Mitigating the Toxic Effects of Cadmium and Total Petroleum Hydrocarbons Contaminated Soil through Plant-Bacterial Interactions and Organic Amendments

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Dedication

I would like to dedicate this thesis to my beloved parents. Without their unwavering support and care I would not have been where I am today.

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Areej Arif

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Abstract

This dissertation describes a unique integrated phytoremediation technique called "phase crop rotation" that addresses the issue of the co-contamination of cadmium (Cd) and total petroleum hydrocarbons (TPH) in contaminated soils. This technique, which consists of two crucial phases, provides a comprehensive solution to the co-contamination problem. The first phase focuses on the simultaneous phytoextraction of cadmium and TPH degradation using two hyperaccumulator grass species, i.e., *Lolium multiflorum* and *Coronopus didymus*, compost, and plant growth-promoting rhizobacteria (PGPR). Following that, in the second phase, the residual contamination is targeted via the phytostabilization process, which is facilitated by the use of TPH degrading bacteria and biochar.

The study's primary goal is to investigate the effect of compost and *Bacillus safencis* on the concurrent uptake of Cd and decomposition of TPH. Furthermore, the effects of compost and bacterial inoculation on plant growth and soil physicochemical parameters are thoroughly investigated. The following phase focuses on determining the impact of *Bacillus cereus* and biochar amendment on cadmium accumulation, TPH breakdown, and maize plant growth.

According to the research findings, the treatment containing spiking soil, 10% compost, *Lolium multiflorum*, and *Bacillus safencis* displayed the maximum efficacy in removing cadmium (Cd) in the first phase (T6), obtaining a 60% removal rate. In contrast, the highest phytostabilization of Cd was reported in the second phase (T5), which used recycled soil from phase 1, 2% biochar, *Zea mays*, and *Bacillus cereus* amendment. Furthermore, with a clearance rate of 94%, this treatment demonstrated remarkable TPH degradation. In terms of plant growth, the treatments T6 in phase 1 and T5 in phase 2 performed very well, with maximum root and shoot weights of 5.5g and 1.5g for T6 and 15g and 28g for T5, respectively. Elevated quantities of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were also found in these treatments, indicating strong plant health and photosynthetic activity.

Biochemical stress indicators revealed significantly lower levels of catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), malondialdehyde (MDA), and hydrogen peroxidase (H2O2) in these treatments, indicating reduced oxidative stress and improved plant resilience. These findings highlight the efficacy of the "phase crop rotation" strategy in addressing co-contamination while encouraging plant development and health.

Key words: Phytoremediation, Phase crop rotation, Co-contamination, Phytoextraction, Phytostabilization.

Chapter 1

Introduction and Literature Review

1.1. Background

Soil is a crucial resource for humans and the ecosystem's lifecycle because it promotes plant development, nutrient cycling, and water filtration. Anthropogenic activities such as mining, oil refineries, and the use of pesticides and other chemicals, on the other hand, are constantly threatening soil integrity. These operations use a variety of chemicals that harm the soil, posing major environmental and human health dangers.

Co-contamination is the presence of numerous contaminants in soil at the same time, where different types of pollutants coexist and interact with one another. This phenomenon is especially concerning in agricultural soils because it can have a cascading detrimental impact on ecosystem health, plant development, and human well-being (Sun et al., 2014).

Soil co-contamination is frequently caused by the mixing of heavy metals with organic and inorganic pollutants (Wu et al., 2020). Heavy metals are elements that exist naturally and have large density and hazardous characteristics. They can enter the environment as a result of a variety of anthropogenic activities such as industrial processes, mining, and the use of fertilisers and pesticides. Cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) are common heavy metals of concern. These pollutants have long-term effects on soil quality and can build in the food chain, causing health concerns to humans.

Petroleum hydrocarbons, polychlorinated biphenyls (PCBs), and pesticides are just a few of the organic pollutants found in soil. Petroleum hydrocarbons, particularly total petroleum hydrocarbons (TPH), constitute a significant class of organic pollutants found in soil. They are formed from crude oil and its refined products, and their presence in soil can be caused by activities such as oil spills, incorrect storage, or underground storage tank leaking (Gan et al., 2009). Pesticides, which are frequently employed in agricultural practices, can pollute soil and remain there for long periods of time, causing threats to both the environment and human health.

Heavy metal and organic pollutant co-contamination of soil has been the focus of in-depth research because it poses special difficulties for remediation techniques. The mobility, bioavailability, and toxicity of several pollutants in soil can be influenced by the interactions between them. For instance, organic pollutants can make it easier for plants to absorb heavy

metals, increasing the likelihood of bioaccumulation and transmission into the food chain. On the other hand, heavy metals can impede microbial activity and enzymatic activities, which can alter how organic pollutants are degraded and detoxified (Yang et al., 2021).

Several research publications and case studies have investigated soil co-contamination with heavy metals and organic or inorganic contaminants, revealing complicated relationships and viable mitigation measures. Lin et al. (2008), for example, evaluated the co-contamination of soil with cadmium and polychlorinated biphenyls (PCBs) and discovered that the presence of cadmium greatly altered the accumulation and transformation of PCBs in soil and plants. Zhao et al. (2021) conducted another investigation on the co-contamination of soil with lead and polycyclic aromatic hydrocarbons (PAHs), demonstrating the interaction effects of both pollutants on soil microbial populations and enzymatic activity. These studies emphasize the significance of knowing soil co-contamination and the implications for environmental and human health.

Among heavy metal contamination in soil, Cadmium (Cd) is quite prevalent. Cadmium is a very hazardous element that accumulates in soil as a result of industrial processes, mining activities, fertiliser use, and sewage sludge. It is especially dangerous due to its persistence in the environment and potential to bioaccumulate (Khan et al., 2017). The World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) have established cadmium in soil threshold levels of 3 parts per million (mg/kg) or less (Kubier et al., 2019). However, cadmium levels in many agricultural soils surpass these limits, threatening food security, ecosystem functioning, and, ultimately, human health.

Aside from heavy metals, agricultural soils are frequently contaminated by organic molecules such as total petroleum hydrocarbons (TPH). TPH contamination is caused by a variety of factors, including oil spills, industrial activity, and inappropriate disposal of petroleum products. TPH contains a complex mixture of hydrocarbons that can stay in soil for long periods of time, limiting plant growth and changing soil microbial ecosystems. The WHO-FAO has established TPH in soil threshold levels ranging from 100 to 300 mg/kg, depending on the individual hydrocarbon components present (Michelsen & Boyce, 1993).

Co-contamination of soil with cadmium and TPH chemicals is a serious barrier for cleanup operations. Conventional remediation procedures frequently focus on individual contaminants and may be ineffective in dealing with the complex interactions of heavy metals and organic compounds in co-contaminated soils. As a result, there is an urgent need to develop innovative,

sustainable, and environmentally friendly techniques to remediating multi-contaminated agricultural soil, with a focus on the removal or reduction of cadmium and TPH.

1.2. Conventional Methods for Soil Remediation

Conventional remediation approaches for co-contaminated soil often comprise individual or sequential treatment techniques that target distinct contaminants. Physical removal, chemical immobilisation, thermal treatment, and soil washing are all typical ways. While these approaches have been successful in treating single contaminants, they frequently fall short of tackling the complexities of co-contaminated soil due to the interactions and synergistic effects of different pollutants. For instance, excavation and landfilling are two popular physical removal processes used to remove contaminated soil from a place. These approaches, however, are costly and disruptive, and they may result in soil erosion and the loss of valuable topsoil. Furthermore, physical clearance ignores the pervasiveness of organic pollutants and the long-term dangers associated with their persistence. Physical removal of contaminated soil from a previous industrial site resulted in significant expenses and disruption to the ecosystem in a case study by Mercier et al. (2001), without entirely addressing the extent of contamination or avoiding potential leakage of toxins into groundwater.

There is another process called chemical immobilisation, which is the process of adding amendments or additions to soil to minimise pollutant mobility and bioavailability. Heavy metals can be immobilised with additions like as lime, phosphate, and activated carbon, for example. Chemical immobilisation, on the other hand, is frequently limited to certain pollutants and may not successfully treat co-contaminants with distinct chemical characteristics. Furthermore, the long-term stability and efficiency of immobilisation procedures can be questionable. Elyamine et al. (2019) used several amendments to immobilise cadmium and polycyclic aromatic hydrocarbons (PAHs) in co-contaminated soil, although the results revealed little success in lowering the mobility of both contaminants.

Thermal treatment procedures, such as incineration and thermal desorption, entail heating contaminated soil to high temperatures in order to volatilize or decompose the contaminants. While heat treatment can effectively degrade organic contaminants, it is energy-intensive, costly, and may result in the discharge of air pollutants. Furthermore, thermal treatment may not fully remove heavy metals, and their potential for volatilization and recontamination remains a worry. Thermal desorption was employed to treat soil contaminated with hydrocarbons like, polychlorinated biphenyls (PCBs) in a study by Vidonish et al. (2016),

although the technique resulted in the production of volatile organic compounds and the inadequate removal of PCBs.

The physical and chemical removal of pollutants from soil particles using water or other solvents is known as soil washing. While soil washing is helpful for removing some organic contaminants, it is less successful for heavy metals due to their strong binding to soil particles. Furthermore, the wastewater generated by soil washing may require additional treatment, and the procedure may result in the development of secondary waste. Zhang et al. (2022) investigated the viability of soil washing for co-contaminated soil with heavy metals and petroleum hydrocarbons, but the results showed that this method had limited success in removing heavy metals without enhancing soil washing method with bio-surfactants.

The limits of standard co-contaminated soil remediation approaches are highlighted in these examples. Interactions between pollutants, variances in their chemical characteristics, and differences in their mobility and permanence all represent important issues. Conventional approaches frequently fail to remove or remediate co-contaminants simultaneously, resulting in partial treatment or the introduction of new environmental issues. As a result, there is a need for new and integrated techniques that take into account the intricacies of co-contaminated soil and address various contaminants at the same time.

1.3. Phytoremediation and Phyto Strategies for Contaminated Sites

This problem of co-contamination may be solved through phytoremediation, a promising green technology. Phytoremediation is a sustainable and environmentally acceptable method of remediating contaminated soil that employs plants and their related microbes. In terms of cost-efficiency, long-term effectiveness, and environmental damage, it outperforms traditional soil remediation approaches. Phytoremediation uses plants' natural capacities to absorb, detoxify, and stabilise pollutants, making it a potential option for co-contaminated soil remediation (Liu et al., 2018).

Depending on the pollutants present in the soil, various phytoremediation strategies can be used. In phytoextraction, plants are used in this method to absorb heavy metals from soil through the uptake and accumulation of pollutants in their roots and above-ground biomass. Hyperaccumulators are plants with high metal tolerance and accumulation capacity that are particularly useful in phytoextraction. For example, Xu et al. (2019) conducted research on the utilisation of hyperaccumulator plants for the cleanup of heavy metal-contaminated soil and exhibited successful removal of cadmium, lead, and zinc. Rhizofiltration uses plant roots to

remove pollutants from water or soil solutions. It effectively removes dissolved organic and inorganic contaminants like as metals and organic chemicals. Cristaldi et al. (2017) explored the use of rhizofiltration for co-contaminated soil with petroleum hydrocarbons and heavy metals in a case study. The study found that combining plant species with effective root systems and specialised microbial consortia improved pollutant removal. Phytostabilization tries to minimise pollutants' mobility and bioavailability by immobilising them in the soil, inhibiting their migration into groundwater or uptake by plants. Certain plants can release chemicals that aid in the binding and precipitation of pollutants, resulting in their immobilisation. Ye et al. (2017) evaluated the usage of grasses and legumes in co-contaminated soil with heavy metals and metalloids and found that the plants effectively reduced the bioavailability of pollutants, reducing their potential for environmental and human exposure.

Phytoremediation has a number of advantages over traditional soil remediation approaches Liu et al. (2018). For starters, it is less expensive than costly physical removal or heat treatment treatments. It also has the potential for long-term efficacy, as plants can continuously remediate soil over lengthy periods of time without the need for recurrent applications or interventions. Phytoremediation is a gentle and non-destructive method that minimises ecological disruption while keeping soil structure and fertility. Furthermore, it may be used in situ, eliminating the requirement for soil excavation and shipment, which can be both logistically and environmentally demanding.

Case studies have shown that phytoremediation for co-contaminated soil is effective. For example, Sun et al. (2014) conducted research on the phytoremediation of soil co-contaminated with heavy metals and petroleum hydrocarbons, revealing the efficacy of specific plant species in lowering the concentrations of both contaminants. Jeong et al. (2018) evaluated the utilisation of phytoremediation for co-contaminated soil with polycyclic aromatic hydrocarbons (PAHs) and heavy metals, highlighting plants' potential to decrease the dangers associated with these pollutants.

To summarise, phytoremediation is a promising technique for co-contaminated soil remediation. Its various kinds, such as phytoextraction, rhizofiltration, and phytostabilization, offer a variety of alternatives based on the unique contaminants and site conditions. Phytoremediation is cost-efficient, long-term effective, causes low environmental disruption, and has the potential to be used in situ. These benefits make it a better option to traditional soil remediation procedures, demonstrating its promise for sustainable and effective co-contaminated soil restoration.

Plants have the ability to withstand and collect toxins, and their root-associated bacteria can help with detoxification and breakdown (Shah & Daverey, 2020). The use of plant-bacterial interactions in conjunction with organic amendments has significant promise for improving the efficiency and effectiveness of phytoremediation procedures, particularly in the case of co-contaminated soil with cadmium and TPH (Luo et al., 2023).

By harnessing the natural capacities of plants and their associated microbes, this phytoremediation aims to reduce the negative effects of cadmium and TPH in polluted soil, restore its fertility, and reduce the risks to human and environmental health. Implementing creative and sustainable co-contaminated agricultural soil remediation solutions can assist to secure food security, preserve ecosystem integrity, and protect human well-being. In recent decades, there has been substantial progress in the field of phytoremediation research. While significant progress has been made, major uncertainties remain. Plants that thrive in uncontaminated habitats are dependent on a variety of elements, including soil composition, temperature, sunlight, precipitation, wind, and nutrient availability. However, because of the existence of soil contamination, phytoremediation requires additional considerations. These new characteristics are inextricably tied to the sorts of contaminants present at the site of interest, and the various natures of contamination and its impacts is difficult to categorise. Furthermore, rather than living in isolation, these pollutants frequently coexist. Many existing phytoremediation studies are largely concerned with either heavy metal remediation or organic pollutant removal. Understanding the effect of co-contamination on phytoremediation is difficult. Because of the complex interactions between organic and heavy metal pollutants, it is impossible to predict the outcomes of phytoremediation activities. More research is needed to optimise phytoremediation strategies for soils degraded with a combination of organic and heavy metal pollutants.

1.4. Suitability of Phytoremediation for Agricultural Soils Contaminated with Mixed Organic and Heavy Metal Pollutants

The use of phytoremediation in agricultural soils contaminated with a mix of organic and heavy metal contamination is a hotly debated topic. While phytoremediation has demonstrated encouraging results in single-contaminant soil remediation, its effectiveness and viability in co-contaminated agricultural soils need to be investigated further.

Co-contamination of agricultural soils with both organic and heavy metal contaminants is a widespread problem around the world. Organic contaminants including petroleum

hydrocarbons, pesticides, and polychlorinated biphenyls (PCBs) can be caused by agricultural practises, industrial operations, and poor waste disposal. Heavy metals, on the other hand, such as cadmium, lead, and arsenic, are frequently produced by industrial operations, mining, and the use of fertilisers and pesticides. The combination of both organic and heavy metal pollutants in agricultural soils makes remediation operations difficult.

Yan et al. (2020) present a thorough overview of phytoremediation, delving into the numerous mechanisms involved in heavy metal uptake, accumulation, and detoxification by plants. Lombi et al. (2001) investigate the natural processes and practical applications of phytoremediation in heavy metal-contaminated soils, emphasising various techniques, plant selection criteria, and the function of soil amendments and associated microbial populations. Alkorta and Garbisu (2001) provide an in-depth analysis of the phytoremediation of organic pollutants in soils, including mechanisms of uptake, transformation, and degradation by plants, as well as factors influencing their phytoremediation capability. Cristaldi et al. (2017) concentrate on phytoremediation of co-contaminated soils with heavy metals and organic pollutants, giving insights into the interactions between these contaminants, the involvement of plants, and factors influencing phytoremediation success in co-contaminated soils. In terms of case studies, Wan et al. (2013) investigated the phytoremediation capability of the arsenic hyperaccumulator plant *Pteris vittata* in arsenic and lead-contaminated soils. The study emphasises the significance of considering co-contamination effects and the influence of one contaminant on the absorption and tolerance of the other during phytoremediation operations.

Collectively, the mentioned research offers insightful knowledge into the application of phytoremediation in agricultural soils contaminated with combined organic and heavy metal pollutants. These papers examine the mechanisms, contributing variables, and difficulties of phytoremediation, emphasising the need for additional study and improvement of this strategy in co-contaminated agricultural soils.

A review of research studies indicated that *L. multiflorum* has a higher potential of phytostabilization and phytoextraction of heavy metals with a minimum decrease in the yield of biomass, providing higher remediation of soil (Emamverdian et al., 2015; Shivakumar et al., 2011). Another study illustrated the Cd tolerance and hyperaccumulation potential of ryegrass (*Lolium multiflorum* L.) in response to Cd stress. Two ryegrass cultivars with different Cd tolerance levels were tested, and it was discovered that the high Cd-tolerant cultivar had more Cd tolerance and lower root cell mortality than the low Cd-tolerant cultivar. The expression of Cd transport regulatory genes differed between the two cultivars, implying that they are

involved in Cd buildup and translocation (Wang et al., 2020). These findings help to understand the physiological and molecular mechanisms that explain ryegrass responses to Cd toxicity, as well as its potential as a hyperaccumulator for phytoremediation of contaminated soil.

Sidhu et al. (2017), reported the tolerance of *C.didymus* for Cd contamination level of up to 400 mg/kg for the first time. The bioconcentration factor (BCF) values obtained for varied cadmium (Cd) concentrations were larger than one, showing that Cd was successfully deposited in *C. didymus* plant tissues. Translocation factor (TF) values were less than one at lower Cd concentrations but approached one at the highest Cd concentration. This shows that *C. didymus* has the features of a hyperaccumulator plant, as it accumulates high levels of Cd in its tissues while exhibiting minimal translocation to aboveground plant sections. Based on these findings, *C. didymus* appears to be a promising candidate for practical application in the remediation of Cd-contaminated soils, perhaps providing a method to minimise Cd contamination

1.4.1. Complexities with Mixed Contamination

Complexities are introduced by the co-contamination of soil with mixed organic and heavy metal contaminants, which present difficulties for phytoremediation operations. Contrary to situations where a single pollutant occurs, interactions between organic and heavy metal contaminants can affect the toxicity, mobility, and destiny of the contaminants in soil. For efficient phytoremediation solutions to be developed in co-contaminated soils, it is essential to comprehend these complications.

Recent studies have brought attention to the difficulties brought on by co-contamination in soil. To illustrate the complex interactions between these contaminants and their influence on phytoremediation, Petruzzelli et al. (2016) looked into the co-contamination of soil with polycyclic aromatic hydrocarbons (PAHs) and heavy metals. According to the study, PAH availability and degradation were affected by the presence of heavy metals, while heavy metal uptake and translocation in plants were impacted by PAH availability.

Alengebawy et al. (2021) investigation into the co-contamination of soil with heavy metals and pesticides. The presence of pesticides, the researchers discovered, altered the bioavailability and toxicity of heavy metals, changing how they interacted with microbial and plant communities. In order to create efficient phytoremediation strategies, the study emphasised the necessity of taking into account the combined effects of organic and heavy metal contamination.

For example, Moreira et al. (2014), studies the impact of two plant growth-promoting rhizobacteria, *Ralstonia eutropha* and *Chryseobacterium humi*, on the growth and metal uptake of *Zea mays* plants in cadmium-contaminated soils. Bacterial injection enhanced plant biomass by up to 63% and significantly reduced Cd levels in shoots by up to 81%. Furthermore, Cd deposition in the roots rose by up to 186%. The study indicated that the rhizobacteria affected the rhizosphere community structure and recommended that *Z. mays* plants injected with these strains could be used in soil remediation procedures for short-term phytostabilization and biomass production for energy reasons. In another study, TCR05 and TCR20 were isolated as promising Cr(VI)-reducing multifunctional stress-tolerant plant growth-promoting bacterial (MST-PGPB) strains (Vishnupradeep et al., 2022). The researchers discovered that inoculating *Zea mays* plants with these MST-PGPB strains showed favourable effects under combined stress conditions. To begin, the MST-PGPB strains improved *Z. mays* stress tolerance, allowing the plants to better survive the negative impacts of various stresses. Second, inoculation with MST-PGPB strains improved photosystem II (PSII) performance in *Z. mays* plants under combined stressors. This shows that the MST-PGPB strains had a good effect on the plants' photosynthetic activity and general health.

Additionally, the co-contamination of the soil poses problems for plant tolerance and choice. Effects of various pollutants on plant growth, physiological functions, and tolerance mechanisms can differ. For instance, Lin et al. (2008) showed that the presence of cadmium affected the absorption and metabolism of PCBs in plants when cadmium and polychlorinated biphenyls (PCBs) were present in soil. In co-contaminated soils, the study emphasised the necessity for plant species with combined tolerance to both heavy metals and organic pollutants.

Soil microbial populations, which are crucial to the processes of phytoremediation, can be impacted by the intricate interactions between organic and heavy metal pollutants. In their investigation of the effects of co-contamination on soil microbial diversity, community structure, and functional potentials, Czarny et al. (2020) examined the co-contamination of soil with petroleum hydrocarbons and heavy metals. The study emphasised the need for phytoremediation solutions for co-contaminated soils to take into account microbial-mediated processes.

These studies show how complex co-contamination in soil is and how it must be handled if phytoremediation is to be successful. In order to choose the best plant species, optimise remediation techniques, and forecast the results of phytoremediation efforts, it is essential to

have a thorough understanding of the interactions, fate, and transport processes of combined organic and heavy metal pollutants.

1.4.2. Methods to Enhance the Process of Phytoremediation

Numerous techniques can be used to improve the efficacy and efficiency of phytoremediation in agricultural soils. In order to help the remediation process, these techniques strive to improve plant selection, pollutant uptake and accumulation, plant-microbe interactions, and soil conditions. Numerous strategies have been put forth and put into practise to improve the efficacy and application of phytoremediation of agricultural soils.

The choice of suitable plant species and genotypes with favourable features for pollutant uptake, accumulation, and tolerance is one way to improve phytoremediation. The expression of genes involved in contaminant uptake and detoxification mechanisms in plants can be improved via genetic engineering approaches. For instance, Yadav et al. (2018) successful genetic engineering of plants to increase their ability to absorb and accumulate arsenic improved the effectiveness of phytoremediation in arsenic-contaminated soils. Amendments can be applied to the soil to increase pollutant absorption and buildup. Chelating agents, surfactants, and biosurfactants are examples of soil amendments that can improve the solubility and bioavailability of pollutants, making it easier for plants to absorb them. In a study by Garbisu et al. (2002), contaminant clearance significantly improved when biosurfactants were used to support phytoremediation of soils contaminated with hydrophobic organic compounds.

Interactions between plants and microbes are essential to phytoremediation. Beneficial microbial populations can be introduced to improve plant health, encourage nutrient cycling in the soil, and accelerate the destruction and transformation of pollutants.

For instance, inoculating plants with particular microbial consortia, like plant growth-promoting rhizobacteria (PGPR), can increase the effectiveness of phytoremediation. A study by Murray et al. (2019) showed the effective application of PGPR in promoting plant growth and hydrocarbon breakdown to improve phytoremediation of petroleum hydrocarbon-contaminated soils. However, in co-contaminated sites, the presence of one pollutant can facilitate the clearance of another in some biologically-driven processes. According to a study by Ali et al. (2022), when the impacts of PAHs and heavy metals are coupled, specific synergistic responses arise. Plants, for example, can upregulate the production of certain genes and organic acids, whereas microorganisms can create protective extracellular polysaccharides (EPSs). Bacterial biofilms and the release of extracellular polymeric substances (EPS) can

increase bacteria's resistance to heavy metals like Cd, allowing for TPH co-remediation (Mahto et al., 2022). Furthermore, under these co-contaminated conditions, there is an increase in enzyme activity. These combined responses suggest that organisms may use adaptive mechanisms to cope with and remediate mixed-contaminant environments.

When confronted with co-contaminants, bacteria might utilize a variety of methods. They can biosorb and bioaccumulate heavy metals while also biodegrading organic pollutants such as TPH via metabolic pathways (Priya et al., 2022). Several bacterial strains have demonstrated success in the treatment of co-contaminated soils. For example, Pseudomonas and Bacillus species have proven the ability to withstand and remediate environments contaminated with Cd and TPH (Daniel et al., 2022). Bacterial stress responses may be induced by the concomitant presence of Cd and TPH. Some bacterial species, however, employ the stress generated by one pollutant to accelerate the degradation or removal of the other, offering a potential synergistic bioremediation effect (Khanpour-Alikelayeh & Partovinia, 2021).

