Comparative Study of Soil Textures to Assess the Effect of Bacterial Augmentation and Organic Amendments on Degradation of Chlorpyrifos in Soil

By

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Dedicated to my deceased sister Haimma and my deceased maternal and paternal grandparents, who always wanted to see me excel in my studies but did not get a chance to witness this accomplishment

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Truly,

Hooria Ikram Raja

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List of Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
CFUs	Colony Forming Units
Chl a	Chlorophyll a
Chl b	Chlorophyll b
CPF	Chlorpyrifos
DETP	Diethyl thiophosphate
EC	Electrical Conductance
EPA	Environmental Protection Agency
FS	Fresh Soil
FS+P	Fresh Soil + Plant
FW	Fresh weight
GPX	Guaiacol peroxidase
KPK	Khyber Pakhtunkhwa
MDA	Malondialdehyde
NARC	National Agriculture Research Center
OC	Organochlorine
OM	Organic Matter
OOC	Oxidizable Organic Carbon
OP	Organophosphate
POPs	Persistent Organic Pollutants

ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
SS	Spiked Soil
SS+P	Spiked Soil + Plant
SS+P+B1	Spiked Soil + Plant + Bacterial Strain 1
SS+P+B2	Spiked Soil + Plant + Bacterial Strain 2
SS+P+B1+B2	Spiked Soil + Plant + Bacterial Strain 1 + Bacterial Strain 2
SS+C+P	Spiked Soil + Compost + Plant
SS+C+P+B1	Spiked Soil + Compost + Plant + Bacterial Strain 1
SS+C+P+B2	Spiked Soil + Compost + Plant + Bacterial Strain 2
SS+C+P+B1+B2	Spiked Soil + Compost + Plant + Bacterial Strain 1 + Bacterial Strain 2
SS+B+P	Spiked Soil + Biochar + Plant
SS+B+P+B1	Spiked Soil + Biochar + Plant + Bacterial Strain 1
SS+B+P+B2	Spiked Soil + Biochar + Plant + Bacterial Strain 2
SS+B+P+B1+B2	Spiked Soil + Biochar + Plant + Bacterial Strain 1 + Bacterial Strain 2
TCP	3,5,6-trichloro-2-pyridinol
TDS	Total dissolved solid
TMP	3,5,6-trichloro-2-methoxy-pyridine
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Abstract

Contamination of the soil and environment with chlorpyrifos and its metabolites is a serious environmental problem. Its inability to dissolve in water makes it exist in the soil for a longer period. Different soil textures have different rates of degrading chlorpyrifos. So, there is an urgent need to develop a strategy to remove chlorpyrifos for improving environmental health and assess which soil texture supports enhanced degradation in a sustainable way. Therefore, the current study was designed to find out the potential application of bio-augmentation and organic amendments for chlorpyrifos degradation. The wheat plant was inoculated with two different chlorpyrifos degrading bacterial strains named *Pseudomonas aeruginosa* and *Bacillus Vietnamensis*. Compost and biochar were used as organic amendments. Results of this study indicated that the treatment SS+B+P+B2 showed the highest chlorpyrifos degradation in sandy loam soil (75.8%) than in clayey soil (55.5%). Plant growth and development were significantly improved in sandy loam soil than in clayey soil. The maximum shoot length, fresh weight and dry weight in sandy loam soil was 41.1cm, 7.9g, and 3.6g respectively, while in case of clayey soil, the values were 38.1cm, 6.3g, and 1.7g respectively. The maximum root length, fresh weight and dry weight in sandy loam soil was 48.7cm, 10.1g and 6.4g respectively, while in clayey soil, the values were 32.3cm, 9.5g and 6.4g respectively. Changes in biochemical stress indicators depicted significantly reduced MDA and H_2O_2 content with increased Chlorophyll a, b, Total chlorophyll, and Carotenoid content along with a decline in plant antioxidant enzyme levels for ascorbate peroxidase, catalase, superoxide dismutase, and guaiacol peroxidase. Improved plant growth and the rhizospheric microbial count were also detected in the treatment SS+C+P+B2 of both the soils but the values were different as sandy loam soil ($8.47*10^7$) had more microbial count in comparison to clayey soil ($4.10*10^5$). There are no such studies that focused on the integration of these techniques for chlorpyrifos degradation in the rhizosphere of *Triticum aestivum.* Integrated bio-augmentation and organic amendment approach have a great potential for rhizoremediation of chlorpyrifos rather than a single biological method.

Key words: Biochar, Chlorpyrifos, Compost, Phytoremediation, *Triticum aestivum*

Chapter 1
Introduction

1.1. Pesticides

The term “pesticide” combines the meaning of several terms that refer to a wide variety of chemical substances, including fungicides that are used to control fungi, insecticides that are used to control various types of insects, herbicides for the control of weeds, termicides for the control of termites, molluscicides that are used to control mollusks, rodenticides that are used to kill vertebrates, nematicides to kill nematodes, and sanitizers and disinfectants that act as an antimicrobial agent (Kumar et al., 2019).

Pesticides are described by the United Nations for Food and Agriculture (UNFA) as a substance or combination of substances used to exterminate, suppress, or prevent various pests. Pesticides can be applied to farms in one of two different ways: as a liquid in the form of a spray, or as a solid in the form of a powder. It can quickly spread in environmental compartments, regardless of how it is used and can pollute the air, soil, surface water, and groundwater reserves (Kumar et al., 2019).

Pesticides can be divided into two categories: Systemic and Contact, depending on how they attack the targeted pest and interact with its cells. Contact insecticides are those that have an immediate impact on their target. For instance, when the target is heavily overgrown with weeds, herbicides might directly kill the weed. Systemic pesticides are pesticides that can harm their target by transferring them inside treated plants (Kumar et al., 2019).

Because of the significant usage of synthetic compounds in contemporary agriculture over the past half-century, the environment's health is steadily declining. In the last 50 years, a sizable number of pesticides have been developed in agriculture to guard crops against pests and diseases. The use of pesticides in agriculture to prevent or eliminate pests has major financial and agronomic benefits, but they also pose a considerable threat to the ecosystem due to leaching that contaminates soil and water (Nandi et al., 2022).

Pests that attack plants, people, and animals are managed by the use of pesticides on a global scale. As a consequence, they are soaked into the ground and remain there for a long time, harming living things (Ibrahim et al., 2013). The demand for food is rising

along with the rate of global population growth. Modern farming now heavily relies on the usage of pesticides to supply global food demand. Fungicides make up 27% of all pesticides used globally, followed by herbicides (44%), insecticides (22%), and other pesticides (7%).

After World War II, the world's food scarcity needed to be addressed. Therefore, farmers all over the world have employed a variety of weed and pest control programs as a result of the development in the agricultural industry (Gill &Mohhan, 2017). With the techniques of intensive farming and quick modernization, soil quality is gradually declining. Pesticide residues have accumulated in soil over the past few decades due to the extensive and ineffective use of pesticides, which has stifled the capacity of soil to purify itself and resulted in severe soil contamination and deteriorated soil quality (Liu et al., 2018).

Water contamination in the Midwestern United States is primarily caused by agronomic systems, specifically the use of pesticides and nitrogen fertilizers. Persistent organic pollutants, which can enter the water from a range of sources and cannot be removed by conventional water treatment techniques, are regarded as a significant cause of contamination in drinking water supplies. Pesticides are classed as POPs because of their persistent nature and capacity to withstand prolonged periods of time in environmental compartments (Nandi et al., 2022).

Any type of pesticide, whether sprayed directly to the target plant or intended to be sprayed to the top sections of the target plant or pest, is likely to go down in the soil and come into contact with soil organisms. Between 30 and 90% of the pesticides are applied directly to the soil, depending on the delivery method. The impact of a wide range of pesticides on soil food chains, various classes of soil organisms, and soil biochemical pathways varies greatly and depends heavily on the soil's ecology, the quantity and type of pesticide used, and the examined biotic categories (Nandi et al., 2022). Both biotic and abiotic mechanisms can metabolize pesticides. Modifications in the pesticide's lethality, elemental composition, and reactivity take place during biodegradation. Pesticide biodegradation is influenced by soil physiochemical variables, such as pH, organic matter

concentration, and soil temperature, in addition to pesticide solubility and microbiota (Sidhu et al., 2019).

When Rachel L. Carson's book "Silent Spring" reported on examples of short-term toxicity, people began to pay attention to the hazardous consequences of pesticides. Environmental substrates are more susceptible to these pesticides because of their toxic effects, and they eventually lose their viability and strength (Kumar et al., 2019).

Pesticides are widely employed, especially in developing countries, to generate food and energy. Yet, the environmental effects of pesticides are the main consideration, especially when agricultural runoff that passes through surface water comprises toxins from pesticides. It is estimated that 1 to 5 percent of field-applied herbicides are lost to surface discharge (Sidhu et al., 2019). Pesticides in soil have received the most attention since they are frequently employed to manage pests that harm farm crops as well as pests in homes, yards, and gardens. How much pesticide residue may remain in soil depends on several aspects, including the physicochemical characteristics and proportion of the compound applied, the type of organic additives included, the properties of soil, the application method, and the frequency and duration of application (Jaikaew et al., 2015).

The last quarter of the 20th century saw the emergence of environmental problems associated with modern intensive agriculture practices, including significant changes in plant and animal habitats as well as deteriorating air, soil, and water quality. Pesticide residues have been discovered in soil matrices as a result of the use of sludge-derived fertilizer for soil amendments due to contamination. These pesticides have enhanced bioaccumulation in the food chain as a result of overuse, which may be dangerous for both animal and human health (Sidhu et al., 2019). Some organochlorine (OC) insecticides were outlawed in the majority of industrialized economies due to their persistence and potential environmental danger. Organochlorides have been replaced with organophosphates (OP) because they can be quickly destroyed in environmental compartments.

Because of the rising levels of soil and groundwater contamination and the accompanying negative effects on local ecosystems and public health, extensive and repetitive use of some pesticides that have poor biodegradation rates and increased potency has been a

matter of worry. The quality of surface water is at stake in many areas because the fertilizers and pesticides transported by wastewater runoff are discharged straight into waterways or lakes (Nandi et al., 2022). The majority of studies have concentrated on reducing the environmental effects of subterranean discharge, and numerous studies have placed a strong emphasis on creating inline bioreactors to get rid of tile effluents from agrochemicals.

A silent threat to the public and environmental health is soil degradation. Pesticide-related water contamination and decreased soil production have become serious threats to the environment and natural things (Francisco et al., 2019). Because of their plant uptake and eventual entry into the food chain, pesticides can occasionally transform into more harmful byproducts that could result in more severe health issues. So, as a cost-effective and environmentally benign alternative to synthetic crop disease treatment, biocontrol could be the right approach (Akhtar et al., 2018). To control insects, weeds, and fungi, modern agriculture in the United States relies entirely on pesticides.

