals (loids). For instance, Cadmium (Cd), a heavy metal, is one of the most dangerous pollutants in agricultural soils. Cd is a non-essential, more mobile, and bioavailable heavy metal and thus can be a potential threat to plant growth and soil biota. Biochar (a stable carbon obtained by the pyrolysis of organic wastes under anaerobic conditions) has been investigated and recommended to reduce soil contaminants' bioavailability. In the present study, we investigated the interaction of PVC-MPs (0%, 0.25%, and 0.5%, (w/w)), Cd (600mg/kg), and cotton stalk (*Gossypium hirsutum* L.) biochar (0%, and 0.5% (w/w)), in two controlled experiments, on plant growth, physiology, antioxidants defense, soil microbial community and abundance using 16S rRNA, 18S rRNA and PLFA biomarkers. Results revealed that the PVC-MPs at the low dose of 0.25% increased the dry shoot mass with no significant effect on the dry root mass. There was no significant effect on plant antioxidant activity. However, MP's addition reduced soil urease, dehydrogenase activity, soil organic and microbial biomass carbon, and bacterial and fungal abundance. The Co-application of biochar with PVC-MPs reduced the adverse effects of the MPs and increased Urease and dehydrogenase activity, soil organic and microbial biomass carbon, and bacterial and

Dr. Ghulam Haider
Assistant Professor
Deptt of Pla... Biotechnology
Atta-ur-... School of Applied
Biosciences (ASAB) NUST Islamabad

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