According to research, bacteria in the Serratia genus can form biofilms that aid in the adsorption and stability of cadmium while also degrading TPH (Chen et al., 2019; Lee et al., 2022). Some research has found that plant growth-promoting rhizobacteria (PGPR) can reduce Cd toxicity in plants by increasing their ability to absorb Cd from soil (Khanna et al., 2021). They provide a dual remediation technique when combined with TPH degradation capabilities (A. Wang et al., 2022). Advanced genomic research on bacterial species such as Mycobacterium has revealed specific genes and metabolic pathways that are active during co-contamination. This knowledge can be used to improve bioremediation approaches (Li et al., 2023).

Plant growth is known to be aided by PGPR, particularly under stressful situations such as metal contamination. They can promote plant root growth, which can improve TPH rhizodegradation (Zuzolo et al., 2021). Furthermore, many PGPR strains have shown the ability to either immobilize metals or help plants hyperaccumulate them (A. Liu et al., 2022). TPH-degrading bacteria target the degradation of hydrocarbon contaminants directly. When combined with metal resistance, these bacteria are able to successfully treat both contaminants at the same time (Qi et al., 2021). Enzymatic processes that convert hydrocarbons into less hazardous chemicals are commonly used in the degradation process.

While both PGPR and TPH-degrading bacteria are useful in bioremediation, their methods of action are not the same. PGPR largely promotes plant growth and health, hence increasing the plant's innate ability to breakdown TPH and accumulate or immobilize metals (Wang et al.,

2023). TPH-degrading bacteria, on the other hand, act directly on the TPH, breaking them down (Rong et al., 2021). The efficiency of PGPR varies depending on plant species and soil type (DalCorso et al., 2019). Conversely, specialized TPH-degrading bacteria chosen depending on the type of hydrocarbon pollutant can be more directly effective regardless of plant species. A combination of PGPR and TPH-degrading bacteria is frequently the most comprehensive cleanup method. This combines the advantages of increased plant growth and direct TPH breakdown (Wang et al., 2022).

According to research, combining bacterial inoculation (bioaugmentation) with nutrient amendments (biostimulation) in co-contaminated soils can result in increased TPH breakdown and Cd immobilization (Ambaye et al., 2022). For instance, adding organic amendments like compost, biochar, and activated carbon can promote pollutant sorption and degradation, improve soil structure, and increase microbial activity.

Soil additives can also be used to alter soil properties and advance the cleanup procedure. Compost, which is made from organic resources such as animal manure and agricultural waste, is widely recognised as a good supplement for enhancing soil health and boosting plant development. Composting has been found in numerous studies to improve soil characteristics, nutrient availability, and microbial activity. In one study, Rezaenejad (2001) found that applying composted animal dung improved soil fertility and increased the availability of critical nutrients, resulting in greater plant growth and output. Similarly, Scotti et al. (2015) discovered that adding compost resulted in increased soil structure, water-holding capacity, and nutrient retention, resulting in improved plant growth and resilience to environmental challenges posed by the Mediterranean basin, where this technique is used over 200,000 Ha.

Compost has also been found to have a major impact on soil microbial communities, which are important for nutrient cycling and plant-microbe interactions. Auffret et al. (2016) discovered that compost addition boosted microbial biomass and diversity, supporting positive microbial activities and nutrient conversions in the soil. These microbial community alterations may have a positive impact on plant nutrient uptake and overall soil health.

Furthermore, compost application has been investigated as a sustainable strategy for the rehabilitation of petroleum hydrocarbon-contaminated soils. Compost was used in a study by Antizar-Ladislao et al. (2004) to aid in the breakdown of TPH in contaminated soil. The results demonstrated that compost amendment improved TPH breakdown by encouraging the establishment of hydrocarbon-degrading bacteria communities. The compost created an ideal

habitat for the growth of indigenous bacteria capable of metabolising petroleum hydrocarbons, speeding up the bioremediation process.

Furthermore, Tran et al. (2021) investigated the efficacy of composting as a treatment technique for petroleum-contaminated soil. Composting not only lowered TPH concentrations but also enhanced soil physicochemical qualities such as pH, organic matter content, and nutrient availability, according to the findings. The compost amendment promoted the growth of hydrocarbon-degrading bacteria and fungi, which resulted in improved TPH breakdown and overall soil remediation.

Biochar, a carbon-rich byproduct of pyrolysis of organic materials in the absence of oxygen, has emerged as a promising soil addition for remediation. This is especially true for soils that have been contaminated with heavy metals and total petroleum hydrocarbons (TPH) (U. Yousaf et al., 2022). Biochar's wide surface area and numerous functional groups make it an efficient adsorbent in the context of heavy metal pollution (Xiong et al., 2021). Metals like Cd are known to be immobilized by attaching them to its surface or incorporating them into its porous structure, decreasing their bioavailability and mobility in soil. Several studies have demonstrated that applying biochar to Cd-contaminated soils can reduce Cd leachability and phytoavailability greatly (Hamid et al., 2022). This not only minimizes the potential of groundwater contamination, but it also inhibits Cd uptake by plants, making it very useful in agricultural environments.

Biochar serves a dual purpose in terms of TPH pollution. For starters, it works as a sorbent, lowering TPH bioavailability in the soil. Biochar's porosity structure improves its ability to absorb organic pollutants, trapping TPH inside its matrix (Lin et al., 2022). Furthermore, biochar can boost microbial activity, boosting TPH biodegradation in soil (Mukome et al., 2020). According to research, the use of biochar can cause a shift in microbial populations, favoring those capable of TPH breakdown (Tan et al., 2022).

The efficiency of Immobilized Microorganism Technology (IMT) for remediating soil co-contaminated with petroleum hydrocarbons and the heavy metal nickel (Ni) was investigated in a study (Xi et al., 2020). Citrobacter sp., which is recognized for its resistance to Ni and capacity to breakdown hydrocarbons, was immobilized on corncob charcoal and put into the polluted soil. The primary goals were to assess the biodegradability of petroleum hydrocarbons and changes in the mobility and form of Ni in soil, taking into account soil characteristics and dehydrogenase enzyme activity. The petroleum hydrocarbon breakdown rate in soil treated

with immobilized microorganisms (IM) was 45.52%, much higher than free bacteria (30.15%), only biochar (25.92%), and a control group (18.47%). With an increase in residual content of 101.50 mgkg1, IM was more efficient in converting mobile Ni in the soil to a less accessible and more stable form. Notably, carcinogenic nickel sulfide was not present following IM therapy. The soil's dehydrogenase activity, a measure of microbial activity, was higher in the IM-treated soil (0.3956 gmL1h1g1) than in the free bacteria-treated soil (0.2878 gmL1h1g1). The breakdown rate of petroleum pollutants and soil dehydrogenase activity were found to be directly related. An investigation on the use of biochar amendments to boost the phytoremediation of heavy metal-contaminated soils was also conducted by Zhao et al. (2022), showing better plant growth and metal uptake. It is important to note, however, that the performance of biochar in remediation is mostly determined by its physicochemical features, which are regulated by the feedstock and pyrolysis conditions. Higher-temperature biochar, for example, has increased adsorption capabilities for both metals and organic molecules (Kuppusamy et al., 2017; Zhao et al., 2020). Therefore, the choice of biochar for soil application must be analysed prior to its addition in soil.

Optimising plant growth conditions can also improve phytoextraction, one of the key phytoremediation methods. The uptake and transport of pollutants by plants can be affected by changing variables like pH, the availability of nutrients, and water management. The phytoremediation of multi-metal contaminated soils was explored in a study by Muhammad et al. (2009), and it was discovered that optimising these factors considerably improved lead accumulation in plants.

In conclusion, a variety of techniques can be used to speed up the phytoremediation process in agricultural soils. The efficacy of phytoremediation is enhanced by the choice of suitable plant species and genotypes, the inclusion of amendments to enhance contaminant uptake and availability, the encouragement of advantageous plant-microbe interactions, and the adjustment of soil conditions. Through the use of these techniques, phytoremediation's application and effectiveness may be improved, opening the door to successful soil remediation in agricultural settings.

1.5. Purpose of this Research

The purpose of this research, which is to develop a comprehensive strategy for phytoremediation for contaminated sites in Pakistan, is directly related to the difficulties that soil contamination poses for the nation's agricultural industry. Pakistan's economy depends

heavily on agriculture, which makes a considerable contribution to the GDP, employment, and food security of the nation. The Pakistan Economic Survey (2020–2021) states that agriculture contributes over 24% of the nation's GDP and employs roughly 38% of the labour force (Agriculture Statistics | Pakistan Bureau of Statistics, n.d.; Government of Pakistan [Finance Division], 2022). The industry assures food supply for the expanding population, provides crucial raw materials for industries, and generates revenue for rural people.

However, Pakistan's agricultural industry has a number of difficulties, and one of the major reasons affecting its sustainability and production is soil pollution. When dangerous compounds are present in the soil, it is said to be polluted. This can have a negative impact on crop output, soil fertility, and plant growth. These contaminants can come from a number of sources, including industrial processes, the use of chemical pesticides and fertilisers, poor waste management, and irrigation with untreated wastewater.

The effects of soil contamination on Pakistan's agriculture are extensive. Reduced soil fertility, poorer crop yields, and lower-quality agricultural products are the results. In Punjab, Pakistan, a study by Shaheen et al. (2018) found that heavy metals' soil pollution had a substantial impact on crop development and yield, with contaminated soils demonstrating lower nutrient availability and impeding plant growth.

Because of pesticide use, mining, and industrial activity, heavy metals are one of the main pollutants in soil. When ingested in large quantities, they can build up in crops and endanger the health of consumers. An investigation on the levels of heavy metal contamination in agricultural soils in Pakistan's Sindh province by Bux et al. (2021) revealed that soil pollution has a negative impact on the quality and safety of food crops.

Furthermore, as pollutants can seep into groundwater or wash off into rivers and streams, soil pollution in agricultural areas can result in water contamination. This worsens the negative effects on the environment and endangers both human health and aquatic ecosystems. In the Pakistani area of Faisalabad, Mahfooz et al. (2019) study investigated the As contamination of irrigation water and its effects on the quality of the soil and crops. The study brought attention to the relationship between water and soil contamination and their impact on agricultural productivity and food safety.

In Pakistan, soil degradation has severe economic repercussions. Crop losses brought on by soil contamination have a direct impact on farmers' income and way of life, particularly small-scale farmers who depend significantly on agriculture. Farmers and the government are

additionally burdened by the expense of cleaning up contaminated soils and implementing sustainable farming methods. In a study, Abbas et al. (2017) evaluated the financial effects of soil pollution on Pakistan's wheat production and predicted significant economic losses due to lower yields as a result of soil pollution. Joint efforts are needed to lessen the effects of soil contamination on agriculture. Reduce the use of chemical inputs and lessen soil contamination by putting into practise sustainable agricultural practises such as organic farming, integrated nutrient management, and precision farming.

This research tackles the need for long-term remedies to alleviate soil contamination in Pakistan by using an integrated strategy to phytoremediation. A promising method of remediation is the employment of plants and related microbes to remove, decompose, and stabilise pollutants. As phytoremediation avoids or decreases the need for pricey physical removal or chemical treatments, this strategy is in line with the goals of developing a low-cost and organic solution. As phytoremediation can enhance soil fertility and quality, easing the resumption of agricultural operations, it also supports the objective of recovering polluted lands for agricultural use. The efficiency and effectiveness of the remediation process are increased through the combination of various phytoremediation techniques, including phytoextraction and phytostabilization, as well as the use of suitable plant species and additives. The intricacies of co-contamination, the specific types of contaminants found in the soil, and the interactions between organic and heavy metal contaminants are all taken into account by this integrated method. The integrated method that has been suggested addresses these complications and provides a customised remedy for cleaning up polluted areas in Pakistan.

Additionally, the integrated approach of the use of organic amendments and promotion of plant-microbe interactions encourage sustainable practises and reduce the need for chemical inputs (Segura and Ramos, 2013). The integrated phytoremediation approach's focus on sustainable remediation methods is consistent with the study's goal of offering an ecologically beneficial solution. The efficacy of integrated phytoremediation techniques using PGPR and bacteria that can enhance phytoextraction while also promoting plant growth in co-contaminated soils has been proven in studies by Khan et al. (2008) and Sessitsch et al. (2013), showing the potential for restoration and agricultural usage. Further promoting the use of plant-based remediation techniques is the study of Paz-Alberto and Sigua (2013), which shows the effective application of phytoremediation for heavy metal-contaminated locations.

Conclusively, Pakistan's economy depends heavily on agriculture since it creates jobs, boosts GDP, and ensures food security. The agricultural industry is faced with considerable obstacles

from soil contamination, which affects crop productivity, soil fertility, and food safety. Particularly alarming issues include water pollution and heavy metal contamination. The financial ramifications are significant, affecting farmers' livelihoods directly and increasing the expense of using sustainable agricultural methods and remediating the soil. Protecting the productivity and long-term sustainability of Pakistan's agricultural sector requires addressing soil pollution through sustainable soil restoration practises and cutting-edge remediation methods.

1.6. Significance of the Study

The importance of this work lies in its emphasis on the use of compost and biochar as organic amendments and plant growth-promoting rhizobacteria (PGPR) for remediating soil that has been contaminated with both cadmium and total petroleum hydrocarbons (TPH). This work helps to develop effective and long-lasting remediation techniques by examining the potential of PGPR and organic amendments in treating such co-contaminated soils.

Because of how well PGPR interacts with plants, their utilisation is important. These rhizobacteria can aid in the breakdown of organic pollutants, improve nutrient intake, and stimulate plant development. Numerous research has shown how well PGPR works to improve hydrocarbon breakdown and phytoremediation of heavy metal-contaminated soils. In their investigation of the role of PGPR in the phytoremediation of Cd-contaminated soil, for instance, Gkorezis et al. (2020) highlighted the potential of these bacteria to enhance plant growth and Cd accumulation.

Compost and biochar are two important organic additions for soil improvement. Compost boosts microbial activity, improves soil structure, and supplies vital nutrients for plant growth and pollutant breakdown. Similarly, biochar can boost pollutant sorption, increase water retention capacity, and improve soil fertility. Compost and biochar amendments work well together to improve soil quality and lower pollutant availability. In a study by Yao et al. (2021), compost and biochar amendments were investigated for their potential to improve microbial activity and TPH degradation in soil that had been contaminated with TPH.

Insightful information about the use of PGPR and organic amendments in the treatment of co-contaminated soils can be gained from a few case studies. For instance, Ma et al. (2022) looked at the usage of PGPR and compost additions for the remediation of co-contaminated soil with Cd and TPH in a developed nation. The study demonstrated the beneficial effects of the integrated strategy, which improved plant growth, decreased Cd uptake, and aided TPH

breakdown. The current study expands upon information and experiences from industrialised nations. This shows the possibility of applying and modifying these remediation techniques to the setting of soil co-contamination in other places, especially in developing nations like Pakistan. As a result, co-contaminated soils can be remedied in a sustainable and effective manner, enhancing soil health, crop yield, and food safety.

1.7. Novelty Statement

This study introduces a brand-new integrated phytoremediation technique called "phase crop rotation" for cleaning up sites that have been contaminated with both cadmium (Cd) and total petroleum hydrocarbons (TPH). There are two major phases to the strategy. Phase 1 involves the phytoextraction of cadmium and the degradation of TPH utilising a hyperaccumulator grass, compost, and plant growth-promoting rhizobacteria (PGPR). By using PGPR and biochar in Phase 2, the lingering heavy metal contamination is phytostabilized. The goal of this ground-breaking strategy is to effectively target both contaminants using a sequential and complimentary method in order to address the co-contamination dilemma.

1.8. Aim & Objectives of the Research

This study aims to improve the soil quality by mitigating the toxic effect of organometallic complex contamination of Cd-TPH in soil through the plant microbial interactions in order to ensure the sustainable environmental and food security.

Phase I

1. To analyse the effect of compost and *Bacillus safencis* on the uptake of Cd and degradation of TPH.
2. Determine the effect of compost and bacterial inoculation on plant growth and physicochemical properties of soil.

Phase II

1. To check the effect of *Bacillus cereus,* and biochar amendment on cadmium accumulation and TPH degradation, and growth of maize plant.

Chapter 2

Materials and Methods

2.1.Introduction of Materials and Methodology

This chapter of the thesis outlines the methods and approaches employed for examining the phytoremediation of soils contaminated with cadmium (Cd) and total petroleum hydrocarbons (TPH). This chapter explains how samples are collected, the experimental design, and all analyses conducted on plant and soil samples. The measurements used in soil assessments include those for soil texture, electrical conductivity (EC), pH, total dissolved solids (TDS), nitrate-nitrogen, phosphates, metal analysis, and TPH degradation evaluation. Stress enzyme measurement, chlorophyll content analysis, and metal analysis are all included in plant analyses. Furthermore, statistical analysis techniques such as mean, standard deviation, regression, correlation, and analysis of variance (ANOVA) are used to analyse the data produced.

2.2.Soil Collection

Soil samples were collected from an agricultural field near Islamabad's Quaid-i-Azam University, where wheat was grown. Precautions were taken prior to soil collection to reduce the inclusion of excessive plant material or stones. The field was cleared to ensure that the soil samples included mostly soil particles. Soil samples were then gathered by digging the topsoil to a depth of 20 cm. The collected soil samples were spread out and left to air-dry for one week to assist further analysis. This procedure ensured that the soil samples were in good enough condition for subsequent laboratory investigations and analyses.

2.2.1. Spiking Soil with Metal Salt Powder

For soil spiking with Cd, dry metal salt powder was added directly to the soil following a protocol by Chen et al, (2019). 150 mg/kg of Cd was added to the soil by determining its concentration from the cadmium chloride monohydrate ($CdCl_2.H_2O$) salt. To assure the homogeneity of the soil samples, the soil preparation approach included many procedures. Initially, bigger clumps of soil were crushed down into tiny particles using an agate motor. After that, a 2mm sieve was used to further refine the soil texture and eliminate any bigger material.

Metal salts were ground into a fine powder to prepare the metal compounds. Using an agate mortar, around 25-50g of soil was ground into a fine powder, with a general guideline of

grinding the soil 10-20 times the mass of the metal salt. Using rods, the ground metal compounds were thoroughly and uniformly mixed with the finely powdered dirt inside the mortar.

A clean plastic sheet measuring 2m by 2m was prepared for the ensuing mixing process. The plastic sheet was filled with a mixture of pulverised metal compounds and earth. The plastic sheet's corners were then diagonally flipped towards the centre, a process that was repeated 5-8 times. This procedure guaranteed that the mixture was thoroughly blended.

At each stage, approximately 10–20 times the volume of the mixture was added to gradually incorporate additional bulk soils into the mixture. The soil was stirred by diagonally flipping the plastic sheet's corners 5-8 times more. This process was repeated with more clean soil until the entire amount of clean soil was integrated into the homogenous mixture.

For TPH spiking, 20 ml of diesel was sprayed in 1kg of soil and mixed thoroughly unless TPH was homogeneously distributed in the soil. The process was repeated for 15 kg of soil, and as a final step, the soil was spread on a large 10 x 10 m plastic sheet, and the corners of the sheet were flipped diagonally 20 times for thorough mixing.

Finally, the prepared soil was allowed to settle for 30 days. This allowed for any potential particle settling or redistribution within the soil mixture. Before further examination or experimentation, the soil samples were stabilised for one month to ensure consistency. Overall, the goal of this process was to produce a well-mixed and homogenous soil sample by grinding larger clumps, sifting the soil, grinding metal salts, mixing the ground metal compounds with the soil, and gradually integrating additional clean soil. The complete mixing technique, followed by a stabilisation time, ensured the consistency and reproducibility of the soil samples prepared for the study.

2.3.Selection of Plants

For Phase 1 of the experiment, two hyperaccumulator grasses were used, which have previously been reported in phytoextraction studies for remediation of different types of heavy metal contamination in soil. The hyperaccumulator plant species that were selected for this were *Lolium multiflorum* and *Coronopus didymus,* commonly known as rye grass and garden cress, respectively. This choice of plant species was guided by a comprehensive review of the literature, which analysed factors such as yield of biomass and phytoremediation efficacy for various contaminants. The seeds of garden cress were obtained from an online store.

In the second phase, *Zea mays* (maize) was selected for phytoremediation of remaining Cd after an extensive literature review. Maize has been reported in many studies to promote phytostabilization of metals in the rhizosphere of Cd contaminated and other HM contaminated soils. The seeds of maize used were obtained from National Agriculture and Research Center (NARC), Islamabad.

2.4.Selection of Bacteria

For the experiment two pre-isolated bacterial strains were used i.e., *Bacillus safencis* (NCCP-2261) and *Bacillus cereus* (NCCP-2265). The choice of these bacteria was based upon the plant growth promoting traits found in them and the extensive literature review showcasing their role in promoting phytoextraction by enhancing the soil health and biomass of the plants. The presence of plant growth promoting traits, such as, siderophore production, ACC deaminase activity, enhance mineral solubilization, nitrogen fixation etc, was confirmed with the application of some bioinformatics tool and online database to confirm if these genes were present in these bacteria or not.

2.5.Selection of Compost

For the Phase 1 of this experiment, compost amendment was used as a treatment alone, and along with plant growth promoting bacteria *Bacillus safencis* to investigate its role in promoting soil health and plant biomass, ultimately leading to phytoextraction of Cd and degradation of TPH in soil. This compost was also obtained from NARC where it was already being used in agriculture sector (**Error! Reference source not found.**).

2.6.Experimental Design

2.6.1. Phase 1

Pots with dimensions of 5 inches in diameter and 5 inches in height were used in this experiment to plant the ryegrass and garden cress. Each pot was filled with 500 g of prepared soil, and five control pots were set up, with one abiotic control and two biotic controls for both rye grass and garden cress. In addition, for each plant species, three contaminated pots were prepared, one with bacterial inoculation, one with compost amendment, and one with both bacterial inoculation and compost amendment. 30 seeds were sown in each pot and all the pots were set up in three replicates, making a total of 33 individual pots including both, control pots and treatment pots. Individual trays were used for each pot to maintain leachate separation. Table 2 shows the treatment plan for this experiment.

Table 1: Abbreviations for Treatments of Phase 1.

Full Form	Abbreviations
Spiked Soil	SS
Fresh Soil	FS
Coronopus didymus (Garden cress)	P1
Lolium multiflorum (Rye grass)	P2
Compost	C
Bacterial Strain – *Bacillus safencis*	B
Compost + Bacteria	C+B

Table 2: Treatment Plan for Phase 1.

Sr #	Treatment Type	Treatment
1	Abiotic control (C1)	SS
2	Biotic Control (C2)	FS+P1
3	Biotic Control (C3)	FS+P2
4	Biotic Control (C4)	SS+P1
5	Biotic Control (C5)	SS+P2
6	Treatment (T1)	SS+P1+B
7	Treatment (T2)	SS+P1+C
8	Treatment (T3)	SS+P1+C+B
9	Treatment (T4)	SS+P2+B
10	Treatment (T5)	SS+P2+C
11	Treatment (T6)	SS+P2+C+B

The experiment was carried out in the green house of Botanical and Research Garden at Quaid-i-Azam University. This guaranteed that the pots received natural sunshine and were exposed to temperature settings that ranged between 25 and 30 degrees Celsius. Pots were watered when needed by adding 50 ml of water to the trays rather than directly to the soil surface. This method

of watering enables the plants to absorb water via capillary action. Furthermore, the use of fans aided in the direction of hot air away from the plants, thereby preserving appropriate temperature conditions for their growth.

The amendments used in this study were biochar 5% w/w, and compost, 10% w/w. These amounts were chosen based on current literature and previously published research findings for the addition of biochar and compost to soils contaminated with total petroleum hydrocarbons (TPH) (Chirakkara & Reddy, 2015; Yousaf et al., 2021). For treatments involving bacteria, the inoculum was prepared by inoculating nutrient broth with the bacterial strain and incubating it at 37 degrees Celsius for 24 hours (Madariaga-Navarrete et al., 2017). The OD of the inoculum was measured via UV spectrophotometry prior to inoculation to be 1.09. The bacterial inoculum was added in respective treatments at 10^8 cells/ml. During the experiment, the plants were cultivated for 40 days, and their growth was closely monitored. The placement of the pots was changed on a regular basis to ensure that all pots received equal light exposure. After the germination of seeds, the saplings were counted, and equal number of plants in each pot was maintained.

2.6.2. *Phase 2* (Pot Experiment)

For phase 2, soil with similar amendments in the pots were combined to make the composite soil batches. For instance, soil from the pots containing only compost were combined to make a composite soil + compost batch. This was done for all the pots including the controls and treatments. In this phase, only maize was used along with the addition of biochar to help enhance the phyto and rhizo stabilization of remaining Cd in soil. The treatment plan for this phase is shown in Table 4. This experiment was also designed in 3 replicates, with addition of equal number of maize seeds, i.e., 15 in each pot except abiotic control. The plants were grown for 28 days in the same greenhouse under similar conditions except temperature which was set to 35-40 degrees Celsius.

Table 3: Abbreviations for Phase 2.