Over 127 mha of agricultural land is present on approximately 45 percent of US land, according to the US Department of Agriculture. According to estimates, the US agricultural sector spent $12 billion on pesticides in 2008. A wide range of pesticides is employed in the agricultural environment to lessen crop problems brought on by disease, dangerous insects, and weeds. Insecticides were used to treat 40.8 million ha of cropland in 2012, whereas herbicides were used to treat 115.5 million ha, according to the US Department of Agriculture. Even with improved agricultural techniques, pesticides can still get up in water through runoff during irrigation events or severe weather because farmLands are connected to water supplies such as lakes and rivers.If such incidents are not managed correctly, the marine facilities downriver may be damaged (Francisco et al., 2019).

One of the regions in the globe with the highest use of agricultural chemicals is Latin America. Along with Colombia, Brazil, and Argentina, Mexico is one of four Latin American nations that lead the ranking of pesticide users (Góngora-Echeverra et al., 2019). The dosage of pesticides used in farming must be carefully chosen, as doing so

increases the risk of numerous illnesses. Eating crops sprayed with pesticides is challenging due to pesticide traces in those crops (Hegazy et al., 2018).

1.1.1. Use of Pesticides in Pakistan

Pakistan's economy is heavily reliant on the agricultural industry, which contributes about 20.9 percent of the country's GDP and provides employment for nearly 67.5 percent of its entire population. The majority of individuals who reside in rural areas rely heavily on agriculture as a direct or indirect source of revenue. Vegetables and fruit are primarily farmed as horticultural commodities because they are an essential component of the daily diet of the Pakistani populace. Fruits and vegetables make up the majority of Pakistan's exports, which are primarily sent to India, the UAE, Saudi Arabia, and the United Kingdom (Kamran et al., 2014).

As Pakistan is an agrarian nation, 22.2 million ha of its land is used for the cultivation of various crops, and just 4.5 million ha are used for the production of fruits and vegetables. Due to rising domestic food requirements, horticultural productivity has expanded recently. However, much like other crops, fruit and vegetables are susceptible to insect damage. There are 108 insecticides, 39 herbicides, 30 fungicides, 6 rodenticides, and 5 acaricides used in Pakistan to protect against and suppress pest attacks and eradicate weeds.In Pakistan, the percentage of pesticides used in the past was very significant, including 74% insecticides, 14% herbicides, 9% fungicides, 2% acaricides, and 1% fumigants. Pakistan's Punjab province utilized 88.3 percent of these pesticides, followed by Sindh province's 8.2 percent, KPK province's 2.8 percent, and Balochistan province's 0.76 percent usage (Syed et al., 2014).

Water pollution has become a serious concern in Pakistan as a consequence of multiple environmental pollutants. Numerous types of pollutants (organic, biological, and inorganic) constitute the primary source of pollution in 70% of Pakistan's groundwater and surface water (Kamran et al., 2014).

According to Food and Agriculture Organization (FAO) standards based on the "Agriculture Pesticide Ordinance (1971)" and "Agriculture Pesticides Rules (1973),"

Pakistan's first set of laws and regulations governing the manufacturing, import, export, and surveillance of pesticides were created (Syed et al., 2014).

Pesticides assisted in the fight against pests, but many new issues are now emerging, including insect chemical and thermal stability, habitat destruction, damage to biocontrol agents, contamination of the air, soil, and water, and dangerous pesticide residues in the food chain. It is commonly recognized that contact with these pesticides for a little period of time can result in major health problems, such as rashes, convulsions, and gastrointestinal illnesses. According to several pieces of research carried out in Pakistan, people from Punjab, Balochistan, and Sindh have pesticide residues in their blood serum and fat samples. By correctly teaching farmers and ranchers about the management and usage of pesticides and by utilizing Integrated Pest Management approaches, the adverse effects of pesticides can be reduced (Syed et al., 2014).

1.1.2. Transportation and Fate of Pesticides

Numerous natural processes, including precipitation, outflow, thermal decomposition, accumulation, adsorption, and leaching, control the pesticide's fate and transit as it enters the soil environment. Adsorption determines how much pesticide is available in soil solution for more complex processes like leaching or breakdown. The processes of pesticide breakdown, adsorption, or transport as well as the parameters governing them have a significant impact on the likelihood that pesticides will survive in the soil (Jaikaew et al., 2015).

Climatic change, which manifests fluctuations in global or regional climate patterns like rainfall, cloud cover, sea levels, temperature conditions, and vapor pressure, has recently emerged as a global issue. The potential of pesticides leaking into groundwater has grown due to the rise in temperatures and rainfall rates brought on by climate change (Jaikaew et al., 2015).

1.2. Insecticides

Chemicals termed insecticides are used to kill insects or stop them from acting in an unwanted or damaging way. Their composition and mechanism of operation are used to classify them. Some insecticides, such as cholinesterase inhibitors, affect an insect's

nervous system, while others operate as growth regulators or endotoxins. Insecticides are frequently employed in agricultural, healthcare, occupational, domestic, and commercial purposes (e.g., control of roaches and termites). The most popular insecticides are carbamates, pyrethroids, and organophosphates (Nandi et al., 2022).

Insecticides are applied using a variety of formulations and delivery methods such as sprays, baits, and slow-release diffusion, which affect their transformation and movement. Mobilization of pesticides can take place by air deposition, subsurface flow, or runoff (Sidhu et al., 2019). High-intensity agriculture's soil degradation makes it easier for insecticides to enter waterways. Aquatic species can store certain pesticides, which they then pass on to their predators. Aquatic insects are particularly at risk from insecticides because they are intended to be fatal, but other aquatic creatures are also impacted (Jaiswal et al., 2017).

In an aquatic environment, several factors can affect an insecticide's potency. These include regional variations in the quality of water that have an impact on bioavailability such as temperature, suspended sediment, and dissolved organic carbon, as well as associations between pesticides and other contaminants. The effects of temperature on these pathways vary (Ubaid ur Rahman et al., 2021). With rising temperatures, the adsorption of hydrophobic pesticides to particulate organic carbon may be reduced. At reduced temperatures, many pesticides take longer to break down and convert, while the lethality of some insecticides rises as the temperature rises.

1.2.1 Organophosphate Insecticide

A phosphate (or thio- or dithio-phosphate) moiety and an organic moiety make up organophosphate insecticides (OP). The phosphate moiety is often replaced with an O,O-dialkyl group. These insecticides are strong cholinesterase inhibitors. They can either reversibly or irreversibly connect covalently to the acetylcholinesterase active site's serine residue, blocking the enzyme's normal ability to catabolize neurotransmitters (Jaiswal et al., 2017). The ramifications of this activity are not limited to insects; they can also affect both animals and people.

More than 30 percent of the insecticide market segments (including agriculture, home, and garden, industrial, corporate, and governmental) worldwide employ organophosphate insecticides, which have been used extensively in the United States for more than three decades (EPA, 2017). Due to its highly efficient characteristics as an insecticide, organophosphorus has been frequently used. Over 38% of all sales worldwide have been accounted for by it (Rathod and Garg, 2017). The most widely used types of organophosphorus insecticides include malathion, parathion, chlorpyrifos (CPF), and diazinon.

The most common routes of transmission to organophosphate insecticides include inhalation, ingestion, and direct skin contact. Although the Environmental Protection Agency (EPA) has curtailed residential use of the majority of organophosphate insecticides due to their toxicity as strong acetylcholinesterase inhibitors, such as chlorpyrifos and diazinon, their metabolites are still found in urine samples from the general population. Organophosphate insecticides cause lipid peroxidation, which leads to the generation of free radicals and is linked to DNA damage (Venugopal et al., 2021). Several chronic diseases, including cancer, diabetes, coronary heart disease, Parkinson's disease, and Alzheimer's disease, have been linked to exposure to organophosphate insecticides (Nandi et al., 2022).

1.3. Chlorpyrifos

The chemical name for the pesticide chlorpyrifos is O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothionate (CAS No. 2921-89-2). Since 1965, chlorpyrifos has been employed in both agriculture and non-agricultural settings as a pesticide. Agriculture requires it extensively. Remaining contaminants in food, dermal exposure, and air diffusion all expose people to them. After endosulfan, acephate, and monocrotophos, it is regarded as the fourth most extensively used pesticide (Gilani et al., 2016). It has a garlicky or rotten egg scent and is made up of white or translucent crystals. This type of pesticide kills a wide variety of pests, including mites, mosquitoes, lice, termites, fire ants, grubs, flea beetles, and pests associated with commercially significant crops (Rayu et al., 2017).

After soil, the pesticide gradually breaks down in 60 to 120 days, but depending on the setting, it can last up to a year. According to research, under anaerobic conditions, the half-life of CPF is 15 days in loamy soil and 58 days in clayey soil (Jaiswal et al., 2017). Because it is insoluble in water, this substance does not readily contaminate groundwater (Ajaz et al., 2005). In both neutral and acidic environments, it resists hydrolysis. Thus, the likelihood of hydrolysis increases as pH rises (Keerthana et al., 2021). According to reports, the degradation is greater in tropical settings than it is in cold ones. This is mostly explained by the increased photodegradation that occurs in tropical regions (Chai et al., 2009). The rate of application, pH, temperature, and moisture are the main determinants of CPF degradation. The molecule can be said to become persistent in acidic environments, at lower temperatures, with increased soil organic matter, and with less ultraviolet light.

1.3.1. Exposure Routes of Chlorpyrifos

Chlorpyrifos can enter the bodies of living organisms directly from the environment by following various pathways. Living organisms are interacted with CPF either by dermal routes or by inhalation of dietary exposure. Each pathway impact different organs of the body as it gets into contact with them.

1.3.1.1. Environmental Exposure

CPF can infect both humans and animals through the environment, and it can spread mostly through inhalation, ingestion, and the skin (Uchendu et al., 2012). In comparison to those who were not exposed, those who were affected show a higher level of chromosomal alterations, sister chromatid exchanges (SCE), and micronuclei (MN) production. Higher professional or worksite exposure limits are frequently experienced by floriculturists, agrarian farm laborers, fumigators, or pesticide applicators (who spray a variety of pesticides at any particular time and are, therefore, overwhelmingly probable to get subjected to various kinds of agrochemical combinations).According to Bolognesi (2003), the length of exposure, the amount of pesticide used, and whether or not personal protective equipment (PPE) was worn all have an impact on the outcomes of exposure. However, the main cause of incidents of non-occupational exposure is contact with CPF at trace amounts.

1.3.1.2. Dietary Exposure

Because CPF is frequently applied to crops, it leaves residues on food, either in an undissociated form or in a residual form, like TCP (Han et al., 2009). This, unavoidably, exposes people and animals to minute amounts of CPF that are found on wholegrain foods or in a variety of processed food items. It is believed that the main source of non-occupational CPF exposure in humans comes through dietary sources. Livestock can potentially be a key cause of CPF intake for people, in addition to plants. Due to CPF's solubility in water, which ranges from 0.7 to 2.0 mg/L at 20 to 25 °C, it can also seep into groundwater when combined with irrigation or precipitation. After spraying, it begins to bind more and more to the plant and soil components and very little CPF penetrates the water to leach underground. Even if CPF does get into the water, it will evaporate from the surface of the water (Ubaid ur Rahman et al., 2021).

1.3.1.3. Dermal Exposure

When compared to oral or inhaled routes, dermal uptake is typically not considered to be significant from the perspective of CPF exposure. Dermal exposure, however, is a crucial mechanism for chemical toxicity. According to a significant experiment performed by Nolan et al. (1984), only 1% of TCP was found in human urine following dermal exposure while 70% of TCP was obtained from urine after an oral dose of CPF. This significant discovery clarifies why CPF is poorly absorbed through the coetaneous route but is readily digested once eaten. The existence of keratinized layers on the dermis may be the cause. Yet, it also relies on how much skin is exposed and for how long (Ubaid ur Rahman et al., 2021).

1.3.1.4. Inhalation Exposure

Nevertheless, compared to oral and dermal uptake, the onset of exposure indicators was quicker in the case of inhalation. The majority of inhalation exposure to CPF, however, is likely to involve either agrarian consumption or sparsely populated residential areas, as a result of drift, even though the concentration levels are not considerable enough to pose a serious threat to society (Ubaid ur Rahman et al., 2021). This is because the sale of CPF for domestic use has been outlawed in many regions (Eaton et al., 2008). Consequently,

the majority of recent research is solely concerned with the workers exposed to CPF in agricultural activities and pesticide applicators.

1.3.2. Metabolites of Chlorpyrifos

There are two main biodegradation processes connected with the decomposition of CPF, both of which are influenced by aerobic and anaerobic microbes; these include catabolism and incidental metabolism (Racke, 1993). First, oxidative desulfuration of CPF occurs in the metabolic system that produces strong electrophilic intermediates, such as 3,5,6-trichloropyridinol (TCP) or diethyl (3,5,6-trichloropyridin-2-yl) phosphate (CP-oxan). However, diarylation also occurs to produce the metabolite diethylthiophosphate (DETP). The CP is generally comparable to the other OP insecticides and is most frequently oxidized to its main harmless metabolites (Ubaid ur Rahman et al., 2021). AChE enzyme activity is inhibited by the oxon form. Cytochrome P450 (CYP) oxidizes CP-oxan to an unstable intermediate, which is then dynamically hydrolyzed to produce DETP and TCP (Eaton et al., 2008).

These metabolites either leave the body in their natural form or conjugate with glucuronide and sulfate. According to certain research, CPF initiates hydroxylation in the soil to produce 3,5,6-trichloro-2-pyridinol (TCP), which then transforms into 3,5,6-trichloro-2-methoxy-pyridine (TMP) (Singh and Walker, 2006). Diethyl thiophosphate (DETP), a result of hydrolysis, is further broken down into phosphorothioic acid and ethanol. These hydrolyzed metabolites are one of the sources of phosphorous (P), sulfur (S), and carbon (C) as a result of the CP degrading microorganisms' additional actions on them (Ubaid ur Rahman et al., 2021). Chlorine removal from TMP results in 2,3-dihydroxypyridine, which is subsequently dichlorinated to produce 2,5,6-trihydroxypyridine. Following oxidation, the hydroxy metabolites yield carbon, amine metabolites, and inorganic phosphate. Maleamic acid is produced when the oxidation breakdown of 2,3-dihydroxypyridine occurs, opening up the pyruvic acid route (Reddy et al., 2013). From losing HCl, CPF can also create glutathione conjugates. In several tissues, the metabolite TCP or thio-TCP was conjugated with both glucuronic acid and sulfate through the free hydroxyl group (Naime et al., 2020).

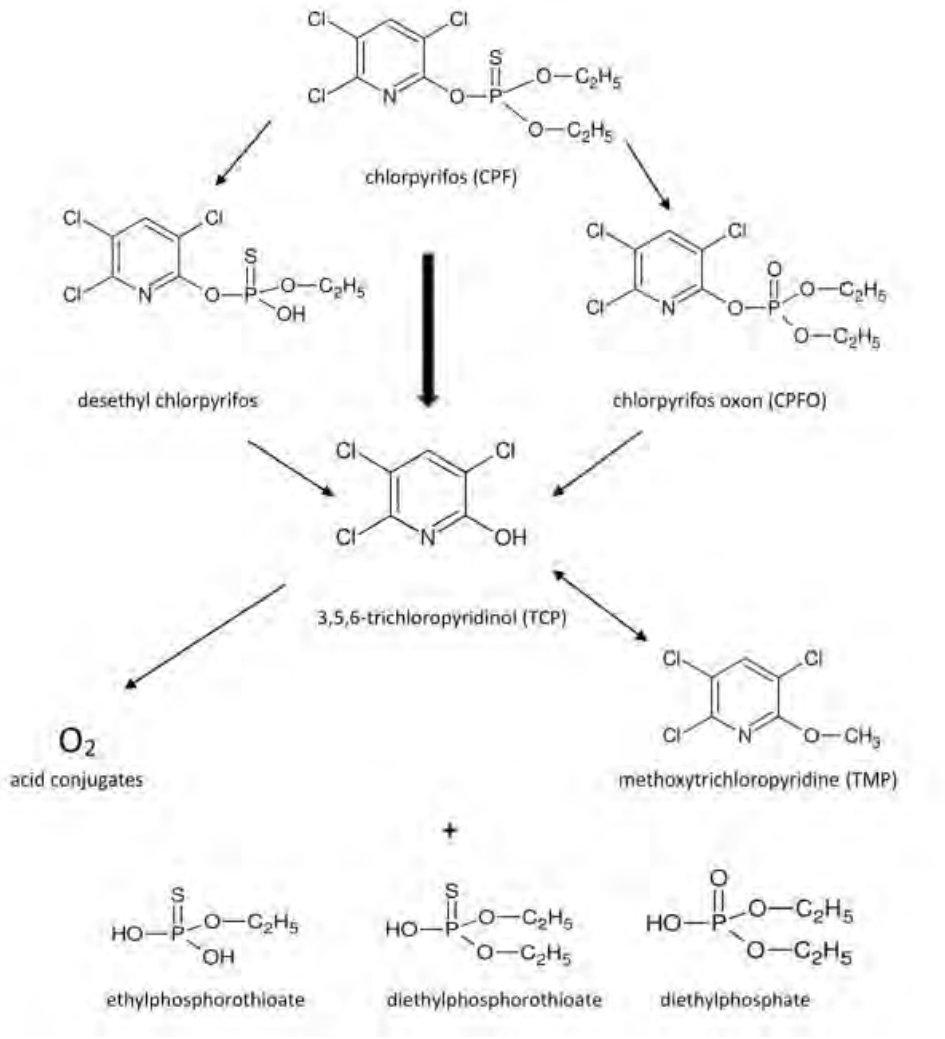

Figure 1.1: Chlorpyrifos Degradation into its Metabolites (Ubaid ur Rahman et al., 2021)

1.3.3. Harmful Effects of Chlorpyrifos

A range of aquatic and terrestrial ecosystems are negatively impacted by the improper use of OP pesticides on non-target crops and non-target animal populations (Sidhu et al., 2019). The bulk of OP is characterized by the US Environmental Protection Agency (USEPA) as having a toxicity class I to IV for inhalation and oral exposures. It is mild to moderately harmful to amphibians and fish and produces photo contact dermatitis, cancer, nausea, and vomiting in humans. It impacts plants by retarding their growth and

reducing chlorophyll content in them. CPF is also known for altering soil quality and mineral content.

1.3.3.1. Adverse Effects of Chlorpyrifos on Humans

Humans who have been exposed to CPF may have acute poisoning, which manifests as dizziness, twitching muscles, headache, increased perspiration, nausea, drowsiness, salivation, convulsions, and mortality. The symptoms start to manifest somewhere between a few minutes and two hours after consuming CPF because of its fast blood absorption. If the patient lives over the first 24 to 48 hours, CPF can be diagnosed. Acute inhalation of CPF causes dizziness, numbness or tingling, tachycardia, motor seizures that resemble seizures, and coma (Nandi et al., 2022). Increased dermal exposure to CPF and an undetermined rise in skin flushing cause the latter process, which culminates in death. Infants, young children, pregnant women, and individuals with pre-existing medical issues are especially susceptible to CPF poisoning.

The ability of CPF to block AChE and cause an elevated cholinergic tone is what determines their immediate neurotoxic effects. The acute systemic toxicity of OP substances results from the phosphorylation-mediated inhibition of the functions of the AChE enzyme. The metabolic disorders hyperglycemia and hypertriglyceridemia, which are common in obesity and type 2 diabetes, have been associated with CPF exposure. Acute and long-term human exposures have been associated with musculoskeletal consequences (Venugopal et al., 2021).

1.3.3.2. Adverse Effects of Chlorpyrifos on Animals

Behavioral changes, respiratory distress, epithelial hyperplasia, hydropic degeneration, erratic swimming, prolonged metamorphosis, curling of secondary lamellae, degeneration & necrosis of renal tubules, and shrinking of the glomerulus are all symptoms of CPF toxicity in aquatic fauna (Valcke et al., 2017). Larvae of the common toad (*Duttaphrynusmelanostictu*) displayed reduced swimming activity, morphological retardation, and postponed metamorphosis when subjected to six different concentrations (1–1500 microgram per liter) of CPF (Wijesinghe et al., 2011). In the species *Planorbariuscorneus*, the effects of OPs like CPF on the performances of carboxylesterases, glutathione S transferases, and ChE were examined for 14 days at two

different doses i.e., 0.4 and 5 micrograms per liter (Abass et al., 2015). With p-nitrophenyl acetate and butyrate, as well as AChE, there was a significant decline in survival, hatching percentage, and suppression of carboxylesterase synthesis (Rivadeneira et al., 2013).