Full Form	Abbreviations
SS	Spiked Soil
FS	Fresh Soil
P	*Zea maize* (maize)

C	Compost
BA	Bacteria – *Bacillus cereus*
BC	Biochar

Table 4: Treatment Plan of Phase 2

Sr #	Treatment Type	Treatment
1	Control 1 (C1)	FAS+P
2	Control 2 (C2)	SS
3	Control 3 (C3)	SS+P
4	Treatment 1 (T1)	SS+P+BA
5	Treatment 2 (T2)	SS+C+P
6	Treatment 3 (T3)	SS+C+P+BA
7	Treatment 4 (T4)	SS+BC +P
8	Treatment 5 (T5)	SS+ BC+P+BA

At harvesting, the shoots were gently chopped at the soil surface. Following that, the roots were carefully removed from soil. Overall, the regulated circumstances for the establishment and development of the planted plants were provided by this experimental design. The specified pot size, meticulous separation of leachate, controlled temperature, and watering practises ensured consistent and controlled environmental conditions, allowing the study to collect valid observations and data.

2.7.Physiological Analysis of Plants

After harvesting, root and shoot lengths were measured, and their fresh weights were also recorded. For measuring the fresh weight of roots, they were gently washed to remove all the attached soil clumps and particles and then air-dried for an hour. For measuring the dry weights, shoots and roots were placed in labelled paper bags and kept in the oven overnight at 70 degrees Celsius till constant weight. Soil samples were also stored at -18°C in zipper bags to be used for further soil analysis.

2.8.Soil Analysis

This section will discuss all the soil analyses conducted for this research, which are as follows:

2.8.1. Soil Texture - Hydrometer Method

The hydrometer method is a popular approach for evaluating soil texture based on particle settling velocities in a water suspension. This approach is based on the idea that particles of varying sizes settle at various rates due to their variable settling velocities. The hydrometer method measures particle settling velocity in grammes per litre (g/L) using a hydrometer with a Bouyoucos scale. All sand-sized particles (0.02 mm and larger) settle out of the suspension after 40 seconds, whereas particles larger than clay (0.002 mm) settle out after 4 hours. The settling velocity is proportional to the square of the particle's radius, according to Stoke's Law. However, the effects of temperature on water density and viscosity must be considered, as higher temperatures result in reduced viscosity and faster particle settling. Corrections for liquid temperature are thus required to achieve accurate findings (Libretexts, 2021).

Sample Preparation

40-gram air-dried soil was carefully weighed and deposited in a 600-mL beaker after being sieved to a 2-mm size. A dispersion solution was prepared by adding 40 g of sodium hexametaphosphate and 10 g of sodium bicarbonate in a 1 litre volumetric flask and bringing the volume up to the mark by adding distilled water. The beaker wass then filled with 60 mL of a dispersing solution, covered with a watch glass, and left to stand overnight. During the suspension preparation process, these chemicals function by lowering surface tension between soil particles, facilitating particle separation, and avoiding re-aggregation. The hydrometer method, when combined with a dispersion agent, can produce reliable and consistent findings for estimating the proportions of different soil texture fractions such as silt, clay, and sand. The following day, the beaker is partially filled with distilled water. The suspension was shaken overnight in a shaker. The suspension was then transferred quantitatively into a 1-L calibrated cylinder, known as a hydrometer jar, and brought to volume with distilled water. Before proceeding with the determination, a blank measurement was performed. The dispersing solution was diluted to 1 L in the hydrometer jar with water, mixed well, and the hydrometer is inserted to obtain the reading, known as Rb.

Determination of Silt plus Clay

The silt plus clay determination was then performed. The suspension in the hydrometer jar was carefully stirred with a specific paddle, and then the paddle was removed, allowing the suspension to settle. The hydrometer was inserted after eliminating any froth with a drop of amyl alcohol, and the reading was obtained 40 seconds after withdrawing the paddle, written

as Rsc. For temperature corrections, a thermometer was also inserted to measure the temperature of the solution.

Determination of Clay Only

The suspension in the hydrometer jar was stirred with the paddle and left undisturbed to determine the clay concentration. The hydrometer was inserted after 6 hours, and the reading was recorded as RC. Temperature was also measure using a thermometer.

Determination of Sand

After the clay and silt measurements, the suspension was put through a 50-m screen to determine the sand concentration. The sieve was washed until the water that passes through it is clear. The sand retained on the sieve was then quantitatively transferred to a 50-mL beaker, and surplus water was decanted after the sand has settled. The sand in the beaker was dried overnight at 105 °C, cooled in a desiccator, and weighed again to achieve the final weight.

Calculation of Soil Texture

The percentages of silt, clay, and sand can be determined using the formulas. Temperature corrections were applied to the following results such that for every 1°C fall in temperature below 20, 0.36 units were subtracted from the final reading, and vice versa. These calculations provide useful information about soil texture, which is necessary for understanding soil attributes.

USDA Textural Triangle

The USDA textural triangle can be used to estimate soil texture after getting the percentages of sand, silt, and clay fractions in the soil. The USDA textural triangle is made up of different soil textures that are defined by the quantities of certain soil fractions.

This triangle allows soil to be classified into twelve distinct textural classes, each having its own composition displayed on the textural triangle. By plotting the measured values on the textural triangle, and following the arrows, an appropriate textural class can be assigned. The crossing point of these lines on the textural triangle indicates the given textural class based on the proportions of sand, silt, and clay fractions. This categorization system characterises soil texture, which gives useful information about soil qualities, water-holding capacity, and agricultural management practices. The soil texture for the soil under study came out to be loam with 36.2 % sand, 20.4% clay, and 43.4% silt.

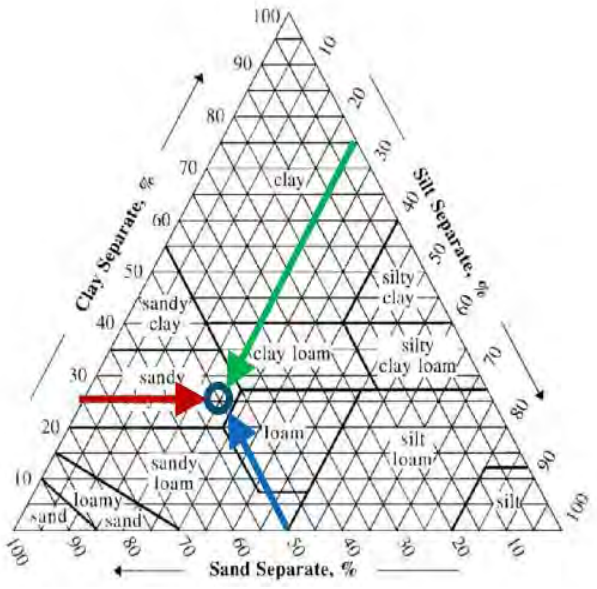

Figure 1: USDA Textural Triangle.

2.8.2. Determination of pH, Electrical Conductivity (EC), and Total Dissolved Solids (TDS) of Soil

For the pH measurement, protocol by Sommer (2017) was followed. The initial stage in this process was to weigh 50 grams of air-dried soil with particle size smaller than 2 mm into a 100 mL glass beaker. The beaker was then filled with 50 mL of deionized (DI) water using a graduated cylinder or a 50 mL volumetric flask. The soil and water were well mixed with a glass rod, and the suspension was allowed to stand for 30 minutes. The suspension was stirred every 10 minutes during this time.

The suspension was stirred again after 30 minutes to ensure thorough mixing. At this stage, the pH metre was calibrated according to the instructions. The pH meter's combination electrode was then put in the suspension, approximately 3 cm deep. The pH reading was taken after 30 seconds and was accurate to one decimal place.

The combined electrode was carefully withdrawn from the suspension after the pH measurement. In a separate beaker, it was thoroughly rinsed with DI water to eliminate any residue. Using a tissue, excess water was carefully dried from the electrode. EC and TDS readings were measured using the probe method (Adwaadmin, n.d.; Corwin & Yemoto, 2019). A 50 g chunk of air-dried spiked soil sample was mixed with 100 ml of distilled water and swirled for a few minutes to ensure appropriate mixing. After then, the mixture was left undisturbed for 15 minutes. Two standard solutions, KCl and NaCl, were created to test the accuracy of the electrical conductivity (EC) probe. The KCl standard solution was made by

dissolving 0.745 g of KCl in distilled water after drying it for 2 hours at 60 °C and adjusting the volume to 1L. When measured with the EC probe, this solution was expected to produce a value of around 1500 S/cm. In contrast, the NaCl standard solution was made by dissolving 1g of NaCl in distilled water and adjusting the volume to 1L. This solution was expected to produce an EC result ranging between 1500 and 2500 S/cm. The EC of both the standard solutions and the soil sample was determined by immersing the probe's electrode in the appropriate samples. When the probe indicated that the measurement was complete, readings were recorded.

The soil sample's total dissolved solids (TDS) were determined by producing a soil sample solution using the same approach as the electrical conductivity (EC) measurement, which entailed blending 50 g of soil with 100 ml of distilled water. After that, the TDS probe was utilised to determine the TDS value of the soil sample solution (Corwin & Yemoto, 2019).

This method allowed for the creation of a soil-water suspension and subsequent pH, EC, and TDS measurements with a calibrated probe. The meticulous handling and techniques used ensured reliable readings and reduced the possibility of contamination or influence during the operation.

2.8.3. Moisture Content Analysis

The ICARDA procedure was used to determine the moisture content of fresh air-dried soil (Sommer, 2017). 40 g air dried soil sample with particle size less than 2 mm was weighed carefully and placed in a glass beaker. The beaker, slightly covered with a glass lid, was then placed in an oven set to 105 °C and dried overnight for roughly 18 hours. After the drying process was completed, the beaker containing the soil sample was taken from the oven and completely covered with a lid. The soil sample in the beaker was weighed again after it had cooled for at least 30 minutes. By subtracting the weight of the oven-dried soil from the initial weight of the soil sample, the moisture content was calculated. The resulting moisture content was discovered to be around 1%.

2.8.4. Quantification of Nitrates-Nitrogen in Soil

The spectrophotometric approach employing chromotropic acid was used to quantify nitrate-nitrogen (NO_3-N) (Sommer, 2017). This approach, which was first designed for water analysis and was later adapted for soil samples, provides a quick and effective alternative to the standard distillation method for determining NO_3-N. To make the required solutions, 4.99 g of $CuSO_4.5H_2O$ was dissolved in deionized (DI) water to make a 0.02 N solution in a final volume

of 2 litres. 0.368 g of chromotropic acid was also dissolved in 200 ml of concentrated H_2SO_4. To maintain the solution's stability, it was kept in a dark bottle for two weeks. It's worth mentioning that the method also made use of 98% pure sulfuric acid. These preparations allowed for precise and dependable measurements.

Standard Stock Solution Preparation

A series of processes were taken to prepare the requisite solutions for measuring nitrate-nitrogen (NO_3-N). Initially, 4-5g of potassium nitrate (KNO_3) was dried in an oven at 100 °C for 2 hours. After drying, the KNO_3 was allowed to cool before being kept in a tightly sealed bottle to preserve its integrity.

The following stage was to make a stock solution. 3.60 g of KNO_3 was dissolved in 500 mL of 0.02 N copper sulphate ($CuSO_4.5H_2O$) solution for this. This solution, which had a known concentration of KNO_3, served as the stock solution. A diluted stock solution was made from the stock solution. In a 200 mL flask, 10 mL of the stock solution was diluted with 0.02 N $CuSO_4.5H_2O$ solution. The resulting solution, dubbed the diluted stock solution, had a NO_3-N concentration of 50 mg/kg.

Various quantities of the diluted stock solution were further diluted to create a series of standard solutions. In numbered flasks, 1, 2, 3, 4, 5, 6, and 7 mL of the diluted stock solution were diluted to a final volume of 100 mL by adding the 0.02 N $CuSO_4.5H_2O$ solution. This procedure yielded a series of standard solutions with NO_3-N concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg/kg. These meticulously prepared solutions provided the calibration standards required for the precise and dependable measurement of NO_3-N levels in the samples.

Procedure

10g of air-dried soil with a particle size of 2mm was carefully weighed and placed in a flask for this process. 50 mL of 0.02 N $CuSO_4.5H_2O$ solution was added to the flask. After that, the mixture was violently shaken for 15 minutes. The suspension was then filtered through a filter paper to produce a clean filtrate.

Following that, 3 mL of the filtrate was carefully pipetted into a 50 mL conical flask, which was then chilled briefly in cold water. Following that, 1mL of a 0.1% chromotropic acid solution was dropped into the solution without being mixed, followed by another cooling period in cold water. After gently mixing the flask, 6 mL of concentrated H_2SO_4 was added along the inner wall of the flask without mixing. All the samples were treated with acid, and

the flask was swirled to ensure full mixing. After about 45 minutes of cooling at normal temperature, the flask developed a yellow colour.

To create a standard curve, 3 mL of each standard solution containing 0.5 to 3.5 mg/kg of NO3-N was pipetted into separate containers using the same process as for the samples. A blank solution was also made by pipetting 3 mL of the 0.02 N $CuSO_4.5H_2O$ solution into a container and repeating the steps used for the samples. After 45 minutes, the absorbance of the blank, standards, and samples was measured with a spectrophotometer at 430 nm. The absorbance measurements were plotted against the matching NO_3-N concentrations in the standards to create a calibration curve. The concentration of NO_3-N in the unknown samples might be calculated by reference to this calibration curve.

2.8.5. Quantification of Phosphates in Soil

This method is based on a complexation reaction, which leads to the creation of a coloured complex between molybdate and phosphorus. When phosphate from a soil sample is heated in the presence of ammonium molybdate, acid, and excess ascorbate ions, this reaction happens. The ascorbate ions serve to avoid colour deterioration as the molybdate progressively oxidises. The intensity of the coloured complex generated is related to the initial phosphate content in the sample. The protocol followed for this analysis was retrieved online from University of Canterbury's website (University of Canterbury, n.d.).

To calculate the phosphate concentration in the soil sample, the blue colour produced is compared to established standards of phosphate that have experienced the same reaction with the molybdate reagent. The content of phosphate in the soil can be properly measured by creating this comparison.

Sample Preparation

To begin the soil analysis process, a properly collected sample of soil was heated overnight at around 50°C to guarantee thorough drying. To prevent dust from being inhaled, the dried dirt was covered with a lid.

A total of 50 mL of water was added to a 250 mL volumetric flask. 0.75 g of ammonium sulphate was carefully added and dissolved. Following that, 5 mL of concentrated sulfuric acid was progressively added to the flask, causing the solution to generate heat. The mixture was allowed to cool before being diluted with distilled water to the volume specified by the flask mark. The previously dried soil was then combined with 200 mL of the produced sulfuric acid

and ammonium sulphate mixture in a plastic flask. To facilitate the reaction, the flask was shaken intermittently for 30 minutes.

The soil sample was filtered through fine filter paper and left aside after shaking. The resultant filtrate should be clear; however, it may have a slight brown hue. This stage allowed the desired components to be separated and any solid particles from the sample to be removed.

Preparation of Standard

The following process was used to prepare standard phosphate solutions. To begin, a 300 mg/L solution was made by precisely weighing 0.220 g of solid KH_2PO_4 and dissolving it in a 500 mL volumetric flask. To bring the solution up to standard, distilled water was added. Aliquots of the standard phosphate solution were then pipetted into volumetric flasks of various diameters. 10 mL of the solution was pipetted into volumetric flasks of 200 mL, 250 mL, 500 mL, and 1 L. The flasks were then filled to the specified level with distilled water. Phosphate solutions with concentrations of 15 mg/kg, 12 mg/kg, 6 mg/kg, and 3 mg/kg were produced as a result of this method. In addition, 4.5 mg/kg solution was prepared by pipetting 15 mL of the standard solution into a 1 L volumetric flask. Each solution was meticulously labelled with its concentration and the date it was created to ensure accurate identification and tracking.

Preparation of Complex

The following process was used to prepare the necessary reagents for the phosphate analysis. 5 g of ammonium molybdate was first dissolved in 100 mL of water. This solution was then transferred to a volumetric flask of 500 mL. 160 mL of concentrated sulfuric acid was progressively added to this solution, with caution due to the heat generated during the reaction (please see safety considerations). If the flask became too hot, the acid addition was stopped and allowed to cool for around 15 minutes. After adding all of the acid, the solution was diluted to 500 mL with water, which was added gently while stirring.

A volume of 10 mL was taken in a 150 mL conical flask for sample analysis. This was mixed with 20 mL of water, 2 mL of molybdate solution, and a little amount of ascorbic acid crystals. The mixture was then steadily heated until it reached boiling point, at which point the reaction was expected to produce a rich blue/green colour. The flask was allowed to cool after boiling. This was done for each of the typical solutions.

Colorimetric Analysis

Following that, a sample tube was filled with the solution with the lowest concentration (3 mg/kg) obtained from the prepared standards, and an absorbance reading was taken. The tube was cleansed after recording the absorbance to guarantee reliable results for following samples. This procedure was performed for each of the standards, starting with the least concentrated and working up to the most concentrated.

Finally, the sample was placed in a colorimetric tube, and an absorbance reading was collected and recorded. This absorbance measurement revealed important information about the concentration of the target component in the sample. Using the colorimeter and this systematic methodology, absorbance values for both the standards and the sample were collected, allowing quantification of the target substance based on the established calibration curve.

2.8.6. Organic Matter and Total Organic Carbon

Organic content determination in soils is an important test that offers information on the quantity of organic matter in relation to dry soil solids. The steps below were taken to carry out this test (Gowda, n.d.).

Procedure

To begin, an empty, clean, and dry crucible was chosen, and its mass was meticulously measured and recorded as MP. The porcelain dish was then filled with 5g of the oven-dried test specimen produced from the moisture content experiment. The total mass of the dish and soil samples was calculated and reported as MPDS.

The crucible containing the soil specimen was then placed in a muffle furnace, and the ramping temperature was set to 7°C. The crucible was kept in the furnace for 2 hours at 800°C to allow the organic matter in the soil to decompose.

When the heating process was finished, the porcelain dish was carefully removed from the furnace using tongs. After that, the dish was allowed to cool to room temperature. The mass of the ash-filled dish, which represented the burned soil, was measured and recorded as MPA.

Finally, the dish was properly cleaned and emptied in preparation for future testing or investigations. By effectively eliminating organic matter by combustion and quantifying the leftover ash, these methods ensured the precise estimation of organic matter and total organic carbon in the soil.

Calculation

1. Mass of dry soil (MD) = MPDS - MP

2. Mass of the ashed soil (MA) = MPA - MP
3. Mass of Organic Matter (MO) = MD - MA
4. Percentage of Organic Matter (OM) = (MO/MD)*100

2.8.7. Gravimetric Analysis

An improved gravimetric analysis was followed for determination of TPH degradation in soil (Villalobos et al., 2008).

Sample Preparation

The soil samples were initially sieved through 2mm sieves. The sieved samples were then dried for 12 hours at 105°C. To guarantee homogeneity, the soil samples were mechanically homogenized for many hours after drying. Subsamples of 10 grams were then accurately weighed and put into round flasks that had been previously dried (at 105°C). To make a free-flowing powder, 10 grams of anhydrous Na_2SO_4 were added to each flask.

TPH Extraction and Quantification

The soil samples were then extracted using n-hexane in an ultrasonic bath. The settings of the ultrasonic bath were tuned to achieve optimum extraction efficiency. The extraction process required a total of 35 mL of hexane. The extracted solutions were then passed through a column filled with n-hexane treated cotton and filter paper. The column was washed with an additional 25 mL of hexane, yielding a final liquid extract volume of 60 mL for further analysis. To concentrate the extract the flasks were left overnight for complete evaporation of n-hexane. Finally, the evaporation residues were weighed with an analytical scale and labeled as Total Petroleum Hydrocarbons (TPH), which served as a measure of the hydrocarbon content in the soil samples (Villalobos et al., 2008).

2.8.8. Cadmium Analysis

Certain heavy metals, particularly Cd, represent considerable environmental hazards in agricultural settings. Wet oxidation is a method used to examine the release of mineral components from soil and sediments. Wet oxidation uses oxidizing acids such as a di-acid mixture of HNO_3-$HClO_4$. The di-acid oxidation process is popular because it is simple, efficient, and time saving. It is crucial to note, however, that di-acid digestion is not a complete or total digestion process because it does not totally dissolve all soil components, particularly silicate minerals. As a result, di-acid digestion is also known as faux digestion or incomplete digestion (Sommer, 2017).

Sample Preparation

To begin the analysis, 0.5 g of air-dried soil was precisely placed into a 250 mL flask. Following that, 3 mL of concentrated HNO_3 was added to it in a fume hood while taking necessary safety precautions, and the contents were gently swirled. The flasks were then placed one by one on a heated plate, with a glass funnel inserted into the neck of each flask to aid in the process. The temperature of the hot plate was gradually increased to around 145 °C, and the samples were digested for 1 hour. Each flask received 4 mL of concentrated $HClO_4$, and the temperature was then elevated to 240 °C for an additional hour of digestion.

To ensure safety, the process was handled with care throughout. When the digestion process was finished, the flasks were removed from the hot plate and set aside to cool to room temperature. The contents of the flasks were filtered through double filter paper after cooling, and the filtrate was adjusted to a volume of 15 mL.

Blank Preparation

To ensure the accuracy of the analysis, each batch of samples had at least one reagent blank (free of soil). This functioned as a check to see whether there was any potential contamination or influence.

Atomic Absorption Spectrophotometry

An Atomic Absorption Spectrophotometer was used to determine Cd amounts. This apparatus measures the absorbance of light at certain wavelengths precisely, allowing quantification of the target constituents in the sample.

2.9.Post-Harvest Plant Analysis

Following plant analysis were conducted post harvesting phase:

2.9.1. Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoid Contents

To generate a homogenous leaf extract, 40 mg of fresh leaf samples were immersed in roughly 2 ml of an 80% acetone solution to prepare the extract needed to evaluate the concentration of chlorophyll and carotenoids. The extract was then centrifuged for 5 minutes at 5000 rpm. The supernatant that resulted was carefully preserved in a new clean falcon tube. The pellet was then vortexed with 1 ml of 80% (v/v) acetone in water and centrifuged at 5000 rpm for 5 minutes. For further examination, the freshly obtained supernatant was mixed with the previously collected supernatant. After measuring absorbance (A) values at various wavelengths, i.e., 663 and 645 and 470 nm, the formulae proposed by Lichtenthaler (1987) for

determining photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were used.

2.9.2. Quantification of Lipid Peroxidation

Malondialdehyde (MDA) content of the sample was used for the quantitative determination of lipid peroxidation following protocol by Senthilkumar et al. (2020). 0.1g of fresh leaf sample was macerated to obtain homogeneous mixture in pre-chilled 1ml 5% of TCA (w/w) in an ice bath. After that homogenized leaf samples were subjected to centrifugation for 10 minutes at 10,000 rpm and the resulting supernatant was mixed with TBA solution (0.67%) at the ratio of 1:1. The resulting mixture was heated for 30 minutes at 95°C and immediately placed in an ice bath for 1 minute after heating. Chilled mixture will then be centrifuged at 10,000 rpm for 10 minutes. The absorbance (A) of developed samples was noted at 450, 532, and at 600 nm wavelengths and the total lipid peroxidation value was presented in µM of malondialdehyde g $^{-1}$ of FW.

2.9.3. Hydrogen Peroxide Production Quantification

Estimation was performed for the formation of the ROS specifically for determining the hydrogen peroxide content by following the same protocol that was used by Khan et al. (2019), with some amendments in the preparation of leaf extract, which was prepared by the method presented by Venkatachalam et al. (2017). 0.1g of leaf sample (fresh) was macerated accurately using pre-chilled extraction buffer (pH 7.4) 1ml, composed of 0.5 mM EDTA with 50 mM potassium phosphate buffer (PPB), and was then be centrifuged for 15 min at 10,000rpm, at 4°C. That resulted in a supernatant of sample, was used as leaf extract in the determination of H2O2 content. To prevent deterioration of the prepared sample, it was kept at 4°C. The reaction mixture for H2O2 content was prepared mixing 40 µl leaf extract with 1ml of 0.05 mM PPB (pH 6.5), and 352.8 µl of 1% $Ti(SO_4)_2$ in 20% H_2SO_4 (*v/v*), subjected to centrifugation for 15 min at 6000rpm. The resulting supernatant was obtained to measure the yellow color intensity, quantified earlier by measuring the absorbance at 410 nm wavelength. By using the molar extinction coefficient (ε) of 0.28 μM^{-1} cm^{-1}, H2O2 content was expressed as µM H2O2 contents g^{-1} of FW.

2.9.4. Plants Enzymatic Activities

The following section introduces a method to quantify enzyme activity. Leaf extract prepared by the method that was performed for H2O2 activity. 1 ml of pre-chilled extraction buffer (pH

7.4) consisting of about 50 mM potassium phosphate (PPB) and 0.5 mM EDTA was used to macerate leaf samples (0.1 g fresh samples) and centrifuged at 10,000 rpm at 4°C for 15 minutes. The obtained supernatant of the homogenized sample was then collected and used as a leaf extract to quantify the enzymatic activity and stored at 4°C to avoid deterioration of the prepared sample. The value was expressed in units of g^{-1} of FW for all enzyme activities of the sample.