1.3.3.3. Adverse Effects of Chlorpyrifos on Soil and Minerals

CPF is known to interact with both the soil's mineral surface and its organic matter content (Sidhu et al., 2019). The cellular bioavailability, bioaccumulation, transportation, and toxicity of pesticides in the environment are strongly influenced by their molecular interactions with soil organic and inorganic components (Morton and Edwards, 2005). A detailed comprehension of the interactions between soil and pesticides is necessary to comprehend the mechanisms involving soil-pesticide-minerals, soil-pesticide-organic matter, soil-pesticide-plants, and soil fertility (Polubesova and Chefetz, 2014). Four main variables affect how pesticides interact with soil: the kind of solute (pesticide), the solvent (usual water), the components of the soil, and pH. The pH is not the only factor to consider when predicting the stability of CPF; other factors like temperature, metal concentration, etc. also have a substantial impact (Nandi et al., 2022).

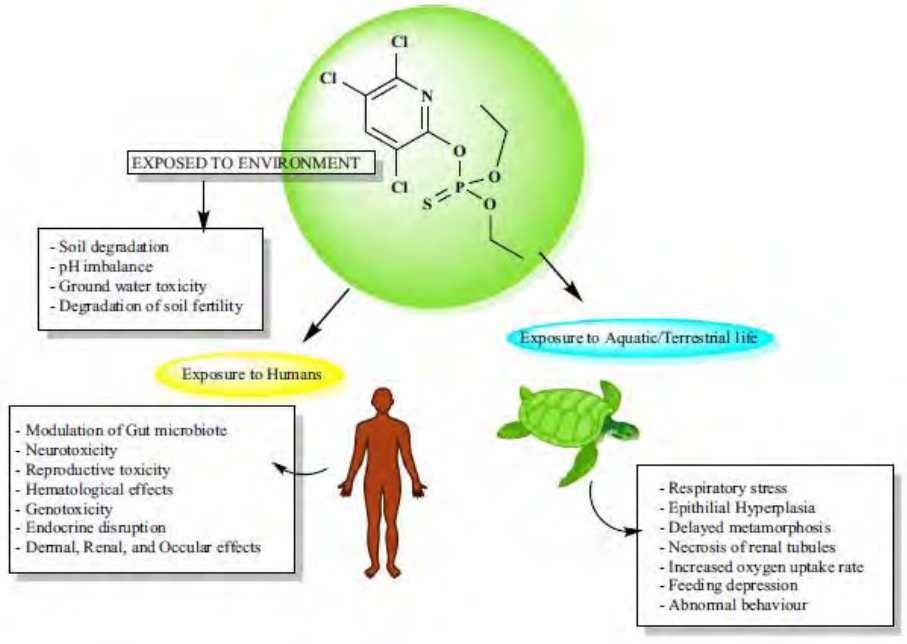

Figure 1.2: Impact of Chlorpyrifos on Environment, Human, Aquatic and Terrestrial Life (Nandi et al., 2022)

1.3.3.4. Adverse Effects of Chlorpyrifos on Plants

OP pesticides have been proven to have a deleterious impact on several biological processes, including photosynthesis, plant mineral nutrition, carbon metabolism, photochemical reactions, chlorophyll biosynthesis, fatty acid synthesis, amino acid synthesis, and nitrogen metabolism, and oxidative stress (Tiwari et al., 2019). It has been demonstrated that CPF inhibits plant development and nitrogen metabolism in *V. radiata* (Sidhu et al., 2019). CPF prevents nitrogen fixation in *Pseudomonas stutzeri* A1501-containing rice-vegetated soil (Lu et al., 2020). The weights of maize plants' shoots and fresh roots were both considerably decreased by CPF. With increasing levels of CPF, maize plants displayed increased residual concentrations of CPF in both shoots and roots.Alfalfa (*Medicago sativa*), clover (*Melilotus alba* and *Trifolium pratense*), *Pinus halepensis*, and *Arabidopsis thaliana*have all been demonstrated to be negatively impacted by CPF (Nandi et al., 2022).

1.3.3.4.1. Adverse Effects of Chlorpyrifos on Wheat Plant

Root and shoot lengths were found to significantly decrease at 20 and 40 mg/kg chlorpyrifos. The root and shoot lengths fell by 24.3 and 13.3 percent, respectively, at 20 mg/kg, compared to the controls. Wheat seedlings were adversely affected by chlorpyrifos; the main symptoms were decreased growth, decreased chlorophyll content, and instability of the antioxidant system. Additionally, wheat treated with chlorpyrifos contained more MDA (a consequence of lipid peroxidation). These findings showed that chlorpyrifos-induced moderate to severe oxidative stress affected wheat seedlings (Wang & Zhang, 2017).

1.4. Conventional Methods for Pesticide Remediation

Conventional pesticide treatment methods call for containment, which entails building a wall to stop toxins from moving from one area to another and vice versa. Later, several techniques, including in situ and ex-situ, for restoring pesticide-contaminated soils were devised. Ex situ methods include thermal desorption at low temperatures, soil flushing, evacuation, and cremation. Depending on the kind of technique used, these can also be divided into physicochemical, biological, and thermal strategies (Kumar et al., 2019).

Physicochemical processes, such as air sparging, soil vapor extraction, dechlorination, soil flushing, and stabilization remove contaminants using various physical and chemical approaches. Thermal procedures, such as thermal incineration, thermal desorption, and vitrification, generally use high temperatures to disintegrate or burn contaminants or use various strategies to cause compounds to volatilize (Kumar et al., 2019).

1.5. Modern Techniques for Chlorpyrifos Degradation

Numerous studies on CPF removal from soil have been conducted in recent years, and several techniques, including bioremediation, phytoremediation, and physical adsorption, have been developed to treat polluted soil. Among the prevalent physical, chemical, and biological approaches for removing CPF from soil and water, biological mechanisms have been preferred above other content (Sidhu et al., 2019). When compared to traditional approaches, the conventional biological approach is irreversible, environmentally friendly, economical, and non-intrusive. Therefore, both industry and academia place great importance on the biodegradation of environmental toxins at ecologically relevant levels (Aziz et al., 2021). To biodegrade and eliminate chemical compounds from soil and convert them into less complex non-toxic chemicals, several microbes and plants are required.

1.5.1. Bioremediation

The safe and affordable bioremediation method breaks down xenobiotic chemicals or toxins by microorganisms (fungi and bacteria) primarily as a means of ensuring their existence. Ex-situ bioremediation uses bioreactors, biofilters, soil cultivation, and composting, whereas in-situ bioremediation uses bio-rehabilitation, biosparging, and composting (Kumar et al., 2019).

1.5.2. Phytoremediation

When it comes to storing, absorbing, sequestering, eliminating, or degrading contaminants from sediment, soil, groundwater, and surface water, phytoremediation is generally referred to as biotechnology (Salamanca et al., 2015). Because it uses naturally grown plant species and does not produce secondary contaminants, phytoremediation has a reputation for being a cheap, environmentally benign, and aesthetically pleasing technique. Pesticides are broken down by plants through adsorption, degradation,

volatilization, accumulation, or by enhancing rhizosphere activity in the soil (Kumar et al., 2019).

It is possible to remove soil, freshwater, and groundwater toxins from plant tissues using a process called phytoextraction or to trap them in the roots using a process called rhizofiltration. By using plant enzymes, some pollutants can be converted into less hazardous forms, a process known as phytotransformation, or they can be released into the atmosphere through a process known as phytovolatilization (Aziz et al., 2021). Rhizoremediation is the term for the procedure that occurs when microorganisms in the plant's root zone break down a soil contaminant.

Although plant species were employed to treat radionuclide-contaminated soils in the 1950s, the idea did not become popular until the 1980s. Contrary to phytoremediation, traditional soil remediation techniques can result in secondary pollution and are costly. In 1991, the term "phytoremediation" and the burgeoning field were established. Because they are significantly safer for life, growth, and development under specific stress situations, native species of plants must be used for phytoremediation. Many experts advise using native plants with quick growth and improved biomass properties for phytoremediation (Kamran et al., 2014).

A plant's ability to absorb and translocate organic molecules depends on the physiochemical characteristics of pollutants, such as solubility, hydrophobicity, and polarity, as well as the species of plant, molecular weight, and ambient factors.There is a direct correlation between pesticide use and vegetation in a field. Organic pollutants are often removed more quickly in cultivated soils than in soils with fewer plants. Thus, using plants in contaminated areas can address some of the clear issues with the biological clean-up technique, such as a low microbial population or inadequate biological growth (Nandi et al., 2022).

Using methods like stiff-grass hedges, riparian buffers, vegetated drainage ditches, and artificial wetlands, phytoremediation is applied in agricultural settings. Numerous studies have demonstrated how vegetation can reduce pollutant loads, notably pesticide content (Sidhu et al., 2019). Only a few researches have demonstrated the importance of plant rhizosphere in promoting biological xenobiotic breakdown.

1.5.3. Rhizoremediation

Strong microbiological activity in the rhizosphere, a thin layer of soil near the plant roots, is brought on by root exudates that transport organic acids, amino acids, and carbohydrates. Rhizoremediation is the term used to describe the removal of pesticides through the rhizosphere, which includes the actions of the roots, the soil surrounding them, and the microbial population that lives there (Nandi et al., 2022). Pesticides are degraded or removed from the soil by several metabolic activities using this strategy. The genetic make-up and strain of the microorganisms existing in the roots are mostly responsible for removing pesticides from the soil.

1.6. Soil Texture and Effects on Plant Growth

Different regions' soil has different texture, which is largely determined by the size of the particles (Sangitaet al., 2015). The impact of soil texture on root penetration and aeration is evident. It has an impact on the soil's nutrient level as well. Sandy soils are lightweight soils with a low nutrient concentration, a low capacity to retain moisture, a low cation exchange capacity, a low capacity for buffering, and a high rate of permeability. Sustaining moisture retention potential and nutrient insufficiency are the key issues with sandy textured soils (Dipti et al., 2013). There is little organic matter in sandy soil. Al-Omran claimed that the introduction of clay deposits to sandy-textured soil enhanced the production of the squash crop (Al-Omran et al., 2005).Deep-rooted crops are less suited to sandy loam textures, which offer less resistance to root penetration (Nwachokor et. al., 2009). Carter claims that clay content added to sandy soil reduces hydrophobicity and boosts crop yield. Because they have low permeability and are thus forced to stay wet for a longer time than soils with a lighter texture, clayey soil is undesirable for crops that cannot survive prolonged soil wetness (Moody & Phan, 2008). Clay has greater content of organic matter in comparison to other soil textures.