2.9.4.1.Catalase Activity

The catalase activity (CAT) was measured according to the protocol of Maehly and Chance (1954), and the reduction of H2O2 was quantified by monitoring A240 after 1 minute. The reaction mixture consisted of 2.5 ml reaction buffer (50 mM PPB, pH 7.4), with 100 µl 1% H2O2, and 50 µl leaf extract (partially diluted to maintain observations within the linear range of the analysis). Activity of catalase was determined by the ε value of 39.4 mM^{-1} cm^{1}.

2.9.4.2.Guaiacol Peroxidase Activity

The method of Upadhyay et al. (2019) to quantify the activity of guaiacol peroxidase (GPX) will be applied. The reaction mixture will be prepared by mixing 20µl of leaf extract with 2.5ml reaction buffer (50 mM PPB, pH 6.1), 1 ml 1% Guaiacol and 1 ml 1% H2O2. A420 was examined for 1 minute to determine the changes. The activity was calculated, using ε equal to 26.6 mM^{-1} cm^{-1}.

2.9.4.3.Ascorbate Peroxidase Activity

The reaction mixture with a volume of 1 ml consisting of a 50 mM phosphate buffer pH (7.0) (containing 0.1 mM EDTA, 0.5 mM ascorbate, 1.54 mM H2O2) and 50 µl enzyme extract. The decrease in absorbance at 290 nm (ε = 2.8 mM^{-1} cm^{-1}) was accompanied by the oxidation of ascorbate. These values are expressed in units of g^{-1} for the weight of the new sample.

2.9.4.4.Calculation for APX, CAT, and GPX

The concentration of enzyme unit were calculated by using Beer's law, given below:

$$C\ (Units\ ml^{-1}) = A / \varepsilon.L$$

Where, C = concentration, A = Absorbance, ε = Molar extinction coefficient, and L = Length of cuvette (1 cm)

Then for each, expressing the values for gram of fresh weight C is multiplied with DF:

$$C\ (Units\ g^{-1}) = (C) \times (W/1000) \times B$$

Where, C = Concentration derived from Beer's Law, V = µl of enzyme extract used for assay, and W = plant sample per ml of extraction buffer (0.1 g per ml of extraction buffer).

2.9.5. Soil Bacterial Analysis - Colony Forming Units

For all treatments containing bacterial inoculum, bacterial colony forming units (CFU) were measured, and aggregated bacterial strains' survivability in soil samples that could survive heavy metals was evaluated (*Colony-forming Unit (CFU)*, n.d.; Sieuwerts et al., 2008). Plate counting was used to collect bacterial isolates. Soil suspensions were made by combining 9 ml of 0.1% (w/v) sterile saline solution with 1 ml of the previous dilution of 0.9 N saline solution of NaCl (10 grams of soil in 90 ml of normal saline). 100 µl of diluent ranging from 10^{-1} to 10^{-4} was put onto a nutrient agar plate containing 50 mg.kg cadmium and 0.55 TPH. Following that, the plates were incubated at 30°C for 24 hours, and the colonies on each plate were counted. The CFU/ml value is calculated by multiplying the number of visible colonies (CFU) on an agar plate by the dilution factor.

2.10. Statistical Analysis

The results from soil, plant, and bacterial studies were statistically analyzed using Microsoft Excel and SPSS software. Several statistical tests were used, including mean and standard deviation calculations, as well as correlation and regression analysis. Furthermore, the analysis included the use of the one-way and two-way analysis of variance (ANOVA) test based on Tuckey's model to detect the presence of any significant differences between the result sets. A significance level of 0.05 was used for validation purposes.

Chapter 3

Results

3.1.Properties of Fresh Soil, Compost, and Biochar

A series of analysis were conducted to determine the physicochemical properties of fresh soil and compost prior to the experiment. The analysis involved the determination of pH, EC, TDS, nitrates, phosphates, organic matter, total organic carbon, and soil texture (**Table 5**). Properties of biochar, reported previously in a study, are also mentioned in **Table 5**.

Table 5: Physicochemical properties of fresh soil, compost, and biochar used for the experiment.

Physicochemical Properties	Fresh soil	Compost	Biochar
Textural Class	Silty clay	-	-
pH	7.05	9.5	8.77 ± 0.08
EC (µS/cm)	250	1784 ± 23	1670 ± 70
TDS (mg/kg)	160	1140 ± 14.7	1070 ± 44.8
OM (%)	17	72	19.3
TOC (%)	10	42	11.2
Nitrates (mg/kg)	38.6	30	-
Phosphates (mg/kg)	15	N/D	-
Nitrogen (%)	-	-	0.65
Cadmium (mg/kg)	0.02	-	-
Total Petroleum Hydrocarbons (%)	N/D	-	-

3.2.Post-Harvest Analysis of Phase 1 – Phytoextraction

3.2.1. Physicochemical Properties of Soil

In this study, we looked at how different treatments affected the levels of pH, electrical conductivity (EC), total dissolved solids (TDS), organic matter (OM), total organic carbon (TOC), nitrates, and phosphates, in soil.

Table **6** shows the physicochemical characteristics of soil in phase 1, together with the standard deviations that indicate the variability of the results. The primary goal was to identify any significant differences between the treatment and control groups. IBM SPSS 10.1 was used to perform the Analysis of Variance (ANOVA) test.

The treatments were compared to their respective controls to see how they affected **soil pH**. The results of one-way ANOVA reveal that the means of pH for all the treatment are significantly different (P = 0.0001). However, P value of Brown-Forsythe test is very large i.e, 0.7604 suggesting that the variability among treatments (difference between standard deviations) is not significant. Thus, different treatments had no significant impact on pH value. The variability in mean values observed was in the range of 7.83 to 7.93 pH.

Table 6 illustrates the changes in electrical conductivity and total dissolved solids of different treatments due to application of amendments. The **EC** and **TDS** of C4 was observed to be 326 µS/cm and 208 mg/kg, which significantly increased to 381 µ S/cm and 243 mg/kg in T2 after the addition of compost. However, in T3 with the combined amendment, the EC of the soil was 302.6 µS/cm, whereas TDS was 178 mg/kg. A different trend was observed in control and treatment with *L. multiflorum*, where biotic controls of spiked soil, i.e., C5, showed EC of 201 µS/cm. In contrast, treatments with bacterial inoculum (T4), compost (T5), and combined amendment (T6) showed declining EC values. Similarly, in control and treatment with *L. multiflorum*, incorporating compost in T5 showed a slight increase in the TDS value compared to T4 with bacterial inoculation only. However, the overall trend that was observed in both plant species was that adding compost and inoculum reduced the TDS values, with the greatest decrease observed in combined treatments. T6 displayed the best results with an average TDS of 81.35 mg/kg. These P value for these results was calculated to be 0.0001 showing a significant difference among means of treatments.

It was observed that addition of compost to soil (T2 & T5) resulted in a significant rise in both **OM** and **TOC** as compared to the application of combined amendment (T3 & T6), with respect to their spiked soil controls (C4 and C5). Thus, adding compost only as an amendment showed the highest increase in both, organic matter content and total organic carbon, whereas inoculating bacteria with compost showed the second highest increase in these values. On the other hand, addition of bacteria observed to increase the concentration of **nitrates** and **phosphates** in soil with highest concentration of nitrates and phosphates in T4 and T6, respectively, with the P value of 0.0001 **(Table 6)**. Mei et al. (2021) in his study categorized

B. safencis as phosphate solubilizing bacteria. Moreover, addition of compost to soil also provides added nitrogen and phosphorus. Therefore, the peaks observed can be attributed to the use of compost and phosphate solubilizing PGPR bacteria.

Table 6:Physico-chemical characteristics of soil in Phase 1.

Treatment	Description	pH	EC (µS/cm)	TDS (mg/kg)	OM (%)	TOC (%)	Nitrates (mg/kg)	Phosphates (mg/kg)
C1	**Spiked soil**	7.855±0.024^{bc}	394.5±2.5^{a}	252±0.7^{a}	18±0.5^{f}	10.4±0.2^{f}	16.14±0.2^{f}	2.95±0.1^{j}
C2	**Fresh soil + *C. didymus***	7.85±0.01^{bc}	278.5±0.5^{f}	138±0.2^{g}	15±0.2^{h}	8.7±0.2^{h}	23±0.4^{d}	9.695±0.03^{g}
C3	**Fresh soil + *L. multiflorum***	7.935±0.015^{a}	216±0^{g}	128±0.2^{h}	17±0.2^{g}	9.9±0.2^{g}	17.71±0.4^{e}	8.5±0.05^{h}
C4	**Spiked soil + *C. didymus***	7.875±0.025^{b}	326±1^{c}	208±0.2^{c}	19±0.3^{e}	11±0.2^{e}	25.5±0.6^{c}	1.31±0.03^{k}
C5	**Spiked soil + *L. multiflorum***	7.86±0.01^{bc}	201±2^{h}	193±0.5^{e}	18±0.5^{f}	10.4±0.2^{f}	8.07±0.4^{h}	3.445±0.02^{i}
T1	**Spiked soil + *C. didymus* + *B. safencis***	7.835±0.004^{c}	315.5±0.5^{d}	202±0.4^{d}	14±0.3^{i}	8.1±0.2^{i}	32.14±0.5^{b}	20.81±0.01^{b}
T2	**Spiked soil + Compost + *C. didymus***	7.91±0.009^{a}	381±1^{b}	243±0.2^{b}	33±0.5^{b}	19.1±0.2^{b}	14.78±0.3^{g}	18.895±0^{d}
T3	**Spiked soil + Compost + *C. didymus* + *B. safencis***	7.865±0.015^{b}	302.6±1.4^{e}	178±0.3^{f}	28±0.5^{c}	16.2±0.2^{c}	15.78±0.4^{f}	20.545±0^{c}
T4	**Spiked soil + *L. multiflorum* + *B. safencis***	7.915±0.014^{a}	181.6±1.4^{j}	116±0.3^{j}	12±0.3^{j}	7±0.2^{j}	35.35±0.4^{a}	15.15±0.15^{e}
T5	**Spiked soil + Compost + *L. multiflorum***	7.86±0.01^{bc}	186.7±1.3^{i}	119±0.1^{i}	38±0.2^{a}	22±0.2^{a}	17.6±0.4^{e}	10.23±0.01^{f}
T6	**Spiked soil + Compost + *L. multiflorum* + *B. safencis***	7.925±0.015^{a}	127±2^{k}	81.35±0.4^{k}	22±0.2^{d}	12.8±0.2^{d}	17.07±0.3^{e}	24.75±0.35^{a}

Note: Data was presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means. Similar small letter in same columns is non-significant.

3.2.2. Effect of Amendments on the Physiological Response of Plants during Remediation of Cd and TPHs through Phytoextraction

Table 7 show the results of a Phase 1 inquiry on the effects of various treatments on plant development. We identified patterns in different physical plant parameters, such as, shoot and root length (cm), their fresh weights (g), and dry weights (g) with different treatments, highlighting occasions where significant departures from their biotic controls occurred.

Fresh soil control, C2 with P1, demonstrated significant plant development under no stress, with a shoot length of 17 cm and root length of 8 cm. However, other parameters like fresh and dry weights of roots and shoots were significantly low as mentioned in **Table 7** with no significant improvement due to any of the amendments. This shows that 121 mg/kg Cd and 2% TPH stress may have a deleterious impact on *C. didymus* growth, despite being reported as a hyperaccumulator of Cd (Sidhu et al., 2017). *L. multiflorum* (P2) performed very well under the stress of co-contamination showcasing significant improvement in all physical parameters (Rasool et al., 2021). T6 showed the highest values compared to its spiked control (C5) i.e., 48 cm shoot length, 31 cm root length, 2.5 g shoot fresh weight, 5.1 g root fresh weight, 1.1 g shoot dry weight, and 3.7 g root dry weight **(Table 7)**.

Table 7: Effect of different treatment on growth parameters of plants in Phase 1.

Treatment	Description	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
C2	**Fresh soil + *C. didymus***	17±1.7^{d}	0.2±0.2^{d}	N/M	8±0.5^{g}	0.4±0.05^{f}	0.1±0.1^{e}
C3	**Fresh soil + *L. multiflorum***	36±3.5^{b}	2.3±0.8^{a}	1±0.2^{ab}	22±0.7^{c}	2.5±0.3^{c}	0.3±0.1^{e}
C4	**Spiked soil + *C. didymus***	10.5±2.8^{e}	0.1±0^{d}	N/M	4±0.5^{hi}	N/M	N/M
C5	**Spiked soil + *L. multiflorum***	27±3^{c}	0.7±0.3^{cd}	0.3±0.1^{a}	14.3±0.6^{d}	1.5±0.1^{e}	0.8±0.3^{d}
T1	**Spiked soil + *C. didymus* + *B. safencis***	16±0.8^{de}	0.1±0.1^{d}	N/M	3.5±0.7^{i}	N/M	N/M
T2	**Spiked soil + Compost + *C. didymus***	13.5±5.6^{de}	0.1±0.1^{d}	N/M	5±0.5^{h}	N/M	N/M
T3	**Spiked soil + Compost + *C. didymus* + *B. safencis***	15±4.3^{de}	0.1±0^{d}	N/M	10.5±0.7^{f}	N/M	N/M
T4	**Spiked soil + *L. multiflorum* + *B. safencis***	44±0.4^{a}	1.5±0.4^{b}	0.8±0.2^{ab}	25±0.9^{b}	2±0.2^{d}	1.4±0.1^{c}
T5	**Spiked soil + Compost + *L. multiflorum***	38±2.1^{b}	1.3±0.4^{bc}	0.7±0.3^{b}	12±0.7^{e}	3.2±0.3^{b}	2±0.2^{b}
T6	**Spiked soil + Compost + *L. multiflorum* + *B. safencis***	48±3.7^{a}	2.5±0.3^{a}	1.1±0.2^{a}	31±0.8^{a}	5.1±0.4^{a}	3.7±0.5$_{a}$

Note: N/M represents "non-measurable" parameter due to lack of biomass detectable above 0.001 g. Data was presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means. Similar small letter in same columns is non-significant.

This table provides a thorough examination of the harvested plants, including physical characteristics such as shoot length, root length, and the fresh and dry weights of both shoots and roots for each treatment in phase 1.

3.2.3. Effect of Cd and TPHs Co-contamination on Pigment Content of Plants

The results of the two-way ANOVA revealed that co-contamination had a significant impact on changing pigment concentrations in plants, which include, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids with P value 0.0001. Moreover, changes in the concentration of pigments is independent of the type of pigment but depends upon the type of treatment being studied (P = 0.0001).

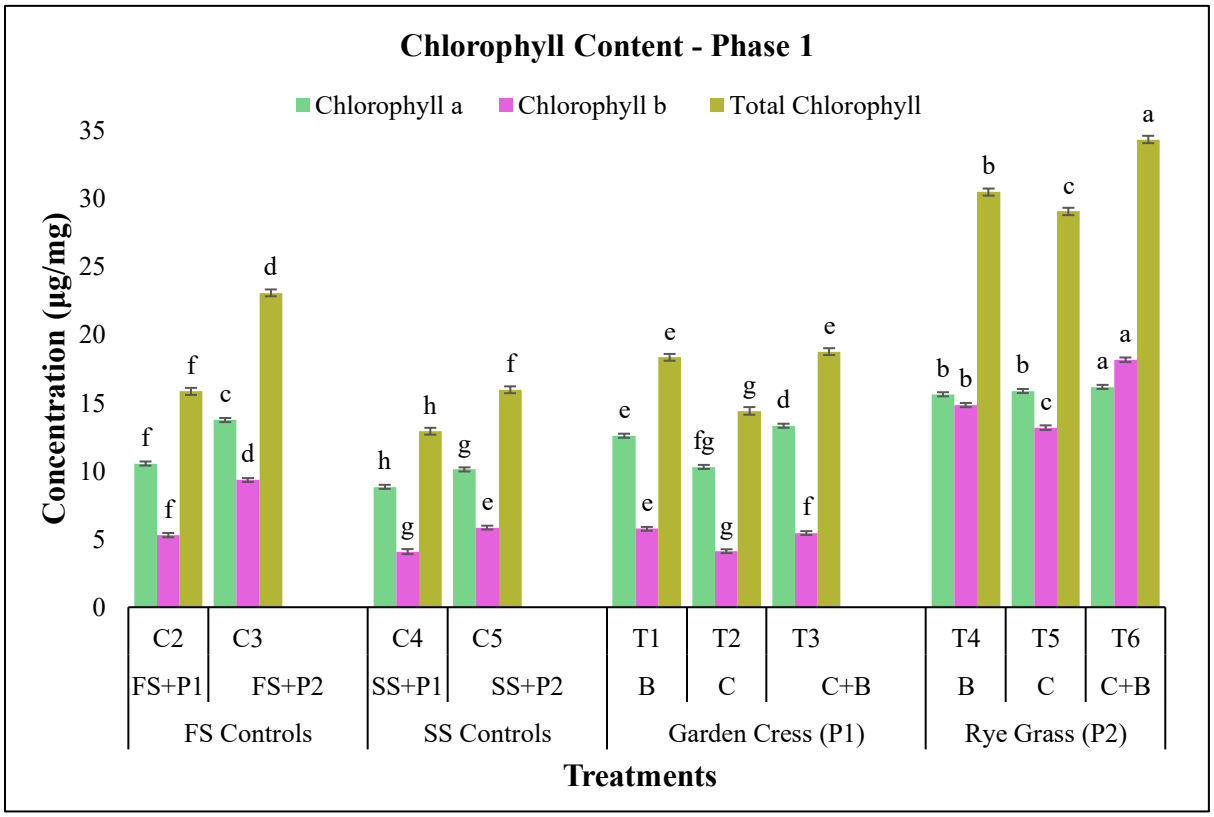

Figure 2: Changes in pigments' concentrations across various treatments in Phase 1.

Figure 2 and **Figure 3** show leaf pigments depicting the effect of co-contamination on *C. didymus* (P1), as well as its effect on chlorophyll levels across multiple treatments. When compared to the spiked biotic control (C4), the treatments improved significantly, with T3 being more effective than T1 > T2 > C4. However, in the case of T2, the increase in pigment concentration was not judged statistically significant, as evidenced by a P-value of 0.6. In treatments with *L. multiflorum,* the addition of amendments in T4, T5, and T6 increased the pigment contents in subsequent treatments.

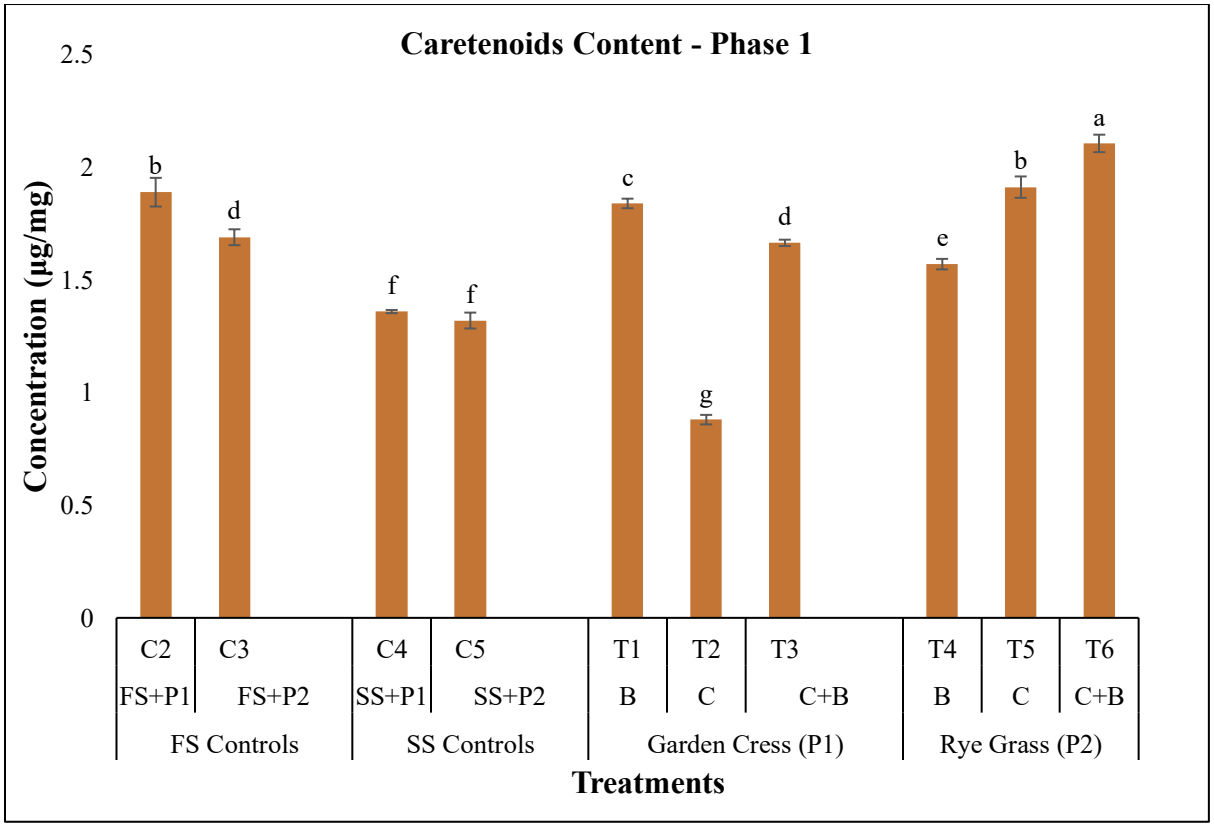

Figure 3: Carotenoid concentration in Phase 1.

T6 showed the highest concentrations of chlorophyll a, b, total chlorophyll, and carotenoids whereas C5 had the lowest concentrations due to co-contamination (Rasool et al., 2021). The difference in chlorophyl content between *L. multiflorum* and *C. didymus* can be seen in the **Figure 2**.

3.2.4. Quantification of Stress Markers in Plants under the Stress of Co-contamination

Figure 4 shows the levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) produced in response to different treatments.

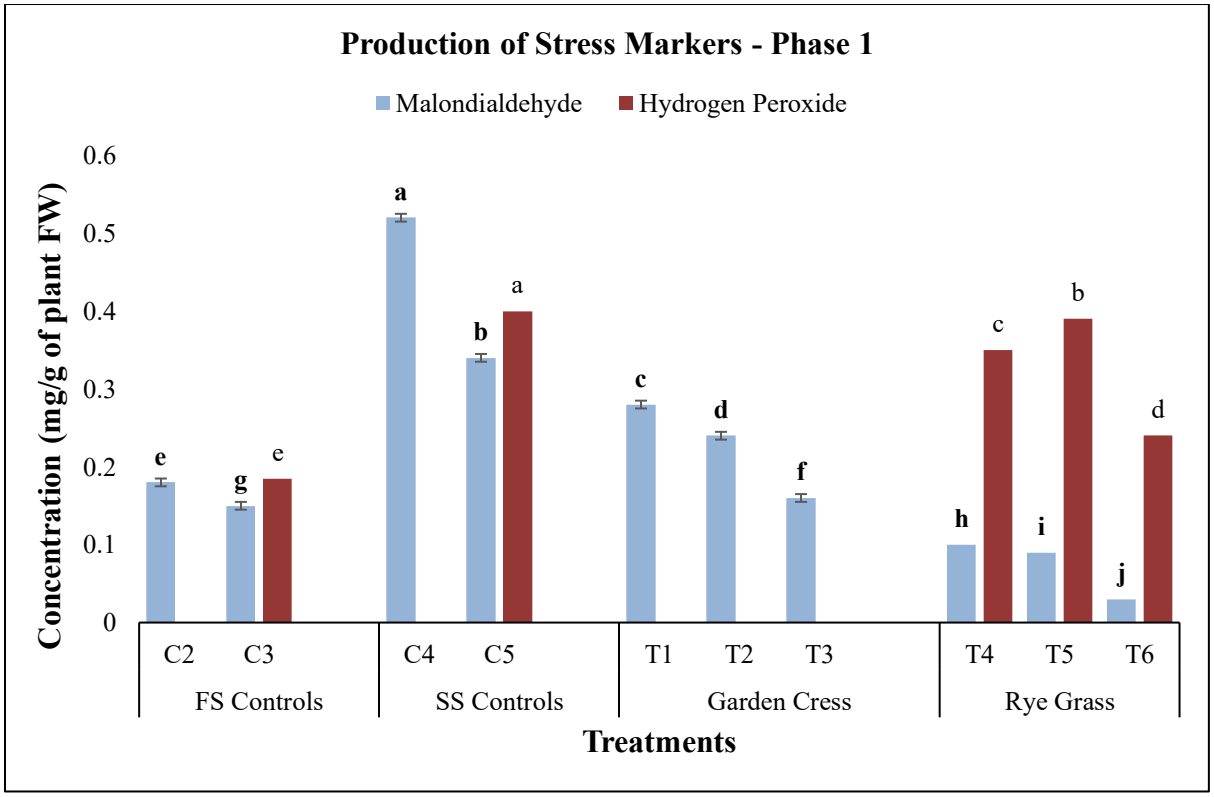

Figure 4: Production of malondialdehyde and hydrogen peroxide in plants with different amendments in Phase 1.