1.7. Soil Amendments as a Bio-Stimulating Agent

By improving adsorption on the top layer of soil, soil amendments can reduce the movement of pesticides while also strengthening soil qualities and maintaining cultivation. Nevertheless, the effect of the amendment on pesticide adsorption and degradation differs depending on the pesticide's qualities, the soil's parameters, the origin

of the modifier, farming techniques, and the environment. Effective, affordable, and locally available modifications have evolved; thus, it is crucial to think about how these soil amendments may affect the breakdown of pesticides (Ghosh et al., 2016).

1.7.1. Compost

In several studies, the usage of compost was found to provide several advantages, including an increase in the amount of soil organic matter (SOM), an increase in the availability of plant nutrients, a decrease in bulk density, and an increase in overall stability. Additionally, there have been cautions on the negative impacts of compost use, which are related to probable contamination worries as a result of increasing levels of very toxic trace elements in some composts made from municipal waste (Paradelo et al., 2019).

1.7.2. Biochar

The ability of biochar to remediate soil as a new soil additive has drawn a lot of attention. Biomass pyrolysis produces the biochar in the absence of oxygen, either completely or partially. Biochar is frequently employed as a soil amendment or adsorbent because of its high cation exchange power, high surface area, aromatic, and condensed form. Presently, research has shown a rise in the potential and effectiveness of biochar-amended soils for the sorptive technique for various pollutants, including pesticides, antibiotics, and other hydrophobic organic compounds. Thus, it is suggested that these changes be used to regulate the mobility of the pollutant CPF by immobilizing it at a contaminated site (Aziz et al., 2021).

According to several earlier studies, the use of biochar and compost is a viable method for combining immobilization with bacterial remediation. With their special nutrients and components, biochar and compost be efficient soil remediators and will have a significant effect on the microbiological habitat of soil as well as on morphologic structures. According to some researchers, applying biochar or compost at relatively modest levels promoted the growth of enzymes and microorganisms and increased microbial diversity, which improved the soil ecosystem's capacity to break down organic contaminants (Aziz et al., 2021).

1.8. Integrated Approach for Chlorpyrifos Degradation

A range of microorganisms, including bacteria and fungi, were used in several studies to degrade CPF from various media. There have been cases where the only thing employed to clean up CPF contamination was plants (Nayak et al., 2021).

Both of these sorts of remediation techniques bring benefits to both sides and drawbacks as well. CPF-polluted soils have been effectively remedied using these biological approaches, both in-situ and ex-situ. The issue with using microorganisms to cure contaminated sites is their associated time because it takes months to many years to obtain the necessary eradication of contaminants, and because of the contaminant's harmful effects on the microbial strain and the microbial strain's responsiveness to breakdown either one or a few contaminants, which makes a significant contribution to the sustenance of the majority of CPF residues throughout the environment (Nayak et al., 2021).

A sustainable alternative known as phytoremediation leaves no long-term environmental secondary contamination in its wake. Additionally, it has been demonstrated that organic contamination is typically eliminated more quickly in cultivated soils than in non-cultivated soil. By using the vegetation at contaminated sites, it may be possible to get around some major limitations associated with the biological cleaning procedure. It is ordinarily employed as an attractive and affordable option (Aziz et al., 2021).

By overcoming the constraints of individual processes, it has been claimed that the continuous application of several therapies improves the potential for repair. To improve the CPF remediation process, it is necessary to research the combined application of bacteria, plants, and organic amendments.

1.9. Problem statement

Nowadays contamination of the soil, water, and groundwater with Chlorpyrifos and its metabolites is a serious environmental problem. Due to its long half-life, less biodegradability, human carcinogenicity, soil fertility disruption, and a threat to aquatic and terrestrial life; chlorpyrifos persists in the environment for a longer period. Further, plant growth varies in soils of different textures because of their properties. So, there is

an urgent need to develop a strategy for CPF removal and enhanced plant growth in different soil textures.

1.10. Research Objectives

The aim of this study is:

- To develop an environmentally sustainable, fast, and efficient technique for the cleanup of CPF from contaminated soil to ensure food safety and public health
- To compare the effect of two soil types on CPF degradation, plant growth and bacterial count
- To examine the influence of *Bacillus vietnamensis* and *Pseudomonas aeruginosa* on CPF degradation and the growth of the wheat plant (*Triticum aestivum*) in CPF contaminated soil
- To assess the role of organic amendments, i.e., compost and biochar, in degrading chlorpyrifos in soil and the growth of the wheat plant

Chapter 2
Materials and Methods

2.1. Collection and Preparation of Soil Samples

The soil of two different textures was collected from two different locations. For experiment 1, the soil was collected from a nursery in Bara Kahu, and for experiment 2, the soil was collected from farms in Chak Shahzad. To remove all forms of debris and plant residues, the collected soil samples were air dried and sieved using a 2mm sieve. To attain soil concentrations of 250mg/kg of chlorpyrifos (CPF), it was physically spiked with a liquid solution of CPF. As CPF is not completely soluble in water, therefore, 1kg soil was sprayed with 49.5mL of water and then, a solution of CPF with 0.5mL of acetone was added and mixed thoroughly. It was then kept undisturbed for 15 days for stabilization. Chemical Stress Ecology Lab, Quaid-i-Azam University, provided CPF.

2.2. Soil Amendment with Compost and Biochar

The spiked soil used for some treatments was amended with 5% (w/w) compost and 5% (w/w) biochar. The compost made of animal manure and biochar composed of wheat straw were used in the experiments and both of them were obtained from the University of Agriculture, Faisalabad (Naveed et al., 2021).

2.3. Minimum Inhibitory Concentration (MIC) and Bacterial Inoculum Preparation

Two pre-isolated bacterial strains including *Bacillus Cereus* (KM248376) and *Bacillus Vietnamensis* (KY933463) were taken from Environmental Microbiology and Bioremediation Lab, QAU, Islamabad. Seven bacterial strains including *Sphingobacteriumpakistanensis* (NCCP-246), *Rhodococcus sp.* (NCCP-309), *Stenotrophomonas sp.* (NCCP-614), *Cellulomonas pakistanensis* (NCCP-11), *Alcaligenes pakistanensis* (NCCP-650), *Brachybacterium paraconglomeratum* (NCCP-801) and *Pseudomonas aeruginosa* (NCCP-821) known for degrading pesticides were taken from NARC, Islamabad. In total, MIC of nine strains was checked to select the best performing ones.

Bacterial strains were checked at 50mg/kg, 100mg/kg, 200mg/kg, and 400mg/kg. A stock solution of 10,000mg/kg was prepared by adding 100mg of chlorpyrifos in 10mL

acetone. The nutrient agar plates were prepared and kept in incubator for 48 hours at 30°C. The growth was visible after 48 hours but no proper difference could be seen. Due to this, all the strains were again tested at 600, 800 and 1000mg/kg using the same methodology. Two strains, i.e., *Bacillus vietnamensis* and *Pseudomonas aeruginosa* were selected for further inoculation because they showed their growth at 1000mg/kg better than other strains.

2.4. Pot Experiment

Pot experiment was designed having fourteen different treatments applied on *Triticum aestivum* L. in two different soil types.

2.4.1. Plant material

Wheat (*Triticum aestivum* L.) seeds of variety Zincol-2016 were taken from the National Agricultural Research Center (NARC), Islamabad. For purification and sterilization, the seeds were soaked in a pre-conditioned solution and finally washed (for three times) with sterile distilled water. The chemical composition of solution used for seed soaking was 5% sodium hypochlorite and 70% ethanol.

2.4.2. Inoculum Preparation

The soil was inoculated with two selected bacterial strains, *Bacillus vietnamensis* and *Pseudomonas aeruginosa***.** Nutrient broth was prepared for both strains. Single colony was picked with a loop and then dipped into the broth and the flasks were placed on a shaker at 30ºC for 24 hours. When the optical density of the inoculum reached 0.9 or above at 600 nm, each flask sample was centrifuged at 10,000 rpm for 15 minutes to obtain bacterial pellet. The pellet was re-suspended in distilled water in a ratio equal to original one. Then 15mL tube was added in each pot. An equal volume of sterile water was used in the un-inoculated control pot (Ren et al., 2019).

2.4.3. Experimental Design

Pots were filled with 500 g pot^{-1} of spiked soil (clay and sandy loam) in the mentioned dimensions (15×7×7cm) and placed in the greenhouse. For compost and biochar treatments (5% w/w) of both were added in soil prior to the sowing of seeds. 30 surface-

sterilized seeds of wheat were planted in each pot. Bacterial inoculum was added to the soil twice during the experiment. For the first inoculation, the prepared bacterial inoculum was used to coat the seeds before sowing them. For this purpose, peat moss was used as a binding agent. 30 seeds were taken, soaked with 10mL bacterial inoculum and a little amount of peat moss for each pot. For the second inoculation, each pot was inoculated with 25 mL of inoculant suspension (10^8 cells mL^{-1}) by evenly spreading the suspension on to the surface of soil in the whole pot. Bacterial suspensions were cultivated in nutrient broth at 30ºC, centrifuged and resuspended in 0.9 % (w/v) NaCl containing one of the strains described above. For control treatments, spiked soil was treated with equal volume of 0.9 % NaCl instead of inoculum suspension. One week after seed germination, seeds were thinned to 25 to maintain equal number in each pot.

Predefined greenhouse conditions 16h light: 8h dark, at 25ºC, and constant moisture levels (after every two days, 20mL of water) were maintained throughout the growth period of ryegrass. Each of the pots was placed on the saucers to avoid contaminant leaching. Complete randomized block design (CRBD) was followed for pots placement in the greenhouse. Each treatment had three replicates. The experiment has the following treatments.

2.4.4. Treatment Plan

Different individual and combination treatments including plant, bacterial strains and organic amendments were performed to analyze the effects of various CPF degrading techniques.

Table 1.1: Description of Treatments

T	Description
T1	Fresh Soil+Plant
T2	Soil+CPF (Spiked Soil)
T3	Spiked Soil+Plant
T4	Spiked Soil+Plant+*Bacillus vietnamensis*
T5	Spiked Soil+Plant+*Pseudomonas aeruginosa*
T6	Spiked Soil+Plant+*Bacillus vietnamensis*+*Pseudomonas aeruginosa*
T7	Spiked Soil+Plant+5% Compost
T8	Spiked Soil+Plant+5% Compost+*Bacillus vietnamensis*
T9	Spiked Soil+Plant+5% Compost+*Pseudomonas aeruginosa*
T10	Spiked Soil+Plant+5% Compost+*Bacillus vietnamensis*+*Pseudomonas aeruginosa*
T11	Spiked Soil+Plant+5% Biochar
T12	Spiked Soil+Plant+5% Biochar+*Bacillus vietnamensis*
T13	Spiked Soil+Plant+5% Biochar+*Pseudomonas aeruginosa*
T14	Spiked Soil+Plant+5% Biochar+*Bacillus vietnamensis*+*Pseudomonas aeruginosa*

The inherent capacity of each treatment was measured individually, and the effectiveness of each procedure was compared. Plants were harvested 60 days after sowing; soil and plant samples were collected for further analysis.

2.5. Soil Analyses

Soil samples from each treatment were taken before sowing and after harvesting the plants. These samples were then used for various analyses, such as determining the soil's physico-chemical properties and the concentration of nutrients. pH, electrical conductivity (EC), total dissolved solids (TDS), extractable phosphate, available nitrates, oxidizable organic carbon (OOC), total organic carbon (TOC), and organic matter (OM) were analyzed. To determine their involvement in nutrient availability and soil remediation, soil enzymes were also examined. The details of the analyses are described in the sections below:

2.5.1. Soil Physicochemical Analyses

Soil physicochemical analysis include basic soil parameters such as soil texture, pH, TDS, and EC along with organic matter content, total organic carbon, oxidizable organic carbon, extractable phosphates, and available nitrates. The details about their measuring procedure are explained below:

2.5.1.1. Soil Texture

40g of dry soil and 60mL of sodium hexa-meta-phosphate dispersion (4:1; ($NaPO_3$)13: Na_2CO_3) was combined in a beaker. The beaker was covered with a watch glass and left overnight. The contents were transferred quantitatively the following day. There were around three-quarters of water in a glass of mixed soil. It was left on shaking overnight. Quantitatively, the suspension was moved to a calibrated 1-liter cylinder, where it was diluted with water to volume. The blank received the same treatment, but without soil. In order to determine the amount of sand, silt, and clay in the soil, the hydrometer method was employed to assess the soil's texture (Estefan et al., 2013).

2.5.1.2. Soil pH, EC, and TDS Quantification

EUTECH PC 510 was used to measure soil pH, EC, and TDS. 10g of soil was added to a beaker with an overhead balancer. After that, 50mL distilled water was added to create an approximate 1:5 weight soil-water suspension. The suspension was stirred on orbital shaker before being allowed to stand for 30 minutes (Khan et al. 2019b). The pH electrode was calibrated to 6.86 using the standard buffer solution, and the temperature was set to room temperature. The electrode was completely cleaned with distilled water, and any water droplets left on the electrode tip were removed with tissue paper. The reading was recorded once the probe has been in the sample for at least a minute. Similar procedure was used for EC and TDS using their respective probe.

2.5.1.3. Soil Extractable Phosphorus Quantification

The conventional “Olsen's sodium bicarbonate method” was used to measure the amount of extractable phosphorus in soil samples (Estefan et al., 2013). 50mL of $NaHCO_3$ was added to a flask containing 2.5g of soil. The pH of $NaHCO_3$ was adjusted to 8.5. For 30 minutes, the flask was covered and placed in an orbital shaker at 150 rpm at 30°C. 10mLfiltrate was taken out, and its pH will be raised to 5. After that, 8mL of Reagent B

was added. Distilled water was added to bring the volume to 40mL. Reagent A and ascorbic acid were mixed to create Reagent B. The solution was placed for ten minutes. The same steps were taken to prepare a blank, but without soil. A UV-Vis spectrophotometer was used to measure absorbance at a wavelength of 882nm. Using values acquired from the calibration curve, a standard was created, and the amount of extractable phosphorus in ppm was computed.

The concentration of extractable phosphorus in ppm was quantified using following formula:

$$P\ (inppm) = Valuederivedfromcalabrationcurveusing Absorbance \times V \times V2/Wt \times V1$$

Here, V= Volume of the total extract (mL), V1= Volume of soil extract used for analysis (mL), V2= Volume of flask used in analysis (mL) and Wt= Weight of air-dried soil (g).

2.5.1.4. Soil Nitrates Quantification

Chromotropic acid method was used for the quantification of soil nitrates (Estefan et al., 2013).1g of soil was extracted to test for nitrates, and 5mL of a 0.02N $CuSO_4.5H_2O$ solution was added. After 15 minutes of shaking, 3mL was filtered out. The flasks were placed in an ice bath and 1mL of 0.1 percent chromotropic acid was added drop by drop. The mixture was allowed to cool without being stirred. 6mL of concentrated $H_2SO_4$was added after it has been stirred for a few minutes. The same steps were taken to prepare a blank, but without soil. After 20 minutes, a yellow color appeared, and a UV-Vis spectrophotometer was used to measure the absorbance values at a wavelength of 430nm. Using values acquired from the calibration curve, a standard was created and the amount of NO_3 in ppm was measured.

The concentration of NO_3 in ppm was quantified using following formula:

$$NO_3(inppm) = Valuefromcalabrationcurveusing A\text{bsorbance} \times V \times V2/Wt \times V1$$

Here, V= Volume of the total extract (mL), V1= Volume of soil extract used for analysis (mL), V2= Volume of flask used in analysis (mL) and Wt= Weight of air-dried soil (g).

2.5.1.5. Soil OOC, TOC, and OM

OOC, TOC, and OM in the soil were measured using the "Walkley-Black method" (Estefan et al., 2013). 5mL of 1 N potassium dichromate was combined with 0.5g of air-dried soil. After adding 10mL of concentrated H_2SO_4 to the resultant solution, it was kept for 30 minutes. After 30 minutes, 100mL of distilled water and 5mL of conc. $H_3PO_4$was added to the solution. A few drops of diphenylamine were added to the resultant solution before being titrated with a 0.5 M solution of ferrous ammonium sulphate. After titrating, the transition from violet to green was carefully watched. The blank was prepared by the same protocol with the exception of soil. The concentrations of OOC, TOC, and OM were measured by using the following equations:

$$OOC\ (\%) = (VB - VS) \times 0.3 \times 10Wt \times VB$$

$$TOC\ (\%) = 1.334 \times OOC\ (\%)$$

$$OM\ (\%) = 1.724 \times \mathrm{T}OC\ (\%)$$

Here, VB= Volume of ferrous ammonium sulphate solution used for the titration of the blank (mL), VS= Volume of ferrous ammonium sulphate solution used for the titration of the sample (mL) and Wt.= Weight of soil taken (g).

2.5.2. Soil Enzyme Analyses

Using established procedures, the enzyme activities of soil (phosphatase, dehydrogenase, catalase, and urease) will be measured. The geometric mean of enzymatic activities was computed to provide a summary of the various enzymes:

2.5.2.1. Soil Phosphatase Activity

The soil phosphatase activity was quantified by the standard method of Tabatabai and Bremner (1969). 0.1g of dried soil was mixed with 4mL of modified universal buffer that was prepared with 12.1g Tris, 11.6g of maleic acid, 14g citric acid and 6.3g boric acid in 0.5 l of 1 M NaOH and diluted the solution to 1000mL with distilled water, pH 6.5; 1 mL 0.115 M p-nitrophenyl phosphate solution and 0.25mL of toluene will be mixed and swirled, and incubated at 37°C for one hour, after which 1mL 0.5 M $CaCl_2$ and 4mL of 0.5 M NaOH was mixed with the prepared solution. The resulted solution was filtered using filter paper. Blank was also prepared similarly without soil. The absorbance of the

filtrate was measured at 430nm. The phosphatase activity in terms of concentration of p-nitrophenyl was calculated by the help of a standard curve of p-nitrophenol in water, and the results are stated as µg p-nitrophenol g^{-1} h^{-1}.

2.5.2.2. Soil Catalase Activity

By back titrating the residual H_2O_2 in with $KMnO_4$, catalase activity of samples was quantified. Briefly, 2g dry soil sample was added with 40mL distilled water, and then mixed with 5mL of 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 5mL of 1.5 M H_2SO_4 was added. The resulted solution was filtered and titrated using 0.02 M $KMnO_4$. The quantity of 0.02 M $KMnO_4$ per gram of dry soil was used to express the activity of catalase.

2.5.2.3. Soil Dehydrogenase Activity

By using the method adopted by Chander and Brookes (1991) and Kaczynski et al., (2016), dehydrogenase activity was quantified. 6g dry soil was mixed in 1mL of 3% triphenyl tetrazolium chloride (TTC) aqueous solution, and 3mL of distilled water was added. The prepared samples were incubated 37°C for 20 hours. After incubation, samples were filtered and filtered soil extracts were acidified using two drops of conc. H_2SO_4. It was followed by the addition of 20mL of toluene. The samples were placed at 250 rpm in an orbital shaker for 30 minutes. The dehydrogenase activity of the samples was calculated by comparing absorbance with the standard curve plotted for standards of 0-500µmol of TPF, with a concentration interval of 50µmol. Absorbance at 485nm was measured. A blank was also prepared. All analytical results were expressed on the basis of dry weight of soil in µgtriphenylformazan g^{-1} h^{-1}.

2.5.2.4. Soil Urease Activity

Urease activity was quantified as done by Türker and Yakar (2017). 1g soil was mixed with 1mL of urea solution (0.01 g urea mL^{-1}), and incubated at 37°C for 5 hours. After incubation, 10mL of 2 M KCl solution having 5mg L^{-1} phenyl mercuric acetate was mixed in the prepared sample. Samples were placed on orbital shaker for 1 hour, and filtered afterwards using Whatman filter paper no. 42. Resulted filtrate of 2mL was mixed with 2mL of 2 M KCl-phenyl mercuric acetate solution and 6mL of coloring agent. Coloring agent was prepared by mixing 10mL of 0.25% thiosemicarbazide and

25mL of 2.5% diacetylmonoxime in a mixture, 300mL 85% phosphoric acid, 10mL of conc. sulphuric acid, and 190mL of distilled water. The reaction mixture was placed for 30 minutes in water bath at 85°C and then immediately placed in ice cold water for 15 minutes. Absorbance of solution was determined at 527nm. The urea content of the sample solution was calculated by comparing with the standard curve plotted on the basis of standards; 0, 200, 400, 600, 800 and 1000ppm of urea, and urease activity was represented as μg urea hydrolyzed g^{-1} h^{-1}.

2.6. Soil Microbial Count and Bacterial Survival

The measurement of bacterial colony forming units (CFU) and the survival of aggregated bacterial strains in each soil were performed in the soil for all treatments that contained bacterial inoculum. Bacterial isolates were obtained by plate counting. The soil suspension was made with 0.9N saline solution of NaCl (10grams of soil in 90mL of normal saline), and serially diluted by mixing 9mL of 0.1% (w/v) sterile saline solution with 1mL of the previous diluent. For each processing, 100μL of the diluent from 10^{-3} to 10^{-4} was spreaded on a nutrient agar plate containing 250ppm pesticide, then the plates were incubated at 30°C for 24 hours, and the number of colonies on the plate were counted.