MDA levels in C4 increased significantly, indicating oxidative stress in the plants because of the elevated contaminated conditions of the soil. MDA levels in T2 remained relatively low, indicating that oxidative stress may have been reduced. For control with *L. multiflorum* (C5), MDA and H_2O_2 levels were highest, indicating oxidative damage. *B. safencis* considerably lowered MDA and H_2O_2 levels in T4, and in T6 when combined with compost, indicating a potential antioxidative action. It is worth mentioning that further plant analysis including quantification of H_2O_2 production could be done for *C. didymus* because the plant did not survive under such high level of co-contamination as evident by the higher production of MDA in all treatments with garden cress.

3.2.5. Effect of Amendments on Production of Antioxidant Enzymes in Plants in Response to Stress

Production of catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) was only calculated for treatments with *L. multiflorum* **(Figure 5)**.

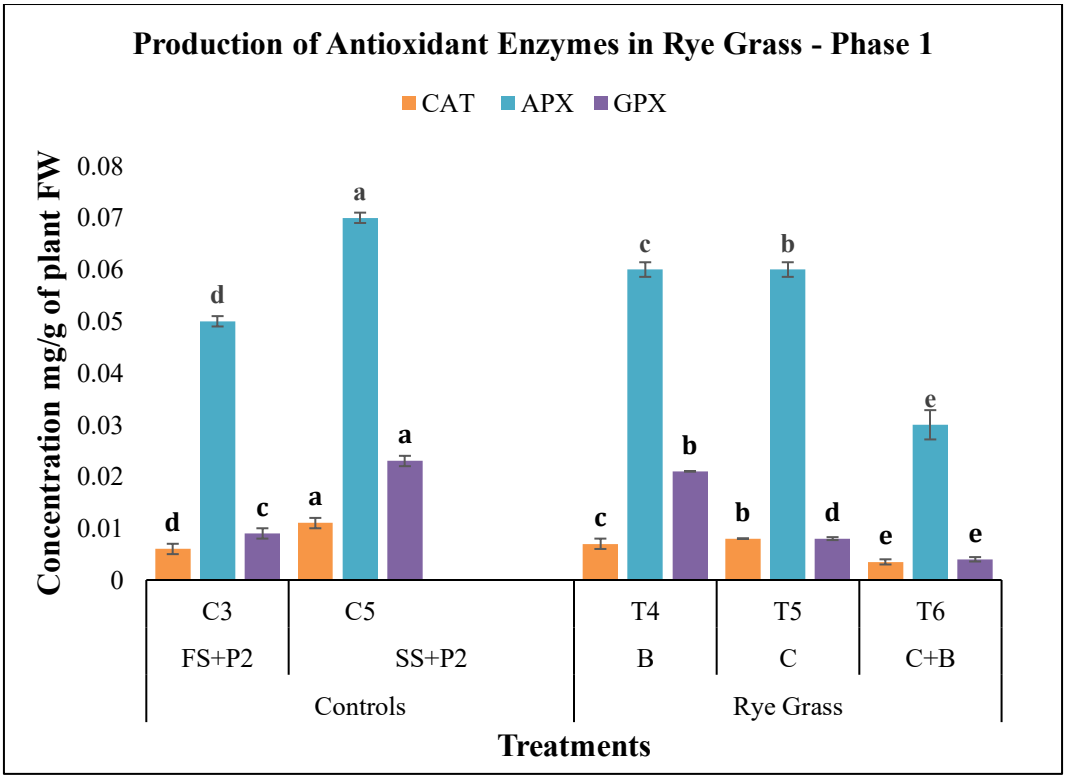

Figure 5: Production of catalase (CAT), ascorbates peroxidase (APX), and glutathione peroxidase (GPX) in plants with different amendments in Phase 1.

The levels of these enzymes were greater in C5, showing an improved antioxidative response to spiked soil conditions. A decrease in enzyme synthesis in T4 shows that *B. safencis* may have a role in strengthening antioxidative defense systems. The increasing APX levels in T5 may indicate greater tolerance to oxidative stress, presumably because of the compost addition. A considerable drop in CAT and APX levels in T6 may indicate a combination of the antioxidative actions of compost and *B. safencis*.

3.2.6. Removal of Cadmium and Total Petroleum Hydrocarbons from Soil

The purpose of this study was to evaluate the efficacy of various treatments for lowering Cd and TPH contents in soil **Table 8**. *Coronopus didymus,* although reported to be a hyperaccumulator of Cd loading in soil of upto 300 mg/kg, did not survive the co-contamination of 121 mg/kg of Cd and 2% TPH in soil. However, *Lolium multiflorum* showed promising results with 30% removal of Cd in biotic control of spiked soil **Figure 6**.

Table 8: Cadmium and Total Petroleum Hydrocarbons concentration in soil after Phase 1.

Treatment	Description	Cadmium in soil (mg/kg)	TPH in soil (%)
C1	**Spiked soil**	110.4±0.05[a]	1.885±0.08[a]
C2	**Fresh soil + *C. didymus***	N/D	N/D
C3	**Fresh soil + *L. multiflorum***	N/D	N/D
C4	**Spiked soil + *C. didymus***	108.55±0.07[b]	1.74±0.18[ab]
C5	**Spiked soil + *L. multiflorum***	75.9±0.14[f]	1.76±0.04[ab]
T1	**Spiked soil + *C. didymus* + *B. safencis***	94.7±0.1[d]	1.72±0.15[ab]
T2	**Spiked soil + Compost + *C. didymus***	98.2±0.1[c]	1.74±0.07[ab]
T3	**Spiked soil + Compost + *C. didymus* + *B. safencis***	88±0.15[e]	1.64±0.16[b]
T4	**Spiked soil + *L. multiflorum* + *B. safencis***	54.1±0.05[g]	1.66±0.13[b]
T5	**Spiked soil + Compost + *L. multiflorum***	51.6±0.1[h]	1.68±0.04[b]
T6	**Spiked soil + Compost + *L. multiflorum* + *B. safencis***	44±0.1[i]	1.14±0.1[c]

Note: Data was presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means. Similar small letter in same columns is non-significant.

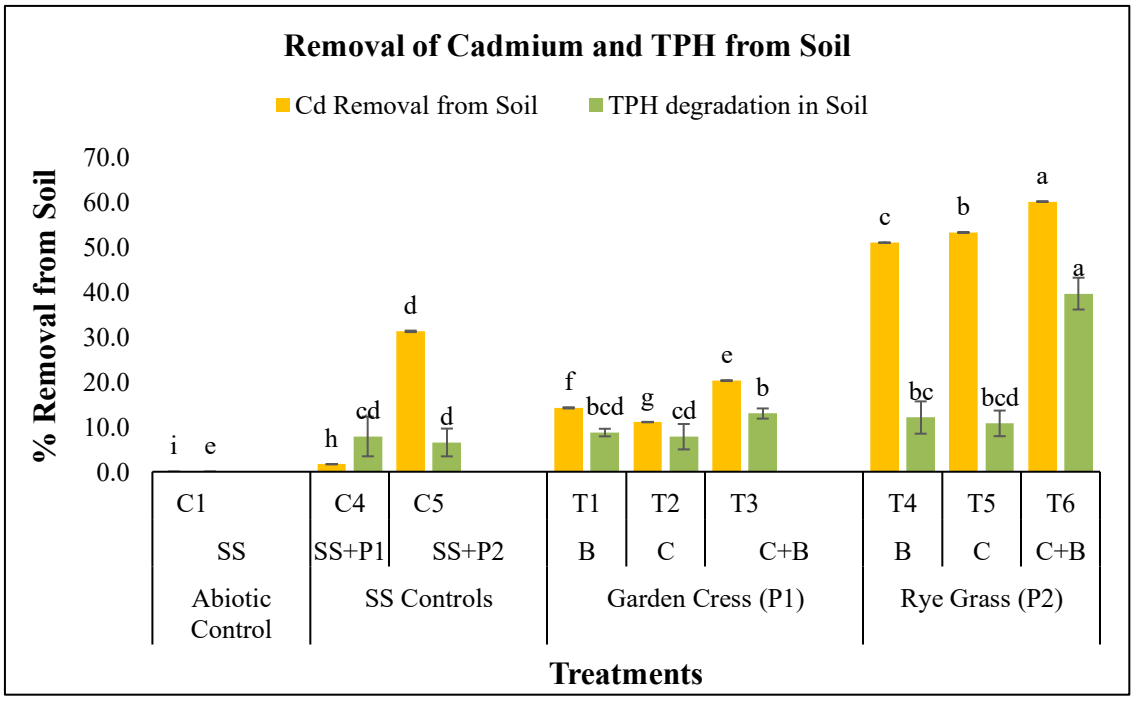

Figure 6: Percentage elimination of Cd and TPH from soil in Phase 1.

T6 with the combined amendment of compost with *B. safencis* showed significant increase in Cd removal of upto 59%, as also reported by Mushtaq et al. (2020) in his study of remediating multi metal contaminated soils, including Cd. T5 and T4 showed 52% and 50% removal efficiency, respectively, with no significant difference among these two treatments. While comparing the results with respect to differences in plant species, *L. multiflorum* displayed its immense potential as a hyperaccumulator. Significant TPH degradation was only observed in T6 with combined amendments, whereas, in all other treatments no considerable impact was observed concluding the contamination in soil was too high for *B. safencis* to survive alone without added nutrition from the compost. Thus, adding 10% compost with *B. safencis* shows moderate potential to TPH degradation in soil (Escobar-Alvarado et al., 2015; Hussain et al., 2018).

3.2.7. Effect of Amendments on Plants' Phytoextraction Ability

Figure 7 illustrate the removal of Cd by *L. multiflorum* through phytoextraction. C5 exhibits moderate Cd accumulation in roots but comparatively modest accumulation in shoots, indicating some phytoextraction capability (Zhang et al., 2019). T4 accumulates more Cd in both shoots and roots, implying improved phytoextraction helped by *B. safencis*. T5 has significant Cd buildup in both shoots and roots, whereas T6 accumulates the highest concentration of Cd in shoots and roots, indicating that it has both Phyto stabilization and phytoextraction actions (Zhang et al., 2019).

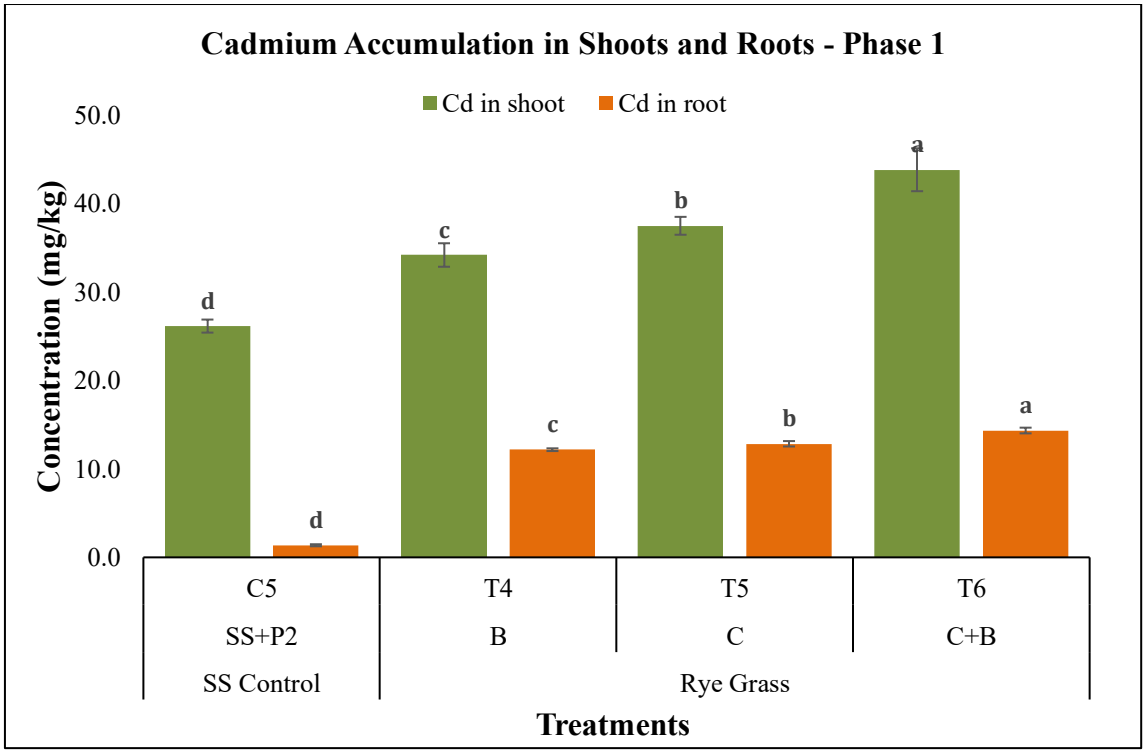

Figure 7: Percentage removal by shoots and roots of L. multiflorum in Phase 1

3.3.Post-Harvest Analysis of Phase 2 – Phytostabilization

3.3.1. Physicochemical Properties of Soil

There was no statistically significant difference observed in the means of **pH** values among different treatments (P = 0.3044). The pH of the soil remained within the range of 7.80 (C1) to 7.91 (C3). The overall pattern revealed that treatments including the addition of organic amendments, such as compost, biochar, and specific plant species, such as *C. didymus*, *L. multiflorum*, and maize tended to raise soil pH as shown in **Table 9** (Carrasco et al., 2009; Ho et al., 2022, Rees et al., 2013).

The biotic control of maize in spiked soil (C3) had **EC** and **TDS** of 229 µS/cm and 194 mg/kg, respectively. The addition of compost in T2 resulted in a significant increase of both EC and TDS values to 234 µS/cm and 211 mg/kg, respectively. However, amendments in T3, T4, and T5 resulted in the declining EC and TDS values with the lowest value observed in T5 i.e., 175 µS/cm and 169 mg/kg, respectively as illustrated in **Error! Reference source not found.**. Variation in EC and TDS of soil under various kinds of stress and amendments is validated by other studies (Abbas et al., 2017; Meng et al., 2019). Increase in soil TDS could be due to the spiking of soil with contaminants and the addition of amendments which also increases the concentration of organic and inorganic salts such as nitrates, phosphates, total organic carbon,

etc. Organic treatments appear to reduce soil EC in general indicating active remediation of Cd in soil.

The overall trend investigated in the treatments implied that adding compost and compost with bacteria (T2 & T3) greatly contribute to the **OM** and **TOC** content of soil than biochar and biochar with bacteria (T4 & T5) with P = 0.0006. Similar trend has been observed previously in a study highlighting the significant impact of these factors on the organic content of the soil (Cooper et al., 2020).

This phase observed most significant increase in **nitrates** and **phosphates** in T5 with combined amendment of biochar and *B. cereus* inoculum i.e., 42.4 mg/kg and 18.5 mg/kg, respectively, compared to 22 mg/kg and 2.5 mg/kg in the control spiked soil. Previous studies have also explained the impact of adding biochar in soils inoculated with bacteria (Ye et al., 2016; Zhou et al., 2020). An apparent increase in the availability of soil nitrates and phosphates was observed with the addition of biochar along with enhanced diversity of soil microbial communities.

Table 9: Physico-chemical characteristics of soil in Phase 2.

Treatment	Description	pH	EC (µS/cm)	TDS (mg/kg)	OM (%)	TOC (%)	Nitrates (mg/kg)	Phosphates (mg/kg)
C1	**Abiotic Control (Spiked soil)**	7.8±0.04[a]	297.6±1.84[a]	208.4±1.29[a]	20±0.5[e]	11.5±0.3[d]	22±0.3[f]	2.5±0.02[h]
C2	**Biotic Control (Fresh soil + maize)**	7.88±0.07[a]	185±0.7[g]	129.5±0.48[g]	17±0.2[h]	9.9±0.3[e]	25.78±0.38[e]	8.5±0.01[d]
C3	**Biotic Control (Spiked soil + maize)**	7.91±0.19[a]	229±0.2[c]	160.3±0.13[c]	22±0.5[d]	12.8±0.4[c]	25.78±0.58[e]	6.83±0.01[e]
T1	**Treatment 1 (Spiked soil + maize + *Bacillus cereus*)**	7.9±0.18[a]	202±0.6[e]	141.4±0.42[e]	19±0.8[f]	11±0.3[d]	29.5±0.3[c]	9.74±0.01[c]
T2	**Treatment 2 (Spiked soil + compost + maize)**	7.6±0.05[a]	234±0.9[b]	163.8±0.63[b]	36±0.3[b]	20.9±0.4[b]	30.21±0.2[b]	5.45±0.02[g]
T3	**Treatment 3 (Spiked soil + compost + maize + *B. cereus*)**	7.84±0.39[a]	219±0.8d	153.3±0.56[d]	39±0.5[a]	22.6±0.4[a]	27.71±0.21[d]	6.5±0.01[f]
T4	**Treatment 4 (Spiked soil + Biochar + Maize)**	7.82±0.17[a]	190±0.8[f]	133±0.56[f]	23±0.3[c]	13.3±0.3[c]	30.29±0.23[b]	10.14±0.01[b]
T5	**Treatment 5 (Spiked soil + Biochar + Maize + B.cereus)**	7.86±0.32[a]	175±0.4[h]	122.5±0.28[h]	18±0.2[g]	10.4±0.3[c]	42.36±0.25[a]	18.5±0.01[a]

Note: Data was presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means. Similar small letter in same columns is non-significant.

3.3.2. Effect of Amendments on the Physiological Response of Maize during Remediation of Cd and TPHs through Phyto Stabilization

The trend observed in shoot and root lengths, and their fresh and dry weights of plant with various treatments was in the order of T5>C2>T4>T3>T1>T2>C3 (Chen et al., 2023). The addition of *B. cereus* inoculum to T1 resulted in an average shoot length of 42.7 cm, compared to 40.1 cm for the spiked control (C3) with 74 mg/kg Cd and 1.5% TPH. This indicates that this treatment may have improved plant's coping mechanism to the stress and had a positive influence on its development. However, T5, with the addition of 2% biochar and bacterial inoculum in the soil, displayed the highest improvement in plant's physical parameters compared to its biotic spiked control, C3 (**Table 10**).

Table 10: Effect of different treatment on growth parameters of plants in Phase 2.

Treatment	Description	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
C2	**Fresh soil + maize**	49.67±0.85^{b}	24.03±0.55^{b}	17±0.6^{b}	42.1±1.55^{a}	9.86±0.4^{b}	3.86±0.32^{c}
C3	**Spiked soil + maize**	40.09±1.11^{e}	5.2±0.65^{f}	1.9±0.3^{g}	33.6±1.95^{d}	2.8±0.5^{e}	0.95±0.22^{e}
T1	**Spiked soil + maize + *Bacillus cereus***	42.67±1.58^{de}	18.16±0.85^{d}	7.73±0.25^{e}	38±1.9^{bc}	8.86±0.65^{c}	3.33±0.41^{cd}
T2	**Spiked soil + compost + maize**	40.68±1.14^{e}	16.63±0.77^{e}	6±0.91^{f}	35.6±1.7^{cd}	6.73±0.4^{d}	2.8±0.27^{d}
T3	**Spiked soil + compost + maize + *B. cereus***	44.19±1.55^{d}	19.5±0.26^{d}	8.93±0.3^{d}	38.8±2.5^{abc}	8.8±0.4^{c}	3.8±0.2^{c}
T4	**Spiked soil + Biochar + Maize**	47.1±1.58^{c}	20.93±0.94^{c}	12.2±0.36^{c}	40.3±2.8^{ab}	8.86±0.35^{c}	4.53±0.3^{b}
T5	**Spiked soil + Biochar + Maize + *B. cereus***	54.52±1.78^{a}	29.16±1.06^{a}	18.46±0.55^{a}	42.5±2.15^{a}	15.1±0.55^{a}	6.06±0.35^{a}

Note: Data was presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means. Similar small letter in same columns is non-significant.

This table provides a thorough examination of the harvested plants, including physical characteristics such as shoot length, root length, and the fresh and dry weights of both shoots and roots for each treatment in phase 2.

3.3.3. Effect of Cd and TPHs Co-contamination on Pigment Content of Maize during Phyto stabilization

The concentration of pigments i.e., chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, increased significantly in treatments compared to their spiked biotic control C3 in the order of T5>T4>T3>T1>T2>C3 (P = 0.0001). This variation is observed under the influence of different amendments employed for remediation of co-contamination (**Figure 8** and **Figure 9**).

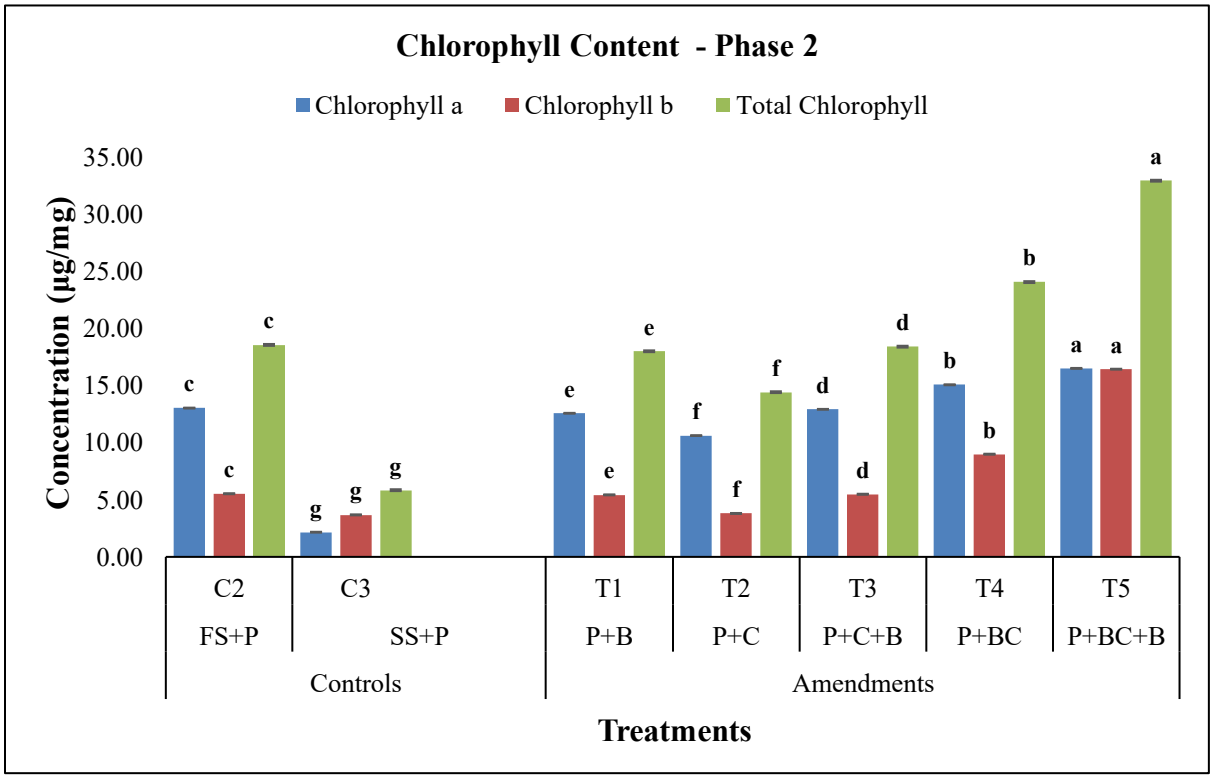

Figure 8: Changes in pigments' concentrations across various treatments in Phase 2.

In T4 and T5 levels of all pigments were raised, indicating a beneficial impact on pigment synthesis and prospective increases in plant life with the addition of biochar and bacterial inoculum (Hussain et al., 2018). In T2, however, chlorophyll content decreased moderately, with lower quantities of chlorophyll a, chlorophyll b, and total chlorophyll in contrast to T1. This indicates a negative impact of excessively high concentration of co-contamination on plant health, hence proving, that addition of compost only is not a suitable amendment to enhance phyto stabilization.

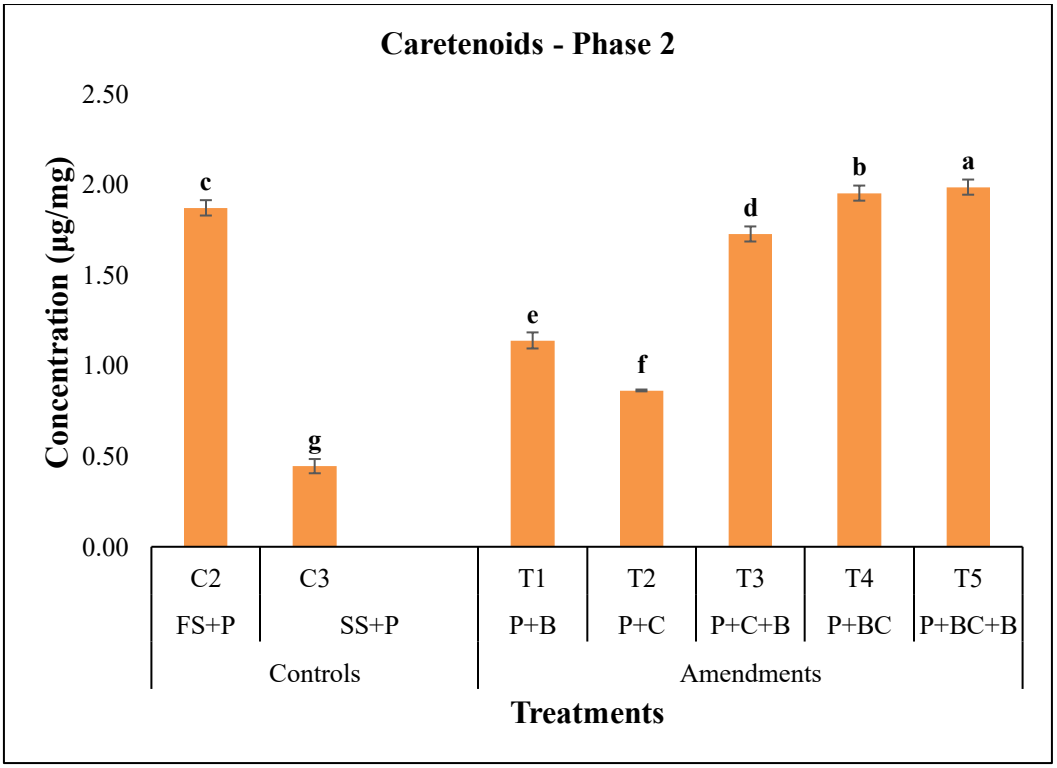

Figure 9: Carotenoids concentration in phase 2.

3.3.4. Quantification of Stress Markers in Maize under the Stress of Co-contamination in Phase 2

MDA and H2O2 levels were significantly raised in C3, indicating oxidative stress caused by the spiked soil. The inoculation of *B. cereus* into T1 resulted in lower MDA and H2O2 levels, indicating a potential antioxidative effect. However, MDA and H2O2 levels in T2 remained high, indicating that plants are still under oxidative stress. T4 and T5 levels of MDA and H2O2 were lowest, indicating most significant antioxidative impact, with the P value of 0.0001 (Sofy et al., 2021).

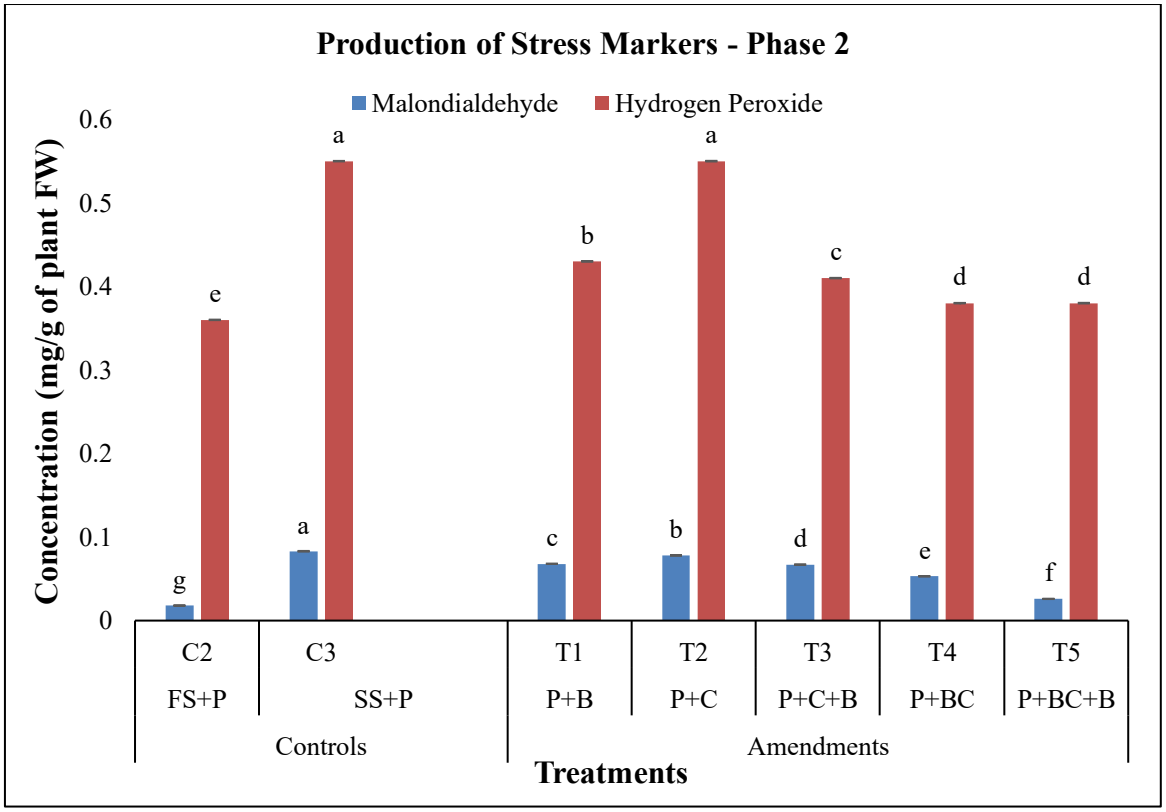

Figure 10: Production of malondialdehyde and hydrogen peroxide in plants with different amendments in Phase 2.

3.3.5. Effect of Amendments on Production of Antioxidant Enzymes in Maize in Response to Stress during Phase 2

Elevated CAT, APX, and GPX levels in C3 compared to C2 may indicate that antioxidative systems are being activated in response to elevated soil conditions (Sofy et al., 2021). Inoculation of *B. cereus* seemed to have contributed to the decrease in APX and GPX levels in T1, indicating an antioxidative response. Lowest levels of GPX in T4 and T5 indicate a possible role for biochar alone and combined with bacterial inoculation in modifying antioxidative defence pathways, respectively **Figure 11**. Treatments incorporating *B. cereus* appear to contribute to improved antioxidative responses, potentially increasing the plants' tolerance to oxidative stress caused by co-contamination (Nawaz et al., 2022).

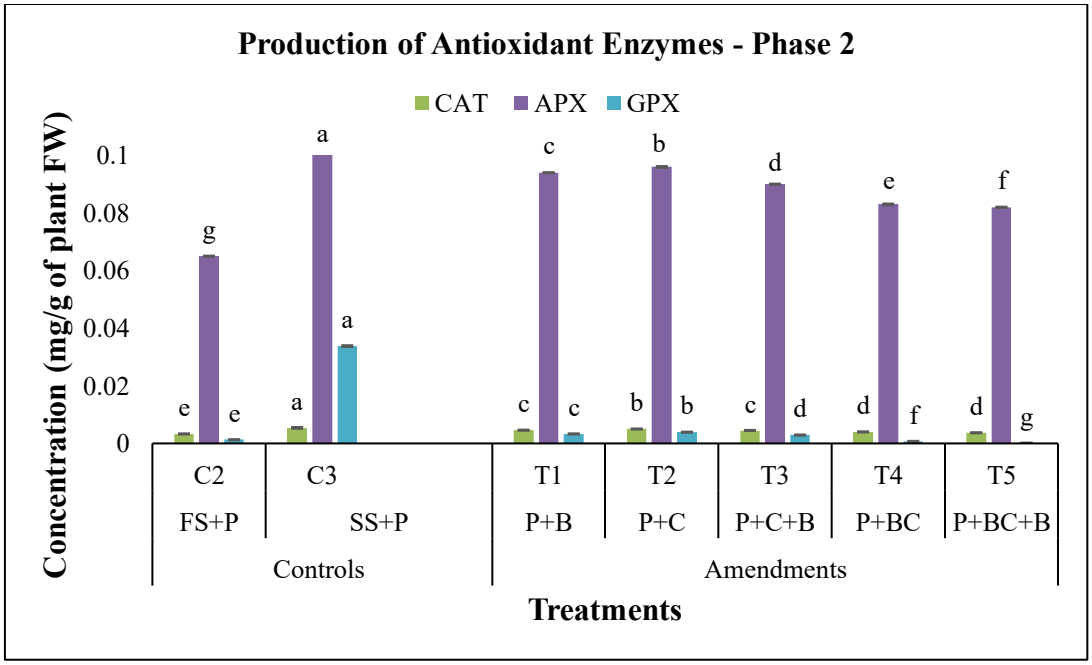

Figure 11: Production of catalase (CAT), ascorbates peroxidase (APX), and glutathione peroxidase (GPX) in plants with different amendments in Phase 2.

3.3.6. Removal of Cadmium and Total Petroleum Hydrocarbons from Soil

The combined effect of biochar and *B. cereus* inoculum in T5 resulted in a significant Cd and TPH reduction in soil up to 27.9 mg/kg and 94% compared to its spiked biotic control C3, revealing considerable Cd and TPH remediation potential (Haider et al., 2022; Hussain et al., 2018) **Table 11**. It outperformed the combined treatment of plant or compost with bacteria and reduced cadmium concentrations significantly, showing their potential for effective phytoremediation as shown in **Figure 12**.

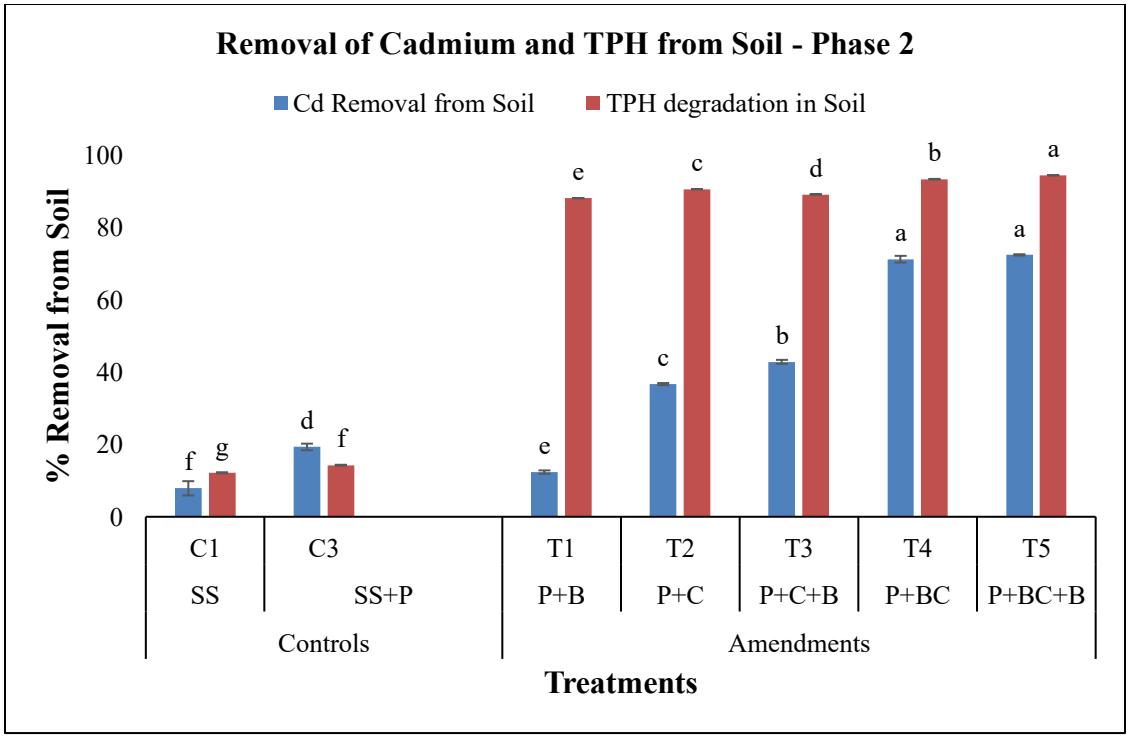

Figure 12: Percentage elemination of Cd and TPH from soil in Phase 2.

Table 11: Cadmium and Total Petroleum Hydrocarbons in soil after Phase 2.

Treatment	Description	Cadmium in soil (mg/kg)	TPH in soil (%)
C1	**Abiotic Control (Spiked soil)**	101.7±2.18^{a}	1.66±0.003^{a}
C2	**Biotic Control (Fresh soil + maize)**	0±0	0±0
C3	**Biotic Control (Spiked soil + maize)**	74.4±0.84^{b}	1.5±0.002^{b}
T1	**Treatment 1 (Spiked soil + maize + *Bacillus cereus*)**	65.2±0.35^{c}	0.2±0.0003^{c}
T2	**Treatment 2 (Spiked soil + compost + maize)**	47.4±0.22^{d}	0.16±0.0005^{d}
T3	**Treatment 3 (Spiked soil + compost + maize + *B. cereus*)**	37.7±0.36^{e}	0.15±0.0001^{e}
T4	**Treatment 4 (Spiked soil + Biochar + Maize)**	29.1±0.94^{f}	0.12±0.002^{f}
T5	**Treatment 5 (Spiked soil + Biochar + Maize + B.cereus)**	27.9±0.19^{f}	0.1±0^{g}

Note: Data was presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means. Similar small letter in same columns is non-significant.

3.3.7. Effects of Amendments on Maize's Phyto stabilization Ability of Cadmium in Soil

Figure 13 shows that C3 exhibits substantial Cd deposition in the roots, indicating potential Phyto stabilization, but only slight accumulation in the shoots. T1 shows enhanced Cd buildup in both shoots and roots, which could be attributed to *B. cereus*-mediated enhancement of Phyto stabilization. T2 and T3 show significant Cd buildup in both shoots and roots, indicating a successful Phyto stabilization-phytoextraction combination due to the presence of compost. However, T4 and T5 significantly lower Cd accumulation in roots.

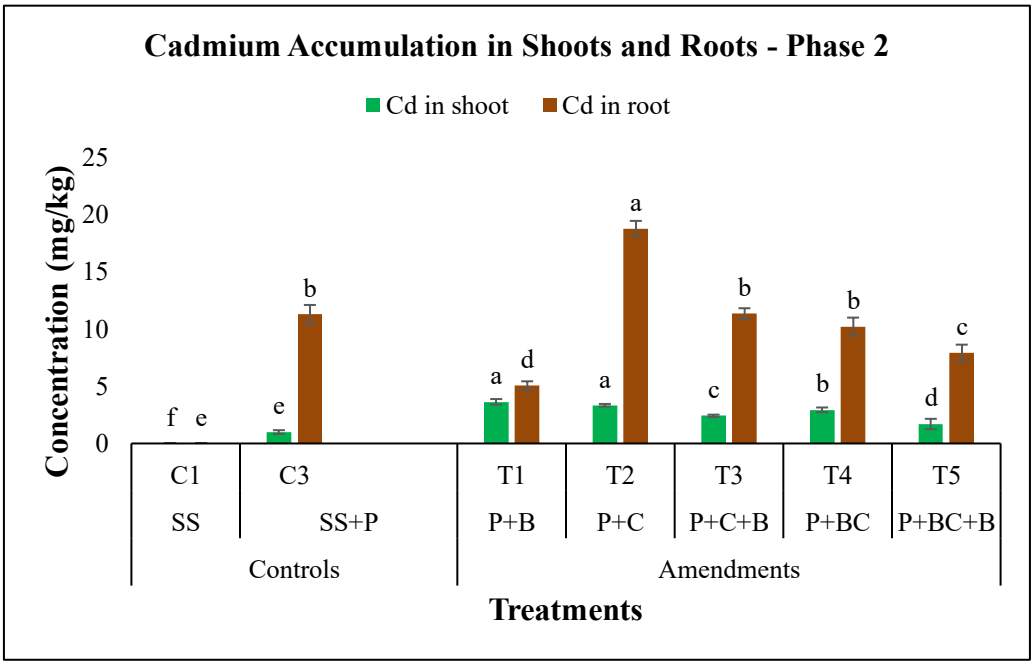

Figure 13: Percentage removal by shoots and roots of maize in Phase 2.

3.4.Bioaccumulation and Translocation Factors

A plant's bioaccumulation factor (BAF) is the ratio of the concentration of heavy metal in its tissues (typically in above-ground sections like leaves or stems) to its concentration in the surrounding soil or environment. In essence, it measures how well a plant absorbs a material from its surroundings. A high BAF shows that a plant has a high capacity for accumulation, implying that it can effectively absorb and retain the substance from the soil or water. This could indicate that a plant is behaving as a "hyperaccumulator" for that specific chemical. A low BAF, on the other hand, indicates that the plant accumulates the material to a lesser amount, signifying a reduced effectiveness in uptake and retention. This could be due to factors such as the plant's species, growing conditions, or the substance's availability in the environment. A high translocation factor (TF), on the other hand, indicates that a plant species'

ability to move metals from its root systems to its above-ground shoots is improved. It means that a significant fraction of the absorbed metal is transported and deposited in the plant's aerial sections. A low translocation factor, on the other hand, indicates that the plant species under that specific treatment has a comparably reduced capacity to move metals from the roots to the shoots.

In phase 1, treatment 6 demonstrated the BCF of 0.33 and TF of 3 suggesting phytoextraction as per the objective of the study **(Table 12)**. In phase 2, on the other hand, all the treatments showed BDF and TF values significantly lower than 1 suggesting phytostabilization with T5 showing the optimum lowest TF of 0.06 **(Table 13)**. These findings offer information on the treatments' varying Cd transport capacities and the implications for metal accumulation in plant tissues.

Table 12: Bioaccumulation and translocation factors of treatments with Lolium multiflorum in phase 1.

Cadmium concentration (mg/kg)						
Treatments	**Description**	**Cd in roots**	**Cd in shoots**	**Cd in soil**	**BAF**	**TF**
C5	**Spiked soil + *L. multiflorum***	1.4	26.1	75.9	0.3	18.6
T4	**Spiked soil + *L. multiflorum* + *B. safencis***	12.2	34.2	54.1	0.6	2.8
T5	**Spiked soil + Compost + *L. multiflorum***	12.8	37.5	51.6	0.7	3
T6	**Spiked soil + Compost + *L. multiflorum* + *B. safencis***	14.3	43.8	44	1	3.1

Table 13: Bioaccumulation and translocation factors of treatments with maize in phase 2.

Cadmium concentration (mg/kg)						
Treatments	**Description**	**Cd in roots**	**Cd in shoots**	**Cd in soil**	**BAF**	**TF**
C3	**Spiked soil + maize**	11.3	1	74.40	0.01	0.08
T1	**Spiked soil + maize + *Bacillus cereus***	5	3.6	65.20		0.72
T2	**Spiked soil + compost + maize**	18.8	3.3	47.40	0.07	0.18

T3	**Spiked soil + compost + maize + *B. cereus***	11.3	2.4	37.70	0.06	0.21
T4	**Spiked soil + Biochar + Maize**	20.6	2.9	29.10	0.1	0.14
T5	**Spiked soil + Biochar + Maize + *B. cereus***	25.2	1.7	27.90	0.06	0.07

Chapter 4

Discussion

The current study attempted to address the issue of co-contamination in soil involving Cadmium (Cd) and Total Petroleum Hydrocarbons (TPHs) using an innovative strategy known as 'phase crop rotation.' This two-phase experimental study was meant to evaluate the efficacy of a dual strategy: phytoextraction in the first phase, followed by phytostabilization in the second phase, as a means of remediating co-contaminated sites and increasing crop output overall.

A variety of remediation approaches were investigated during both phases, including the use of individual and mixed amendments such as microbial strains (bioremediation), specific plant species (phytoremediation), compost, and biochar. Prior to this study, there was little information available on the phase crop rotation strategy. Several obstacles and factors emerged during this integrated remediation technique, including the availability of resources, possible interactions among various remediation methods, such as the impact of Cd and TPHs co-contamination on bacteria, the effect of organic matter on bacterial activity, execution time, environmental implications, and overall sustainability levels. These factors influenced the study's overall direction.

An important discovery in phase 1 was the difference in performance of the two hyperaccumulator plant species, *C. didymus* (garden cress) and *L. multiflorum* (rye grass), even in the presence of combined amendment. Garden cress did not survive in our experiment at 121mg/kg Cd and 2% TPHs contamination levels, despite being a relatively new addition to the group of Cd hyperaccumulators (Sidhu et al., 2017). This means that garden cress may exhibit Cd tolerance but not TPHs tolerance, which may limit its ability to hyperaccumulate. In light of these findings, a careful selection of hyperaccumulators is essential, taking the level of contamination into account. On the other hand, rye grass performed remarkably well, especially in the T6 treatment.

It was predicted that these organic amendments would not only improve the soils and plant’s physical and biochemical characteristics but also hasten the removal of Cd and the breakdown of TPH. pH was not significantly affected and remained within a range of 7.8-7.9 among all treatments. This is most likely because of the high organic matter content in soil which can buffer its acidic or basic character by balancing it (Ho et al., 2022; Rees et al. 2013).

Bacillus safencis, a plant growth-promoting rhizobacterium (PGPR), was used in the first stages of this study with the intention of facilitating rhizodegradation TPH and improving phytoextraction of Cd (Basit et al., 2021). To create an environment that is favorable for plant growth, PGPRs fix atmospheric nitrogen, solubilize phosphates, and produce plant growth regulators. Through improved root development and metabolism, a richer nutritional profile in the rhizosphere can help hyperaccumulators efficiently absorb heavy metals like Cd. Additionally, PGPRs have been linked to an increase in the amount of total organic carbon and organic matter in soil, two crucial factors that affect soil structure, water retention, microbial activity, and general soil health (Borah et al., 2022).

Our initial results with *B. safencis* in terms of TPH degradation, however, weren't the best. Its inoculation unquestionably enhanced soil health parameters such as EC, TDS, organic matter content, total organic carbon, and nutrient availability. However, it was only able to degrade TPH by a meagre 15-20%. This demonstrates the complexity of rhizodegradation processes and the potential sensitivity of bacterial strains to pollutants. Additionally, the potential synergy between the PGPR and the hyperaccumulators to aid in Cd elimination was not fully appreciated as showed in results of physicochemical characteristics of soil for T1 and T4. Following these results, it was decided that the second phase should be used to investigate different bacterial strains that would be able to provide a more effective rhizodegradation of TPH.

The introduction of the bacterial strain *Bacillus cereus* in the second stage of our study was justified by the presence of TPH degrading genes, confirmed by bioinformatics tools (Deng et al., 2020). With the main objective of obtaining improved TPH degradation, second phase evaluated the effectiveness of this strain in both TPH degradation and impacting soil health indices. *Bacillus cereus*, performed far better than *B. safencis*. Up to 90% of TPH was found to be destroyed in the soil, which is an amazing degradation rate. The inherent genetic makeup of *B. cereus*, particularly the presence of genes that facilitate TPH breakdown, is responsible for its potential.

Additionally, to its primary function in TPH breakdown, *B. cereus* had a favorable effect on soil health indices. One of the most important findings was the increased soil availability of nitrates and phosphates as observed in T1. PGPRs, such as *B. cereus*, can solubilize phosphate and fix atmospheric nitrogen, enhancing the soil's nutritional profile. This strengthens the plants' natural capacity to absorb heavy metals like Cd while also promoting plant growth and health (Kumar et al., 2023). This phase of the experiment gave insight on the critical role of

Bacillus cereus as a potent bioremediating agent. Its genetic proclivity for TPH breakdown and positive influence on soil health parameters made it a clear option over *B. safencis* (Adeleye et al., 2021).

TDS and EC of the soil indicate soil's salinity and concentration of dissolved ions. They were observed to increase significantly in treatments with only compost as an amendment. This indicates an overall increase in soil's salinity and the number of dissolved ions because of the mineral salts and nutrients in compost. When added to soil, the soluble salts in the compost dissolve in the soil moisture, raising the EC and TDS values of the soil (Azeez & Van Averbeke, 2012). Furthermore, compost helps boost soil's water-holding ability. Because there is more water in the soil, soluble salts are diluted less, potentially leading to increased EC and TDS levels (Brown & Cotton, 2011). Additionally, the increased organic matter level allowed for a more effective release of nutrients. Particularly, there was a marked increase in the availability of phosphates and nitrates in T2 and T5 in phase 1, and in T2 in phase 2 (Hannet et al., 2021). The highest values of EC and TDS were observed in biotic controls with spiked soil highlighting Cd stress in soil. Whereas their lowest values were observed in treatments with maximum remediation of Cd in soil i.e., T6 in phase 1 and T5 in phase 2.

The introduction of different amendments to Cd-contaminated soils also illustrated a noticeable change in chlorophyll a, b, and total chlorophyll contents which are foundational indicators of plant health. The elevated chlorophyll levels can be attributed to the nutrient-rich environment provided by the amendment, ensuring that plants are not nutrient-deprived and can maintain their photosynthetic apparatus effectively (Rasool et al., 2021). Another type of pigment known as carotenoids functions as an antioxidant and is essential for photoprotection. They can quench reactive oxygen species (ROS) and lessen the possibility of cellular damage, which is critical in the setting of heavy metal stress (Hussain et al., 2018). Plants grown in composted soil showed highest carotenoid content in phase 1. In-depth analysis of the oxidative stress indicators revealed that plants cultivated in the soils with the best treatment produced significantly less MDA (t6 in phase 1 and T5 in phase 2). MDA is a byproduct of lipid peroxidation and a marker of oxidative stress-related cellular membrane damage. Even in the presence of Cd, decreased MDA levels point to a reduction in oxidative stress in both phases. A typical ROS called hydrogen peroxide (H2O2) generation, however, was not suppressed in phase 1 suggesting damage to the cells.