2.7. Quantification of CPF in Soil

To measure the concentration of CPF and its major metabolites, samples of soil were analyzed. CPF derivatives were extracted from soil samples by using an extraction procedure that Copaja et al. (2014) reported. 20g of dried soil was taken and treated three times with 10mL of acetonitrile. It was stirred for 30 minutes and the samples were centrifuged at 4000rpm for 15 min. Supernatants were filtered (0.45μm) and evaporated under vacuum. The residues were dissolved in 4mL of acetonitrile and analyzed by HPLC.

Using HPLC, the initial and final concentrations of CPF were measured, and the percentage of CPF removal was calculated as:

$$X = (CI - CF)/CI \times 100$$

Where, X is the CPF degradation rate, CF is the CPF final concentration (mg/L), and CI is the CPF original concentration (mg/L).

2.8. Plant Analyses

Plant analyses include physical analysis of length and weight, chlorophyll content, presence of MDA and H_2O_2, and plant enzyme activity including ascorbate peroxidase activity, guaiacol peroxidase activity, catalase and superoxide dismutase activity.

2.8.1. Physiological Growth Analysis

Harvested plants were subjected to physiological analysis. The studied plant parameters included root and shoot length, fresh weight of roots and shoots and dry weight of roots and shoots. The lengths of the roots and shoots were measured with a ruler, and the results were given in centimeters. A representative number of fresh leaves were also maintained for biochemical and enzymatic examination. The weight of fresh and dried shoot and root was measured using an electric weighing balance. During harvesting, fresh weights were measured immediately. For dried weights, plant materials were dried at 60°C until a uniform weight was reached. The weights of the fresh roots and shoots were simply added to determine the total plant biomass. By using a conventional approach for plant physiological analysis, all the plant physiological parameters were recorded (Khan et al., 2019a).

2.8.2. Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoid Content

Arnon (1949) protocol was followed for extract preparation needed to approximate the chlorophyll and carotenoid material. In order to prepare the extract needed to determine the content of chlorophyll and carotenoids, 40mg of fresh leaf samples was immersed to obtain a homogeneous leaf extract in about 2mL of 80% acetone solution. The extracts were then centrifuged at 5000rpm for 5 minutes. Supernatant obtained were stored properly in a new, clean falcon tube. The pellet was vortexed with about 1mL of 80% (v/v) acetone in water and then centrifuged for 5 minutes at 5000rpm. The obtained new supernatant was then combined with the previously harvested supernatant for analysis. Lichtenthaler (1987) equations for calculating photosynthetic pigments, including chlorophyll a, chlorophyll b, the total chlorophyll and carotenoids were used after absorbance values were obtained at wavelengths 663nm and 645nm and 470nm.

Following equations were used for the calculations:

$$Chlorophyll\,a = (12.25 \times A663) - (2.79 \times A645)$$

$$Chlorophyll\,b = (21.50 \times A645) - (5.10 \times A663)$$

$$Total\,Chlorophyll = (Chl\,a) + (Chl\,b)$$

$$Carotenoids = ((1000 \times A470) - (1.82 \times Chl\ a) - (85.02 \times Chl\ b))/198$$

2.8.3. Quantification of Lipid Peroxidation

Malondialdehyde levels in samples used for lipid peroxidation measurement were examined. The analysis was performed using the Venkatachalam et al. (2017) technique. Briefly, 0.1g of fresh leaf material was macerated in 1mL of 5% TCA that had been previously refrigerated to create a uniform suspension. The homogenized sample was centrifuged for 10 minutes at 10,000rpm, and the collected supernatant was then mixed 1:1 with 0.67% TBA solution. The combination was heated for 30 minutes in a water bath at 95°C before being immediately transferred to an ice bath for one minute. The cold mixture was then centrifuged once again for 10 minutes at 10,000rpm.Absorbance at 450nm, 532nm, and 600nm wavelengths of the produced samples were observed. The total lipid peroxidation was reported in μM of malondialdehyde g^{-1} of FW using following equation:

$$MDA = ([6.45 \times (A532 - A600)] - [(0.56 \times A450) \times Vt])/W$$

Where, Vt = 0.001 L; and W = 0.1 g.

2.8.4. Hydrogen Peroxide Production

The quantification of the formation of reactive oxygen species (ROS), particularly H_2O_2, was carried out according to Khan et al. (2019b) protocol, with certain modifications made to the leaf extract preparation according to the Venkatachalam et al. (2017) methodological framework. In a nutshell, 0.1g of fresh leaf sample was macerated in 1mL of pre-chilled extraction buffer with a pH of 7.4 made of 50 mM potassium phosphate buffer (PPB) and 0.5 mM EDTA, and then centrifuged at 10,000rpm for 15 minutes at 4°C. To measure the amount of H_2O_2 in the produced supernatant, it was placed in a separate tube and used as a leaf extract. To stop the sample's deterioration, it

was stored at 4°C.The reaction mixture, which was used to determine the H_2O_2 content, was made by combining 40µL of leaf extract, 1mL of 0.05 mM PPB (pH 6.5), and 352.8µL of 1 percent $Ti(SO_4)_2$ produced in 20% H_2SO_4(v/v). Centrifugation was then performed at 6000rpm for 15 minutes. In order to gauge the degree of yellow colour present, the supernatant from the reaction was collected and its absorbance at 410nm measured. With the help of molar extinction coefficient (ε) of 0.28 μM^{-1} cm^{-1}, the value of H_2O_2 content were expressed as µM H_2O_2 contents g^{-1} of FW, which was computed using "Beer-Lambert law" by following equation:

$$A=\varepsilon bc$$

Where, A = Absorbance of sample at selected wavelength, ε = molar extinction coefficient of desired substance, b = the path length of the sample (which was 1 cm in case of cuvette), and c = the compound's concentration in the solution.

2.8.5. Quantification of Enzymatic Activities

This section includes an explanation of the methodology used to measure enzyme activity. The leaf extract was made using the Venkatachalam et al. (2017) method, same like it was for H_2O_2 activity. In a nutshell, 0.1g of fresh leaf sample was taken and dissolved in 1mL of pH 7.4 pre-chilled extraction buffer that contained 50 mM potassium phosphate buffer (PPB) and 0.5 mM EDTA, and it was then centrifuged at 10,000rpm for 15 minutes at 4°C. To measure the enzyme activity, the supernatant from the reaction was collected and used as a leaf extract. To preserve the sample, it was stored at 4°C.Value of results were expressed in Units g^{-1} of FW of sample for all type of enzyme activities.

2.8.5.1. Ascorbate Peroxidase Activity

Using a modified version of Chen and Asada's (1989) procedure, ascorbate peroxidase activity (APX) was measured. For this, a reaction mixture made by mixing 50µL of leaf extract with 1mL of reaction buffer made of 500µM ascorbate, 100µM EDTA, 1.54mM H_2O_2, and 50mM PPB, having pH at 7.0 was used to observe the absorbance at 240nm. To compute the APX activity ε of 2.8 mM^{-1} cm^{-1} was used.

2.8.5.2. Catalase Activity

The catalase activity (CAT) was measured according to the protocol of Maehly and Chance (1954), and the reduction of H_2O_2 was quantified by monitoring A240 after 1 minute. The reaction mixture consisted of 2.5mL reaction buffer made of 50mM PPB, having pH 7.4, with 100µL 1% H_2O_2, and 50µL leaf extract. Activity of catalase was determined by the ε value of 39.4 mM^{-1} cm^{-1}.

2.8.5.3. Guaiacol Peroxidase Activity

The method of Upadhyay et al. (2019) to quantify the activity of guaiacol peroxidase (GPX) was applied. The reaction mixture was prepared by mixing 20µl of leaf extract with 2.5mL reaction buffer made by 50mM PPB at pH 6.1, 1mL 1% Guaiacol and 1mL 1% H_2O_2. A420 was examined after 1 minute to determine the changes. The activity was calculated, using ε equal to 26.6 mM^{-1} cm^{-1}.

Calculation for APX, CAT, and GPX

The concentration of enzyme unit was calculated by using Beer's law, which is

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

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

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 (0.1g per mL of extraction buffer)

2.8.5.4. Superoxide Dismutase Activity

1.5mL reaction mixture having pH 7.8, including 100µM EDTA, 13mM methionine, 75µM nitroblue tetrazolium (NBT), 50mM PPB and 2µM riboflavin was used for superoxide dismutase activity. 50µL of plant extract wasmixed and exposed to room temperature for 15 minutes. The control sample of the reaction mixture was kept in the

dark to avoid color development, and the optical density of the samples was recorded at a wavelength of 560nm after 15 minutes. Following equation was used for quantification of SOD:

$$50\% \text{ inhibition} = (ControlOD - TreatmentOD/ControlOD) \times (100/50) \times (A/1000) \times (B)$$

Where, Control OD = Control reaction Absorbance at 560nm, Treatment OD = Treatment reaction Absorbance at 560nm, A = Volume of Enzyme extract used in the enzyme activity and B = 0.1g of FW of plant per mL of extraction buffer.

2.9. Quantification of CPF in Plant

To measure the concentration of CPF and its major metabolites, samples of plants were analyzed. CPF derivatives were extracted from plant samples by using an extraction procedure that Copaja et al. (2014) reported. 500mg of wheat plant was macerated 3 times in succession with 10mL of acetonitrile using a pestle and mortar. The extracts were centrifuged at 4000rpm for 15 minutes and the supernatants were filtered (0.45µm). They were then evaporated under vacuum; the residues were dissolved in 4mL of acetonitrile and analyzed by the HPLC method.

Using HPLC, the initial and final concentrations of CPF were measured, and the percentage of CPF removal was calculated as:

$$X = (CI - CF)/CI \times 100$$

Where, X is the CPF degradation rate, CF is the CPF final concentration (mg/L), and CI is the CPF original concentration (mg/L).

2.10. Statistical Analysis

Data from all treatments were subjected to one-way analysis of variance, which was followed by the Duncan multiple range post hoc test to compare multiple means. The p-value of less than 0.05 was regarded as significant because all the data were obtained in triplicate. All statistical work was completed using IBM SPSS 21.

Chapter 3
Results

3.1. Physicochemical Properties of Fresh and Spiked Soils

Soil texture of both soils was analyzed (Figure 3.1 and 3.2). Soil that was brought from Bara Kahu was clayey in texture as it had 55% clay, 10% sand and 35% silt content. On the other hand, soil brought from the farms in Chak Shahzad had 15% clay, 60% sand and 25% silt content that made it sandy loam in texture.