The increased activity of antioxidant enzymes in plants can also be linked to the reduction of oxidative stress (Sidhu et al., 2020). The key enzymatic antioxidants catalase (CAT), ascorbate

peroxidase (APX), and glutathione peroxidase (GPX) neutralize ROS and shield plant cells from oxidative harm. These enzymes were also most active in plants growing in T6 of phase 1 and T5 of phase 2, demonstrating how these treatments helped strengthen a plant's natural defences against heavy metal stress (Guarino et al., 2018).

As we focus on the main contaminants, we see that the presence compost significantly increased the Cd removal rate in phase 1 (T5 and T6). This is because compost acts as soil conditioner enhancing nutrient availability for plant growth, ultimately resulting in phytoextraction of Cd from the soil (Mushtaq et al., 2020). When combined with *B. safencis* in T6, PGPR enhanced the solubilization of nitrates and phosphates which is a sign of better soil health and promotes plant growth, which helps the plant's ability to take Cd from the environment, showing best results of phytoextraction. When it comes to TPH degradation, the 45% decrease seen with the combined amendment in T6 is remarkable but falls short of predictions. The increased activity of *B. safencis* in the combination treatment can be explained by the presence of organic waste from compost, which provides substrates that can promote microbial growth. However, only compost amendment in T5 (phase 1) and T2 (phase 2) did not play a significant role in TPH degradation as shown in results. This is due to the absence of any TPH degrading bacteria in soil.

Despite being the best treatment for phytoextraction in phase 1, compost + bacteria treatment in phase 2 showed some limitations. The significant increase in OM and total organic carbon TOC in the soil during phase 2 was a key finding particularly due to plant leftovers from earlier harvest in phase 1. Although an enriched organic profile frequently heralds improved soil health, this study highlights the dangers of having too much organic content. A buildup of organic debris in the soil more than 20% can hinder soil aeration and immobilize vital nutrients, which stunts plant growth. Additionally, a lot of organic matter might have an impact on the soil's microbial dynamics. Despite their adaptability, bacteria perform best in specific environments. The increased organic content may have affected the soil's moisture, aeration, or even direct microbial interactions, which could have impaired the effectiveness of the bacterial strain. This is further supported by the observed decline in Cd removal efficiency, which indicates that while the combined amendment was advantageous in 1st phase, it may have unintentionally generated complications that affected both the performance of the bacterial community and the plant in second phase. Another factor in lower efficiency of this treatment in phase 2 for the removal of Cd could be use of non-hyperaccumulator plant species.

The 10% compost content gave the living plants various advantages in addition to improving the soil's health. The production of chlorophyll was consistently increased. Although oxidative stress indicators such malondialdehyde and hydrogen peroxide concentrations were high, increased antioxidant enzyme activity was showed that defence mechanism of plant is in action (Sofy et al., 2021).

The 10% concentration of compost was selected as a middle ground to maximize the advantages of compost without unintentionally causing any negative effects. However, our findings suggested that even lower concentrations might still provide similar, if not superior, advantages. Compost is unquestionably helpful; however, higher quantities may increase the danger of soil salinization due to increasing salts, or may result in excessive nutrient provision, which may cause nutrient leaching or even prevent some microbial activity necessary for TPH breakdown (Mbarki et al., 2020). Finding a balance is therefore crucial. Future research could hone this ideal concentration, focusing on simpler but still efficient compost incorporations.

The2nd phase of the experiment also evaluated the effects of adding 2% biochar to soils that were co-contaminated with Cd and TPH, making careful consideration to how this would affect soil and plant health indicators and, consequently, the efficacy of the pollutants' removal, adsorption, or degradation, to protect the safety of the edible crop, maize.

It is necessary to explain the rationale behind the 2% biochar concentration selection. Even while biochar is beneficial, using too much of it could pose problems like changing soil pH that is too high or even competition between plants and bacteria for nutrients. Aiming to maximize the advantages of biochar without unintentionally introducing potential drawbacks, the decision of 2% finds a compromise. Its appropriateness is supported by the improvements in soil health and pollution mitigation that have been seen at this concentration (Hannet et al., 2021).

The primary mode of action of biochar is due to its porous composition and large surface area, which increase its adsorptive ability. This was supported by our results, which showed that adding biochar to the soil in T4 of phase 2 effectively removed 75% of the cadmium (Abbas et a., 2017). In the case of an edible crop like maize, stabilization of Cd within the soil becomes crucial. Biochar substantially lowered cadmium's bioavailability by adsorbing it, restricting its uptake by maize and ensuring the yield's safety.

Beyond its function in cadmium sequestration, biochar demonstrated notable advantages for soil health. The improved availability of nitrates and phosphates following the application of

biochar was one notable aspect which could be due to increased cation exchange capacity, reduced nitrogen fixation, and improved soil structure (Cooper et al., 2020). Additionally, the stability and high carbon content of biochar have the potential to enhance microbial activity, soil structure, and water retention. Although reducing cadmium uptake was the main goal, the unforeseen result was a nutrient-rich, favorable environment that might benefit plant development and health (Chen et al., 2023). It is also necessary to discuss the potential effects of biochar on the adsorption of TPH. While the direct contribution of biochar to TPH breakdown may be not modest, its introduction can promote adsorption of TPH. To clarify any direct or indirect functions of biochar in TPH removal, further focused research is needed.

Due to the noticeable advantages in the remediation of Cd and TPH, the combined application of biochar and the bacterial strain, *Bacillus cereus*, emerges as the best solution in phase 2. The significant elimination of Cd, which resulted in a stunning 80% reduction, emphasizes the critical function of biochar in the remediation process. Biochar efficiently lowers the bioavailability of cadmium in the soil by serving as a sink, reducing plant uptake and groundwater migration (Chen et al., 2023). Furthermore, the combination treatment significantly improved the rate of TPH breakdown, which reached an astonishing 90%. This reveals the fascinating interaction between biochar and *B. cereus*. The function of biochar extends beyond Cd adsorption; it may also offer a favorable environment for microbial activity. Its porous structure might be home to microorganisms, providing shelter and aiding in their metabolic functions. However, *B. cereus*, which has hydrocarbon degradation pathways, may effectively break down TPH, especially if it had access to the favorable environment that biochar fosters (Haider et al., 2022; Hussain et al., 2018).

Conclusion

To summarize, the rising environmental issues created by soil contamination caused by human activities demand immediate and inventive solutions to mitigate their negative effects. Multi-contaminated or Co-contaminated sites, which are distinguished by the presence of heavy metals such as Cadmium (Cd) and organic contaminants such as total petroleum hydrocarbons (TPH), represent a significant challenge that necessitates appropriate remediation strategies. This study addressed this question successfully by using a novel and integrated phytoremediation strategy known as "phase crop rotation." This two-phase technique has showed efficacy in concurrently alleviating Cd and TPH contamination within soil, providing a promising path for sustainable and eco-friendly soil restoration.

The study emphasizes the importance of remediating soils contaminated with toxins that exceed the World Health Organization (WHO) and Food and Agriculture Organization (FAO) threshold limits. Co-contamination has serious ramifications for food security, ecological balance, and human well-being. Recognizing the scarcity of complete solutions, the study's emphasis on "phase crop rotation" stands out as a critical contribution to the field of phytoremediation. The technique shows significant potential in the phytoextraction of Cd during the early phase by merging hyperaccumulator grasses, compost, and plant growth-promoting rhizobacteria (PGPR), with the potent interplay between PGPR and the hyperaccumulator species increasing this therapeutic activity. Furthermore, the presence of TPH-degrading bacteria *Bacillus cereus* and biochar in the second phase emphasizes the need of bioaugmentation and phytostabilization in combination with plant-microbe interactions.

The findings support the feasibility of "phase crop rotation" as an environmentally beneficial and long-term solution to soil restoration. The sophisticated arrangement of biological and chemical factors, combined with the careful selection of appropriate plant species and microbes, represents a comprehensive and effective approach to dealing with multi-contaminated soils. This integrated strategy achieves both organic pollutant degradation and heavy metal removal synergistically, highlighting its greater efficacy over single procedures. As the research enhances our understanding of efficient and green soil restoration approaches, it makes a significant contribution to the larger goal of protecting the environment, ensuring food production, and conserving human health.

Future Recommendations

The "phase crop rotation" technique is an intriguing alternative to co-contamination issues affecting industrial and agricultural soils, but its thorough potential can only be realised through additional research, optimisation, and practical implementation on a broader scale.

I. Long-term field trials in a variety of agricultural contexts are required to demonstrate the viability and sustainability of the "phase crop rotation" strategy. To give a rigorous assessment of its real-world application, these experiments should follow soil quality, crop yields, and the permanence of remediation effects across several growing seasons.
II. More research should be conducted to investigate the complex relationships between hyperaccumulator plants and plant growth-promoting rhizobacteria (PGPR). Understanding the mechanisms that improve phytoextraction with microbial help can

lead to more personalised ways for optimising the co-removal of pollutants such as Cd and TPH.

III. The introduction of specialised microbial consortia, such as TPH-degrading bacteria such as Bacillus cereus, can boost the efficacy of the second phase of phytoremediation. These consortia can be fine-tuned for specific soil conditions and pollutants to improve phytostabilization processes.

IV. Efforts should be undertaken to scale up the "phase crop rotation" technique for large-scale agricultural adoption to address the broader issue of co-contamination. This could entail developing low-cost technologies for manufacturing biochar and microbial inoculants.

V. To measure the ecological and financial benefits of using this approach, comprehensive environmental and economic analyses should be done. When compared to standard remediation approaches, it will provide vital insights into its overall viability and benefits.

Conclusively, amid rising soil contamination concerns, this novel technique has the potential to greatly contribute to sustainable environmental practises and food security.

References

Abbas, Q., Rinklebe, J., Liu, G., Zia-Ur-Rehman, M., Ali, M., Munir, M. a. M., & James, N. D. (2017). Evaluating the health risks of potentially toxic elements through wheat consumption in multi-industrial metropolis of Faisalabad, Pakistan. *Environmental Science and Pollution Research*, *24*(34), 26646–26657. https://doi.org/10.1007/s11356-017-0311-9

Abbas, T., Rizwan, M., Ali, S., Adrees, M., Rehman, M. Z. U., Qayyum, M. F., Ok, Y. S., & Murtaza, G. (2017). Effect of biochar on alleviation of cadmium toxicity in wheat (Triticum aestivum L.) grown on Cd-contaminated saline soil. *Environmental Science and Pollution Research*, *25*(26), 25668–25680. https://doi.org/10.1007/s11356-017-8987-4

Adeleye, A. O., Musa, Y., Nkereuwem, M. E., Onokebhagbe, V. O., Sadiq, I., Amoo, A. O., Bate, G. B., Shiaka, G. P., & Raji, M. A. (2021). Bio-enhanced removal of hydrocarbon contents from spent engine oil contaminated soil using Staphylococcus aureus and *Bacillus cereus* co-culture. *Agro-Science*, *20*(3), 80–90. https://doi.org/10.4314/as.v20i3.11

Adwaadmin. (n.d.). *How to test soil EC*. https://www.adwainstruments.com/support/knowledge-base/how-to-test-soil-ec

Agnello, A. C., Bagard, M., Van Hullebusch, E. D., Esposito, G., & Huguenot, D. (2016). Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. *Science of the Total Environment*, *563–564*, 693–703. https://doi.org/10.1016/j.scitotenv.2015.10.061

Agriculture Statistics | Pakistan Bureau of Statistics. (n.d.). https://www.pbs.gov.pk/content/agriculture-statistics

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M. (2021). Heavy Metals and pesticides Toxicity in agricultural soil and plants: ecological risks and human health implications. *Toxics*, *9*(3), 42. https://doi.org/10.3390/toxics9030042

Ali, M., Song, X., Ding, D., Wang, Q., Zhang, Z., & Tang, Z. (2022). Bioremediation of PAHs and heavy metals co-contaminated soils: Challenges and enhancement strategies. *Environmental Pollution*, *295*, 118686. https://doi.org/10.1016/j.envpol.2021.118686

Alkorta, I., & Garbisu, C. (2001). Phytoremediation of organic contaminants in soils. *Bioresource Technology*, *79*(3), 273–276. https://doi.org/10.1016/s0960-8524(01)00016-5

Ambaye, T. G., Chebbi, A., Formicola, F., Rosatelli, A., Prasad, S., Gomez, F. H., Sbaffoni, S., Franzetti, A., & Vaccari, M. (2022). Ex-situ bioremediation of petroleum hydrocarbon contaminated soil using mixed stimulants: Response and dynamics of bacterial community and phytotoxicity. *Journal of Environmental Chemical Engineering*, *10*(6), 108814. https://doi.org/10.1016/j.jece.2022.108814

Antizar-Ladislao, B., Lopez-Real, J., & Beck, A. J. (2004). Bioremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated waste using composting approaches. *Critical Reviews in Environmental Science and Technology*, *34*(3), 249–289. https://doi.org/10.1080/10643380490434119

Auffret, M. D., Karhu, K., Khachane, A. N., Dungait, J. A., Fraser, F., Hopkins, D., Wookey, P. A., Singh, B. K., Freitag, T. E., Hartley, I. P., & Prosser, J. I. (2016). The role of microbial community composition in controlling soil respiration responses to temperature. *PLOS ONE*, *11*(10), e0165448. https://doi.org/10.1371/journal.pone.0165448

Azeez, J. O., & Van Averbeke, W. (2012). Dynamics of Soil pH and Electrical Conductivity with the Application of Three Animal Manures. *Communications in Soil Science and Plant Analysis*, *43*(6), 865–874. https://doi.org/10.1080/00103624.2012.653022

Basit, A., Shah, S. T., Ullah, I., Muntha, S. T., & Mohamed, H. I. (2021). Microbe-assisted phytoremediation of environmental pollutants and energy recycling in sustainable agriculture. *Archives of Microbiology*, *203*(10), 5859–5885. https://doi.org/10.1007/s00203-021-02576-0

Borah, P., Gogoi, N., Asad, S. A., Rabha, A. J., & Farooq, M. (2022). An Insight into Plant Growth-Promoting Rhizobacteria-Mediated Mitigation of Stresses in Plant. *Journal of*

Plant Growth Regulation, *42*(5), 3229–3256. https://doi.org/10.1007/s00344-022-10787-y

Brown, S., & Cotton, M. (2011). Changes in soil properties and carbon content following compost application: results of on-farm sampling. *Compost Science & Utilization*, *19*(2), 87–96. https://doi.org/10.1080/1065657x.2011.10736983

Bux, R. K., Haider, S. I., Batool, M., Solangi, A. R., Sheikholeslami, M., Karimi-Maleh, H., & Şen, F. (2021). Assessment of heavy metal contamination and its sources in urban soils of district Hyderabad, Pakistan using GIS and multivariate analysis. *International Journal of Environmental Science and Technology*, *19*(8), 7901–7913. https://doi.org/10.1007/s13762-021-03691-7

Carrasco, L., Caravaca, F., Azcón-Aguilar, C., & Roldán, A. (2009). Soil acidity determines the effectiveness of an organic amendment and a native bacterium for increasing soil stabilisation in semiarid mine tailings. *Chemosphere*, *74*(2), 239–244. https://doi.org/10.1016/j.chemosphere.2008.09.035

Chance, B., & Maehly, A. (2006). The assay of catalases and peroxidases. In *Methods of biochemical analysis* (pp. 357–424). Wiley. https://doi.org/10.1002/9780470110171.ch14

Chen, L. C., Larson, S. M., Ballard, J. R., Ma, Y., Zhang, Q., Li, J., Wu, L., Arslan, Z., & Han, F. X. (2019). Laboratory spiking process of soil with various uranium and other heavy metals. *MethodsX*, *6*, 734–739. https://doi.org/10.1016/j.mex.2019.03.026

Chen, W., Wu, Z., Liu, C., Zhang, Z., & Liu, X. (2023). Biochar combined with Bacillus subtilis SL-44 as an eco-friendly strategy to improve soil fertility, reduce Fusarium wilt, and promote radish growth. *Ecotoxicology and Environmental Safety*, *251*, 114509. https://doi.org/10.1016/j.ecoenv.2023.114509

Chen, Y., Zhu, Q., Xiang-Zhou, D., Huang, W., Du, C., & Lu, D. (2019). How Serratia marcescens HB-4 absorbs cadmium and its implication on phytoremediation. *Ecotoxicology and Environmental Safety*, *185*, 109723. https://doi.org/10.1016/j.ecoenv.2019.109723

Chirakkara, R. A., & Reddy, K. R. (2015). Biomass and chemical amendments for enhanced phytoremediation of mixed contaminated soils. *Ecological Engineering*, *85*, 265–274. https://doi.org/10.1016/j.ecoleng.2015.09.029

Colony-forming unit (CFU). (n.d.). REVIVE. https://revive.gardp.org/resource/colony-forming-unit-cfu/?cf=encyclopaedia#:~:text=To%20measure%20the%20CFU%2C%20bacterial,x%20106%20CFU%2Fml.

Cooper, J., Greenberg, I., Ludwig, B., Hippich, L., Fischer, D. A., Glaser, B., & Kaiser, M. (2020). Effect of biochar and compost on soil properties and organic matter in aggregate size fractions under field conditions. *Agriculture, Ecosystems & Environment*, *295*, 106882. https://doi.org/10.1016/j.agee.2020.106882

Corwin, D. L., & Yemoto, K. (2019). Measurement of soil salinity: electrical conductivity and total dissolved solids. *Soil Science Society of America Journal*, *83*(1), 1–2. https://doi.org/10.2136/sssaj2018.06.0221

Cristaldi, A., Conti, G. O., Jho, E. H., Zuccarello, P., Grasso, A., Copat, C., & Ferrante, M. (2017). Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. *Environmental Technology and Innovation*, *8*, 309–326. https://doi.org/10.1016/j.eti.2017.08.002

Czarny, J., Staninska-Pięta, J., Piotrowska-Cyplik, A., Juzwa, W., Wolniewicz, A., Marecik, R., Ławniczak, Ł., & Chrzanowski, Ł. (2020). Acinetobacter sp. as the key player in diesel oil degrading community exposed to PAHs and heavy metals. *Journal of Hazardous Materials*, *383*, 121168. https://doi.org/10.1016/j.jhazmat.2019.121168

DalCorso, G., Fasani, E., Manara, A., Visioli, G., & Furini, A. (2019). Heavy Metal Pollutions: State of the art and innovation in phytoremediation. *International Journal of Molecular Sciences*, *20*(14), 3412. https://doi.org/10.3390/ijms20143412

Daniel, A. J., Enzo, E. R., Juliana, M. S., Stefanie, B. C., Alvarez, A., Claudia, S. B., & Marta, A. P. (2022). The current approach to soil remediation: A review of physicochemical and biological technologies, and the potential of their strategic combination. *Journal of*

Environmental Chemical Engineering, *10*(2), 107141. https://doi.org/10.1016/j.jece.2022.107141

Deng, Z., Jiang, Y., Chen, K., Gao, F., & Liu, X. (2020). Petroleum Depletion Property and Microbial Community Shift After Bioremediation Using Bacillus halotolerans T-04 and *Bacillus cereus* 1-1. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00353

Elyamine, A. M., Moussa, M. A., Afzal, J., Rana, M. S., Imran, M., Zhao, X., & Hu, C. (2019). Modified rice straw enhanced cadmium (II) immobilization in soil and promoted the degradation of phenanthrene in co-contaminated soil. *International Journal of Molecular Sciences*, *20*(9), 2189. https://doi.org/10.3390/ijms20092189

Emamverdian, A., Ding, Y., Mokhberdoran, F., & Xie, Y. (2015). Heavy metal stress and some mechanisms of plant defense response. *The Scientific World Journal*, *2015*, 1–18. https://doi.org/10.1155/2015/756120

Escobar-Alvarado, L. E. A., Vaca-Mier, M. V. M., Rojas-Valencia, N. R. V., López, R. L., & Flores, J. F. (2015). Degradation Efficiency and Bacterial Species in Soil Polluted with Used Motor Oils, Treated By Composting with Yard Trimmings and Phytoremediation with Lolium perenne. *Journal of Agricultural Engineering*, *3*(2), 72–78. https://doi.org/10.18005/JAEB0302004

Gan, S., Lau, E., & Ng, H. K. (2009). Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). *Journal of Hazardous Materials*, *172*(2–3), 532–549. https://doi.org/10.1016/j.jhazmat.2009.07.118

Garbisu, C., Hernández-Allica, J., Barrutia, O., Alkorta, I., & Becerril, J. M. (2002). Phytoremediation: A Technology Using Green Plants to Remove Contaminants from Polluted Areas. *Reviews on Environmental Health*, *17*(3). https://doi.org/10.1515/reveh.2002.17.3.173

Government of Pakistan [Finance Division]. (2022). *Pakistan Economic Survey 2021-2022*. Finance Division - Government of Pakistan. https://www.finance.gov.pk/survey/chapter_22/Economic%20Survey%202021-22.pdf

Gowda, M. G. (n.d.). *Experiment 2-Organic content*. Scribd. https://www.scribd.com/document/74754008/Experiment-2-Organic-Content

Guarino, C., Paura, B., & Sciarrillo, R. (2018). Enhancing phytoextraction of HMS at real scale, by combining Salicaceae trees with microbial consortia. *Frontiers in Environmental Science*. https://doi.org/10.3389/fenvs.2018.00137

Haider, F. U., Farooq, M., Naveed, M., Cheema, S. A., Ain, N. U., Salim, M. A., Cai, L., & Mustafa, A. (2022). Influence of biochar and microorganism co-application on stabilization of cadmium (Cd) and improved maize growth in Cd-contaminated soil. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.983830

Hamid, Y., Liu, L., Usman, M., Tang, L., Lin, Q., Rashid, M. S., Ulhassan, Z., Hussain, M. I., & Yang, X. (2022). Organic/inorganic amendments for the remediation of a red paddy soil artificially contaminated with different cadmium levels: Leaching, speciation, and phytoavailability tests. *Journal of Environmental Management*, *303*, 114148. https://doi.org/10.1016/j.jenvman.2021.114148

Hannet, G., Singh, K., Fidelis, C., Farrar, M. B., Muqaddas, B., & Bai, S. H. (2021). Effects of biochar, compost, and biochar-compost on soil total nitrogen and available phosphorus concentrations in a corn field in Papua New Guinea. *Environmental Science and Pollution Research*, *28*(21), 27411–27419. https://doi.org/10.1007/s11356-021-12477-w

Ho, T. T. K., Tra, V. T., Le, T., Nguyen, N., Tran, C., Nguyen, P., Vo, T., Thai, V., & Bui, X. (2022). Compost to improve sustainable soil cultivation and crop productivity. *Case Studies in Chemical and Environmental Engineering*, *6*, 100211. https://doi.org/10.1016/j.cscee.2022.100211

Hussain, F., Hussain, I., Khan, A. H. A., Shad, M. Y., Iqbal, M., Soja, G., Reichenauer, T. G., Zeshan, & Yousaf, S. (2018). Combined application of biochar, compost, and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. *Environmental and Experimental Botany*, *153*, 80–88. https://doi.org/10.1016/j.envexpbot.2018.05.012

Hussain, M. S. (2019). Total Dissolve Salts (TDS). *ResearchGate*. https://doi.org/10.13140/RG.2.2.11858.30406

Khan, M. M., Zaidi, A., Wani, P. A., & Oves, M. (2008). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*, *7*(1), 1–19. https://doi.org/10.1007/s10311-008-0155-0

Khan, M. S., Khan, S., Khan, A., & Alam, M. M. (2017). Soil contamination with cadmium, consequences and remediation using organic amendments. *Science of the Total Environment*, *601–602*, 1591–1605. https://doi.org/10.1016/j.scitotenv.2017.06.030

Khanna, K., Ohri, P., Bhardwaj, R., & Ahmad, P. (2021). Unsnarling Plausible Role of Plant Growth-Promoting Rhizobacteria for Mitigating Cd-Toxicity from Plants: An Environmental Safety Aspect. *Journal of Plant Growth Regulation*, *41*(6), 2514–2542. https://doi.org/10.1007/s00344-021-10445-9

Khanpour-Alikelayeh, E., & Partovinia, A. (2021). Synergistic and antagonistic effects of microbial co-culture on bioremediation of polluted environments. In *Microorganisms for sustainability* (pp. 229–265). https://doi.org/10.1007/978-981-15-7455-9_10

Kubier, A., Wilkin, R. T., & Pichler, T. (2019). Cadmium in soils and groundwater: A review. *Applied Geochemistry*, *108*, 104388. https://doi.org/10.1016/j.apgeochem.2019.104388

Kumar, C., Tomar, A., Pandey, S., & Prasad, M. (2023). Plant Growth Promoting Rhizobacteria Sustaining Saline and Metal Contaminated Soils. *Agroecological Approaches for Sustainable Soil Management*, 437–456. https://doi.org/10.1002/9781119911999.ch19

Kuppusamy, S., Thavamani, P., Venkateswarlu, K., Lee, Y. B., Naidu, R., & Megharaj, M. (2017). Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. *Chemosphere*, *168*, 944–968. https://doi.org/10.1016/j.chemosphere.2016.10.115

Lee, S. Y., Lee, Y., & Cho, K. (2022). Effect of Novosphingobium sp. CuT1 inoculation on the rhizoremediation of heavy metal- and diesel-contaminated soil planted with tall

fescue. *Environmental Science and Pollution Research*, *30*(6), 16612–16625. https://doi.org/10.1007/s11356-022-23339-4

Li, C., Cui, C., Zhang, J., Shen, J., He, B., Liu, Y., & Ye, J. (2023). Biodegradation of petroleum hydrocarbons based pollutants in contaminated soil by exogenous effective microorganisms and indigenous microbiome. *Ecotoxicology and Environmental Safety*, *253*, 114673. https://doi.org/10.1016/j.ecoenv.2023.114673

Libretexts. (2021). 1.6: Particle size Analysis: the Hydrometer Method. *Geosciences LibreTexts*. https://geo.libretexts.org/Bookshelves/Soil_Science/Principles_of_Soil_Science_Exercise_Manual_%28Bowen%29/01%3A_Hands-on_Exercises/1.06%3A_New_Page

Lichtenthaler, H. K. (1987). [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology* (pp. 350–382). Academic Press. https://doi.org/10.1016/0076-6879(87)48036-1

Lin, H., Yang, Y., Shang, Z., Li, Q., Niu, X., Ma, Y., & Liu, A. (2022). Study on the Enhanced Remediation of Petroleum-Contaminated Soil by Biochar/G-C3N4 Composites. *International Journal of Environmental Research and Public Health*, *19*(14), 8290. https://doi.org/10.3390/ijerph19148290

Lin, Q., Shen, K., Zhao, H., & Li, W. (2008). Growth response of Zea mays L. in pyrene–copper co-contaminated soil and the fate of pollutants. *Journal of Hazardous Materials*, *150*(3), 515–521. https://doi.org/10.1016/j.jhazmat.2007.04.132

Liu, A., Wang, W., Zheng, X., Chen, X., Fu, W., Wang, G., Ji, J., Chen, J., & Guan, C. (2022). Improvement of the Cd and Zn phytoremediation efficiency of rice (Oryza sativa) through the inoculation of a metal-resistant PGPR strain. *Chemosphere*, *302*, 134900. https://doi.org/10.1016/j.chemosphere.2022.134900

Liu, L., Li, W., Song, W., & Guo, M. (2018). Remediation techniques for heavy metal-contaminated soils: Principles and applicability. *Science of the Total Environment*, *633*, 206–219. https://doi.org/10.1016/j.scitotenv.2018.03.161

Lombi, E., Zhao, F., Dunham, S. J., & McGrath, S. P. (2001). Phytoremediation of Heavy Metal-Contaminated Soils: Natural Hyperaccumulation versus Chemically Enhanced

Phytoextraction. *Journal of Environmental Quality*, *30*(6), 1919–1926. https://doi.org/10.2134/jeq2001.1919

Luo, H., Xou, C., Chinnathambi, A., Nasif, O., Narayanan, M., Shanmugam, S., Chi, N. T. L., Pugazhendhi, A., On-Uma, R., Jutamas, K., & Anupong, W. (2023). Optimistic influence of multi-metal tolerant Bacillus species on phytoremediation potential of Chrysopogon zizanioides on metal contaminated soil. *Chemosphere*, *311*, 136889. https://doi.org/10.1016/j.chemosphere.2022.136889

Madariaga-Navarrete, A., Rodríguez-Pastrana, B. R., Villagómez-Ibarra, J. R., Acevedo-Sandoval, O. A., Perry, G. M., & Islas-Pelcastre, M. (2017). Bioremediation model for atrazine contaminated agricultural soils using phytoremediation (using*Phaseolus vulgaris*L.) and a locally adapted microbial consortium. *Journal of Environmental Science and Health Part B-pesticides Food Contaminants and Agricultural Wastes*, *52*(6), 367–375. https://doi.org/10.1080/03601234.2017.1292092

Mahfooz, Y., Yasar, A., Sohail, M., Tabinda, A. B., Rasheed, R., Irshad, S., & Rinklebe, J. (2019). Investigating the drinking and surface water quality and associated health risks in a semi-arid multi-industrial metropolis (Faisalabad), Pakistan. *Environmental Science and Pollution Research*, *26*(20), 20853–20865. https://doi.org/10.1007/s11356-019-05367-9

Mahto, K. U., Vandana, Priyadarshanee, M., Samantaray, D., & Das, S. (2022). Bacterial biofilm and extracellular polymeric substances in the treatment of environmental pollutants: Beyond the protective role in survivability. *Journal of Cleaner Production*, *379*, 134759. https://doi.org/10.1016/j.jclepro.2022.134759

Mbarki, S., Skalicky, M., Talbi, O., Chakraborty, A., Hnilička, F., Hejnak, V., Zivcak, M., Brestic, M., Cerdà, A., & Abdelly, C. (2020). Performance of Medicago sativa Grown in Clay Soil Favored by Compost or Farmyard Manure to Mitigate Salt Stress. *Agronomy*, *10*(1), 94. https://doi.org/10.3390/agronomy10010094

Mei, C., Chretien, R. L., Amaradasa, B. S., He, Y., Turner, A., & Lowman, S. (2021). Characterization of Phosphate Solubilizing Bacterial Endophytes and Plant Growth Promotion In Vitro and in Greenhouse. *Microorganisms*, *9*(9), 1935. https://doi.org/10.3390/microorganisms9091935

Meng, W., Chen, S., Chen, L., Wang, D., & Zhao, C. (2019). The responses of a soil bacterial community under saline stress are associated with Cd availability in long-term wastewater-irrigated field soil. *Chemosphere*, *236*, 124372. https://doi.org/10.1016/j.chemosphere.2019.124372

Mercier, G., Sorelli, L., & Blackburn, D. (2001). Prediction of Metal Removal Efficiency from Contaminated Soils by Physical Methods. *Journal of Environmental Engineering*, *127*(4), 348–358. https://doi.org/10.1061/(asce)0733-9372(2001)127:4(348

Michelsen, T. C., & Boyce, C. P. (1993). Cleanup standards for petroleum hydrocarbons. Part 1. Review of methods and recent developments. *Journal of Soil Contamination*, *2*(2), 109–124. https://doi.org/10.1080/15320389309383432

Moreira, H., Marques, A. C., Franco, A. R., Rangel, A. O. S. S., & Castro, P. M. L. (2014). Phytomanagement of Cd-contaminated soils using maize (Zea mays L.) assisted by plant growth-promoting rhizobacteria. *Environmental Science and Pollution Research*, *21*(16), 9742–9753. https://doi.org/10.1007/s11356-014-2848-1

Muhammad, D., Chen, F., Zhao, J., Zhang, G., & Wu, F. (2009). Comparison of EDTA- and Citric Acid-Enhanced Phytoextraction of Heavy Metals in Artificially Metal Contaminated Soil by*Typha Angustifolia*. *International Journal of Phytoremediation*, *11*(6), 558–574. https://doi.org/10.1080/15226510902717580

Mukome, F. N., Buelow, M. C., Shang, J., Peng, J., Rodriguez, M. J., Mackay, D. M., Pignatello, J. J., Sihota, N., Hoelen, T. P., & Parikh, S. J. (2020). Biochar amendment as a remediation strategy for surface soils impacted by crude oil. *Environmental Pollution*, *265*, 115006. https://doi.org/10.1016/j.envpol.2020.115006

Murray, E., Greenberg, B. M., Cryer, K., Poltorak, B., McKeown, J., Spies, J., & Gerwing, P. D. (2019). Kinetics of phytoremediation of petroleum hydrocarbon contaminated soil. *International Journal of Phytoremediation*, *21*(1), 27–33. https://doi.org/10.1080/15226514.2018.1523870

Mushtaq, M. U., Iqbal, A., Nawaz, I., Mirza, C. R., Yousaf, S., Farooq, G., Ali, M. A., Khan, A. H. A., & Iqbal, M. (2020). Enhanced uptake of Cd, Cr, and Cu in Catharanthus roseus (L.) G.Don by *Bacillus cereus*: application of moss and compost to reduce metal

availability. *Environmental Science and Pollution Research*, *27*(32), 39807–39818. https://doi.org/10.1007/s11356-020-08839-5

Nawaz, F., Rafeeq, R., Majeed, S., Ismail, M. S., Ahsan, M., Ahmad, K. S., Akram, A., & Haider, G. (2022). Biochar Amendment in Combination with Endophytic Bacteria Stimulates Photosynthetic Activity and Antioxidant Enzymes to Improve Soybean Yield Under Drought Stress. *Journal of Soil Science and Plant Nutrition*, *23*(1), 746–760. https://doi.org/10.1007/s42729-022-01079-1

Paz-Alberto, A. M., & Sigua, G. C. (2013). Phytoremediation: a green technology to remove environmental pollutants. *American Journal of Climate Change*, *02*(01), 71–86. https://doi.org/10.4236/ajcc.2013.21008

Petruzzelli, G., Pedron, F., Rosellini, I., Grifoni, M., & Barbafieri, M. (2016). Polycyclic aromatic hydrocarbons and heavy metal Contaminated sites: Phytoremediation as a strategy for addressing the complexity of pollution. In *Springer eBooks* (pp. 61–90). https://doi.org/10.1007/978-3-319-41811-7_5

Priya, A., Gnanasekaran, L., Dutta, K., Rajendran, S., Deepanraj, B., & Soto-Moscoso, M. (2022). Biosorption of heavy metals by microorganisms: Evaluation of different underlying mechanisms. *Chemosphere*, *307*, 135957. https://doi.org/10.1016/j.chemosphere.2022.135957

Qi, X., Gou, J., Chen, X., Xiao, S., Imran, A., Shang, R., Wang, D., Wu, Y., Han, M., & Luo, X. (2021). Application of mixed bacteria-loaded biochar to enhance uranium and cadmium immobilization in a co-contaminated soil. *Journal of Hazardous Materials*, *401*, 123823. https://doi.org/10.1016/j.jhazmat.2020.123823

Rasool, M., Akhter, A., Soja, G., & Haider, M. S. (2021). Role of biochar, compost and plant growth promoting rhizobacteria in the management of tomato early blight disease. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-85633-4

Rees, F., Simonnot, M., & Morel, J. (2013). Short-term effects of biochar on soil heavy metal mobility are controlled by intra-particle diffusion and soil pH increase. *European Journal of Soil Science*, *65*(1), 149–161. https://doi.org/10.1111/ejss.12107

Rezaenejad, Y. (2001, January 10). *Effect of organic matter on soil chemical properties and corn yield and elemental uptake*. JWSS - Isfahan University of Technology. https://jstnar.iut.ac.ir/article-1-177-en.html

Rong, L., Zheng, X., Oba, B. T., Shen, C., Wang, X., Wang, H., Luo, Q., & Sun, L. (2021). Activating soil microbial community using bacillus and rhamnolipid to remediate TPH contaminated soil. *Chemosphere*, *275*, 130062. https://doi.org/10.1016/j.chemosphere.2021.130062

Scotti, R., Bonanomi, G., Scelza, R., Zoina, A., & Rao, M. A. (2015). Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. *Journal of Soil Science and Plant Nutrition*, *ahead*, 0. https://doi.org/10.4067/s0718-95162015005000031

Segura, A., & Ramos, J. P. F. (2013). Plant–bacteria interactions in the removal of pollutants. *Current Opinion in Biotechnology*, *24*(3), 467–473. https://doi.org/10.1016/j.copbio.2012.09.011

Senthilkumar, M., Amaresan, N., & Sankaranarayanan, A. (2020). Estimation of malondialdehyde (MDA) by thiobarbituric acid (TBA) assay. In *Springer protocols* (pp. 103–105). https://doi.org/10.1007/978-1-0716-1080-0_25

Sessitsch, A., Kuffner, M., Echevarria, G., Vangronsveld, J., Wenzel, W. W., Fallmann, K., & Puschenreiter, M. (2013). The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology & Biochemistry*, *60*, 182–194. https://doi.org/10.1016/j.soilbio.2013.01.012

Shah, V., & Daverey, A. (2020). Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil. *Environmental Technology & Innovation*, *18*, 100774. https://doi.org/10.1016/j.eti.2020.100774

Shaheen, A., Iqbal, J., & Hussain, S. (2018). Adaptive geospatial modeling of soil contamination by selected heavy metals in the industrial area of Sheikhupura, Pakistan. *International Journal of Environmental Science and Technology*, *16*(8), 4447–4464. https://doi.org/10.1007/s13762-018-1968-4

Shivakumar, C. K., C, H., Swamy, B. T., & M, K. (2011). Effect of Inoculation with Arbuscular Mycorrhizal Fungi on Green Gram Grown in Soil Containing Heavy Metal Zinc. *Journal of Experimental Sciences*, *2*(10), 17–21. https://worldveg.tind.io/record/47857

Sidhu, G. P. S., Bali, A. S., Singh, H. P., Batish, D. R., & Kohli, R. K. (2020). Insights into the tolerance and phytoremediation potential of *Coronopus didymus* L. (Sm) grown under zinc stress. *Chemosphere*, *244*, 125350. https://doi.org/10.1016/j.chemosphere.2019.125350

Sidhu, G. P. S., Singh, H., Batish, D. R., & Kohli, R. K. (2017). Tolerance and hyperaccumulation of cadmium by a wild, unpalatable herb *Coronopus didymus* (L.) Sm. (Brassicaceae). *Ecotoxicology and Environmental Safety*, *135*, 209–215. https://doi.org/10.1016/j.ecoenv.2016.10.001

Sieuwerts, S., De Bok, F., Mols, E., De Vos, W. M., & Van Hylckama Vlieg, J. (2008). A simple and fast method for determining colony forming units. *Letters in Applied Microbiology*, *47*(4), 275–278. https://doi.org/10.1111/j.1472-765x.2008.02417.x

Sofy, M. R., Mohamed, H. I., Dawood, M. F. A., Abu-Elsaoud, A. M., & Soliman, M. H. (2021). Integrated usage of Trichoderma harzianum and biochar to ameliorate salt stress on spinach plants. *Archives of Agronomy and Soil Science*, *68*(14), 2005–2026. https://doi.org/10.1080/03650340.2021.1949709

Sommer, R. (2017, November 15). *Methods of Soil, Plant, and Water Analysis: A manual for the West Asia and North Africa Region: Third Edition*. https://repo.mel.cgiar.org/handle/20.500.11766/7512

Sun, L., Liao, X., Yan, X., Zhu, G., & Ma, D. (2014). Evaluation of heavy metal and polycyclic aromatic hydrocarbons accumulation in plants from typical industrial sites: potential candidate in phytoremediation for co-contamination. *Environmental Science and Pollution Research*, *21*(21), 12494–12504. https://doi.org/10.1007/s11356-014-3171-6

Tan, S., Narayanan, M., Huong, D. T. T., Ito, N., Unpaprom, Y., Pugazhendhi, A., Lan, N. T., & Liu, J. (2022). A perspective on the interaction between biochar and soil microbes: A way to regain soil eminence. *Environmental Research*, *214*, 113832. https://doi.org/10.1016/j.envres.2022.113832

Tran, H. M., Lin, C., Bui, X., Ngo, H. H., Cheruiyot, N. K., Hoang, H. S., & Shih, Y. J. (2021). Aerobic composting remediation of petroleum hydrocarbon-contaminated soil. Current and future perspectives. *Science of the Total Environment*, *753*, 142250. https://doi.org/10.1016/j.scitotenv.2020.142250

University of Canterbury. (n.d.). https://www.canterbury.ac.nz/media/documents/science-outreach/phosphate.pdf. In *https://www.canterbury.ac.nz*. https://www.canterbury.ac.nz/media/documents/science-outreach/phosphate.pdf

Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N. P., Rene, E. R., Sharma, N., & Sahi, S. T. (2017). Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in Leucaena leucocephala seedlings: A physiochemical analysis. *Plant Physiology and Biochemistry*, *110*, 59–69. https://doi.org/10.1016/j.plaphy.2016.08.022

Vidonish, J. E., Zygourakis, K., Masiello, C. A., Sabadell, G., & Alvarez, P. J. J. (2016). Thermal Treatment of Hydrocarbon-impacted soils: A review of Technology Innovation for Sustainable Remediation. *Engineering*, *2*(4), 426–437. https://doi.org/10.1016/j.eng.2016.04.005

Villalobos, M., Avila-Forcada, A. P., & Gutiérrez-Ruiz, M. (2008). An improved gravimetric method to determine total petroleum hydrocarbons in contaminated soils. *Water Air and Soil Pollution*, *194*(1–4), 151–161. https://doi.org/10.1007/s11270-008-9704-1

Vishnupradeep, R., Bruno, L. B., Taj, Z., Karthik, C., Challabathula, D., Tripti, Kumar, A., Freitas, H., & Rajkumar, M. (2022). Plant growth promoting bacteria improve growth and phytostabilization potential of Zea mays under chromium and drought stress by altering photosynthetic and antioxidant responses. *Environmental Technology and Innovation*, *25*, 102154. https://doi.org/10.1016/j.eti.2021.102154

Wan, X., Lei, M., Chen, T., Zhou, G., Yang, J., Zhou, X., Zhang, X., & Xu, R. (2013). Phytoremediation potential of Pteris vittata L. under the combined contamination of As and Pb: beneficial interaction between As and Pb. *Environmental Science and Pollution Research*, *21*(1), 325–336. https://doi.org/10.1007/s11356-013-1895-3

Wang, A., Fu, W., Yu, F., Liu, Z., & Song, D. (2022). Synergetic effects of microbial-phytoremediation reshape microbial communities and improve degradation of petroleum contaminants. *Journal of Hazardous Materials*, *429*, 128396. https://doi.org/10.1016/j.jhazmat.2022.128396

Wang, J., Zhao, J., Feng, S., Zhang, J., Gong, S., Qiao, K., & Zhou, A. (2020). Comparison of cadmium uptake and transcriptional responses in roots reveal key transcripts from high and low-cadmium tolerance ryegrass cultivars. *Ecotoxicology and Environmental Safety*, *203*, 110961. https://doi.org/10.1016/j.ecoenv.2020.110961

Wang, P., Wei, H., Tan, K. W., Fu, Y., Zeng, Y., Chen, C., & Chen, L. (2023). Characterization and genome analysis of Acinetobacter oleivorans S4 as an efficient hydrocarbon-degrading and plant-growth-promoting rhizobacterium. *Chemosphere*, *331*, 138732. https://doi.org/10.1016/j.chemosphere.2023.138732

Wu, J., Li, Z., Huang, D., Liu, X., Tang, C., Parikh, S. J., & Xu, J. (2020). A novel calcium-based magnetic biochar is effective in stabilization of arsenic and cadmium co-contamination in aerobic soils. *Journal of Hazardous Materials*, *387*, 122010. https://doi.org/10.1016/j.jhazmat.2019.122010

Xi, L., Wang, Y., Luo, T., Ma, Y., Wang, B., & Huang, Q. (2020). Remediation potential of immobilized bacterial strain with biochar as carrier in petroleum hydrocarbon and Ni co-contaminated soil. *Environmental Technology*, *43*(7), 1068–1081. https://doi.org/10.1080/09593330.2020.1815858

Xiong, Z., Zheng, H., Wu, J., Chen, W., Chen, Y., Gao, X., Yang, H., & Chen, H. (2021). Physicochemical and adsorption properties of biochar from biomass-based pyrolytic polygeneration: effects of biomass species and temperature. *Biochar*, *3*(4), 657–670. https://doi.org/10.1007/s42773-021-00102-5

Xu, L., Xing, X., Liang, J., Peng, J., & Zhou, J. (2019). *In situ* phytoremediation of copper and cadmium in a co-contaminated soil and its biological and physical effects. *RSC Advances*, *9*(2), 993–1003. https://doi.org/10.1039/c8ra07645f

Yadav, S., Rastogi, A., Shukla, A., Srivastava, S., & Yadav, S. (2018). Prospects of genetic engineering utilizing potential genes for regulating arsenic accumulation in plants. *Chemosphere*, *211*, 397–406. https://doi.org/10.1016/j.chemosphere.2018.07.152

Yan, A., Wang, Y., Tan, S. T., Yusof, M. L. M., Ghosh, S., & Chen, Z. (2020). Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00359

Yang, L., Wang, T., Zhou, Y., Shi, B., Bi, R., & Meng, J. (2021). Contamination, source and potential risks of pharmaceuticals and personal products (PPCPs) in Baiyangdian Basin, an intensive human intervention area, China. *Science of the Total Environment*, *760*, 144080. https://doi.org/10.1016/j.scitotenv.2020.144080

Ye, J., Rui, Z., Nielsen, S., Joseph, S., Huang, D., & Thomas, T. (2016). A combination of Biochar–Mineral complexes and compost improves soil bacterial processes, soil quality, and plant properties. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00372

Ye, S., Zeng, G., Wu, H., Zhang, C., Dai, J., Liang, J., Yu, J., Ren, X., Yi, H., Cheng, M., & Zhang, C. (2017). Biological technologies for the remediation of co-contaminated soil. *Critical Reviews in Biotechnology*, *37*(8), 1062–1076. https://doi.org/10.1080/07388551.2017.1304357

Yousaf, S., Khan, A. H. A., Muhammad, Y. S., & Iqbal, M. (2021). Soil conditioners improve rhizodegradation of aged petroleum hydrocarbons and enhance the growth of Lolium. . . *ResearchGate*. https://www.researchgate.net/publication/354046294_Soil_conditioners_improve_rhizodegradation_of_aged_petroleum_hydrocarbons_and_enhance_the_growth_of_Lolium_multiflorum

Yousaf, U., Khan, A. H. A., Farooqi, A. H. A., Shad, M. Y., Barros, R., Tamayo-Ramos, J. A., Iqbal, M., & Yousaf, S. (2022). Interactive effect of biochar and compost with Poaceae and Fabaceae plants on remediation of total petroleum hydrocarbons in crude oil contaminated soil. *Chemosphere*, *286*, 131782. https://doi.org/10.1016/j.chemosphere.2021.131782

Zahed, M. A., Salehi, S., Madadi, R., & Hejabi, F. (2021). Biochar as a sustainable product for remediation of petroleum contaminated soil. *Current Research in Green and Sustainable Chemistry*, *4*, 100055. https://doi.org/10.1016/j.crgsc.2021.100055

Zhang, J., Yang, N., Yani, G., Zhou, J., & Ji, L. (2019). Effects of the combined pollution of cadmium, lead and zinc on the phytoextraction efficiency of ryegrass (*Lolium perenne* L.). *RSC Advances*, *9*(36), 20603–20611. https://doi.org/10.1039/c9ra01986c

Zhang, X., Zhang, X., Wang, S., & Zhao, S. (2022). Improved remediation of co-contaminated soils by heavy metals and PAHs with biosurfactant-enhanced soil washing. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-07577-7

Zhang, Y., Li, F., Xu, W., Ren, J., Chen, S., Shen, K., & Zhang, L. (2019). Enhanced Phytoextraction for Co-contaminated Soil with Cd and Pb by Ryegrass (Lolium perenne L.). *Bulletin of Environmental Contamination and Toxicology*, *103*(1), 147–154. https://doi.org/10.1007/s00128-019-02661-7

Zhao, K., Wang, N., Jiang, S., Li, F., Luo, S., Chen, A., Li, H., Lin, X., Zhang, J., Zhang, L., Huang, H., & Luo, L. (2022). Potential implications of biochar and compost on the stoichiometry-based assessments of soil enzyme activity in heavy metal-polluted soils. *Carbon Research*, *1*(1). https://doi.org/10.1007/s44246-022-00029-x

Zhao, M., Dai, Y., Zhang, M., Feng, C., Qin, B., Zhang, W., Zhao, N., Li, Y., Ni, Z., Xu, Z., Tsang, D. C., & Qiu, R. (2020). Mechanisms of Pb and/or Zn adsorption by different biochars: Biochar characteristics, stability, and binding energies. *Science of the Total Environment*, *717*, 136894. https://doi.org/10.1016/j.scitotenv.2020.136894

Zhao, Y., Duan, F., Cui, Z., Hong, J., & Ni, S. (2021). Insights into the vertical distribution of the microbiota in steel plant soils with potentially toxic elements and PAHs contamination after 60 years operation: Abundance, structure, co-occurrence network and functionality. *Science of the Total Environment*, *786*, 147338. https://doi.org/10.1016/j.scitotenv.2021.147338

Zhou, C., Heal, K., Tigabu, M., Xia, L., Hu, H., Yin, D., & Ma, X. (2020). Biochar addition to forest plantation soil enhances phosphorus availability and soil bacterial community

diversity. *Forest Ecology and Management*, *455*, 117635. https://doi.org/10.1016/j.foreco.2019.117635

Zuzolo, D., Guarino, C., Tartaglia, M. C., & Sciarrillo, R. (2021). Plant-Soil-Microbiota combination for the removal of total petroleum hydrocarbons (TPH): an In-Field experiment. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.621581