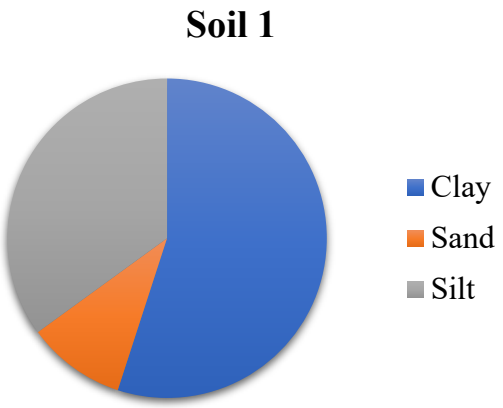

Figure 3.1: Constituents' Proportion of Soil 1

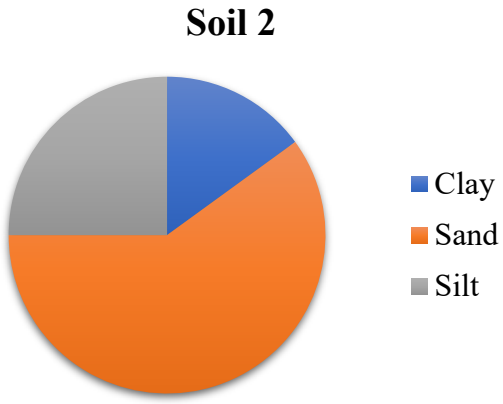

Figure 3.2: Constituents' Proportion of Soil 2

Initial and final physicochemical parameters of fresh soil and spiked soil used in different treatments of were measured for both clayey and sandy loam soil. These treatments were T1: FS+P, T2: SS, T3: SS+P, T4: SS+P+B1, T5: SS+P+B2, T6: SS+P+B1+B2, T7:

SS+C+P, T8: SS+C+P+B1, T9: SS+C+P+B2, T10: SS+C+P+B1+B1, T11: SS+B+P, T12: SS+B+P+B1, T13: SS+B+P+B2, T14: SS+B+P+B1+B2. The results of clayey soil are presented in Table 3.1, while the results of sandy loam soil are present in Table 3.2.

The physicochemical characteristics of fresh and spiked soil for clayey soil were measured before and after the experiment; no significant differences were found in a few parameters while some showed significant difference (Table 3.1). The pH of the soil was in range of 7.24 to 7.44. The significant differences were noted for soil EC (dS/m) and the lowest EC (200.33) was noted with FS. Maximum EC was noted in treatment SS (590.67). The significant differences were noted for soil TDS (mg/kg). Lowest TDS value (121.00) was noted for FS while maximum TDS (254.33) was noted in SS treatment, In context of available nitrates, the treatment SS+B+P+B2 had the highest value (7.12), while the lowest value of available nitrates (1.08) was noted for SS treatment. Extractable phosphorous also showed the significant differences among different treatments. The highest value of extractable phosphorous (mg/kg) was found for the treatment SS+B+P+B2 which was 999.19mg/kg while the lowest value of extractable phosphorous was observed for the SS treatment, which was 493.74mg/kg. The significant differences were observed for soil OOC, TOC and OM. The highest levels for OOC, TOC and OM (%) were noted for treatment SS+B+P+B2 that were 3.09, 4.12 and 5.33, respectively.

The physicochemical characteristics of fresh and spiked soil for sandy loam soil were also measured before and after the experiment (Table 3.2). The pH of the soil was in the range of 6.85 to 7.87 The significant differences were noted for soil EC (dS/m) and the lowest EC value (80.33) was noted for FS. Maximum EC was noted in SS (395.67). The significant differences were noted for soil TDS (mg/kg) and initial lowest TDS value (101.67) was noted for FS while maximum TDS value (301.00) was noted in SS treatment. In context of available nitrates, the treatment SS+B+P+B2 had the highest value (19.46), while the lowest value of available nitrates (3.43) was noted for SS treatment. Extractable phosphorous also showed the significant differences among treatments. The highest value of extractable phosphorous (mg/kg) was found for the treatment SS+B+P+B2 which was 911.12mg/kg while the lowest value of extractable

phosphorous was observed for the SS treatment, which was 439.18mg/kg. The significant differences were observed for soil OOC, TOC and OM. The highest levels for OOC, TOC and OM (%) were noted for treatment SS+B+P+B2 that were 2.90, 3.86 and 4.99 respectively.

Overall, in terms of nutrients and organic matter content, clayey soil has more quantity of phosphates, OOC, TOC and OM in comparison to the sandy loam soil. On the other hand, sandy loam soil had greater quantity of nitrates. There is difference between the treatments of same soil type, but significant difference in values is observed in the values of both soil types for the nutrient content, though the trend within the treatments remained the same.

Table 3.1: Physicochemical Properties of Clay Soil

	Treatments	pH	EC dS/m	TDS mg/kg	Nitrates mg/kg	Phosphates mg/kg	OOC %	TOC %	OM %
Initial	FS	7.33 ± 0.02^{cd}	200.33 ± 1.15^{l}	121.00 ± 1.00^{l}	2.48 ± 0.01^{g}	995.50 ± 1.75^{b}	0.34 ± 0.04^{j}	0.45 ± 0.05^{j}	0.58 ± 0.07^{j}
	SS	7.29 ± 0.02^{de}	590.67 ± 0.58^{b}	254.33 ± 1.15^{b}	1.08 ± 0.09^{h}	714.80 ± 1.75^{n}	0.43 ± 0.03^{j}	0.57 ± 0.04^{j}	0.74 ± 0.05^{j}
Final	FS+P	7.24 ± 0.03^{efgh}	201.33 ± 1.53^{l}	133.33 ± 0.58^{k}	4.40 ± 0.27^{de}	939.36 ± 1.75^{i}	1.49 ± 0.08^{f}	1.99 ± 0.10^{f}	2.57 ± 0.13^{f}
	SS	7.19 ± 0.01^{h}	641.00 ± 1.00^{a}	271.00 ± 1.00^{a}	2.32 ± 0.06^{g}	493.74 ± 1.75^{o}	0.39 ± 0.06^{j}	0.51 ± 0.08^{j}	0.66 ± 0.10^{j}
	SS+P	7.19 ± 0.02^{gh}	484.67 ± 0.58^{c}	254.33 ± 0.58^{b}	3.36 ± 0.03^{f}	882.17 ± 0.35^{m}	0.62 ± 0.09^{i}	0.82 ± 0.12^{i}	1.06 ± 0.16^{i}
	SS+P+B1	7.23 ± 0.03^{fgh}	456.67 ± 1.53^{e}	196.33 ± 0.58^{d}	4.25 ± 0.16^{e}	928.84 ± 1.75^{k}	1.30 ± 0.06^{g}	1.73 ± 0.08^{g}	2.24 ± 0.10^{g}
	SS+P+B2	7.24 ± 0.02^{efgh}	396.33 ± 0.58^{f}	194.33 ± 0.58^{e}	4.39 ± 0.18^{de}	934.45 ± 0.35^{j}	1.40 ± 0.04^{f}	1.87 ± 0.06^{f}	2.41 ± 0.07^{f}
	SS+P+B1+B2	7.21 ± 0.06^{fgh}	463.00 ± 1.00^{d}	226.33 ± 1.15^{c}	3.40 ± 0.21^{f}	921.82 ± 1.75^{l}	0.77 ± 0.08^{h}	1.02 ± 0.11^{h}	1.32 ± 0.14^{h}
	SS+C+P	7.37 ± 0.04^{bc}	395.33 ± 1.15^{f}	193.33 ± 0.58^{e}	4.64 ± 0.23^{cde}	942.87 ± 1.40^{h}	1.50 ± 0.02^{f}	1.99 ± 0.03^{f}	2.58 ± 0.04^{f}
	SS+C+P+B1	7.41 ± 0.04^{ab}	325.00 ± 1.00^{h}	175.00 ± 1.00^{g}	4.91 ± 0.01^{cd}	956.91 ± 1.75^{f}	1.99 ± 0.04^{d}	2.65 ± 0.05^{d}	3.43 ± 0.07^{d}
	SS+C+P+B2	7.44 ± 0.06^{a}	283.67 ± 1.15^{i}	167.00 ± 1.00^{h}	5.09 ± 0.11^{c}	963.92 ± 1.75^{e}	2.18 ± 0.07^{c}	2.90 ± 0.10^{c}	3.75 ± 0.13^{c}
	SS+C+P+B1+B2	7.40 ± 0.02^{ab}	375.33 ± 0.58^{g}	181.67 ± 0.58^{f}	4.80 ± 0.26^{cd}	953.40 ± 1.40^{g}	1.62 ± 0.04^{e}	2.15 ± 0.05^{e}	2.78 ± 0.07^{e}
	SS+B+P	7.25 ± 0.03^{ef}	211.33 ± 1.53^{j}	164.33 ± 1.53^{i}	5.57 ± 0.79^{b}	970.94 ± 1.75^{d}	2.23 ± 0.05^{c}	2.97 ± 0.06^{c}	3.84 ± 0.08^{c}
	SS+B+P+B1	7.25 ± 0.02^{efg}	150.33 ± 1.53^{m}	163.00 ± 1.00^{i}	5.74 ± 0.62^{b}	997.08 ± 1.58^{ab}	2.76 ± 0.05^{b}	3.68 ± 0.07^{b}	4.76 ± 0.09^{b}
	SS+B+P+B2	7.26 ± 0.05^{ef}	130.33 ± 0.58^{n}	152.00 ± 1.00^{j}	7.12 ± 0.14^{a}	999.19 ± 0.53^{a}	3.09 ± 0.02^{a}	4.12 ± 0.02^{a}	5.33 ± 0.03^{a}
	SS+B+P+B1+B2	7.25 ± 0.02^{efgh}	208.00 ± 1.73^{k}	163.00 ± 1.00^{i}	5.60 ± 0.02^{b}	977.61 ± 0.70^{c}	2.73 ± 0.04^{b}	3.64 ± 0.06^{b}	4.70 ± 0.07^{b}

FS= Fresh soil, SS=CPF spiked soil, FS+P=Fresh soil + Plant, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

EC= Electrical conductance, TDS= Total dissolved solids, OOC= Oxidizable organic carbon, TOC= Total organic carbon, and OM= Organic matter, Data is presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means.

Table 3.2: Physicochemical Properties of Sandy Loam Soil

	Treatments	Ph	EC dS/m	TDS mg/kg	Nitrates mg/kg	Phosphates mg/kg	OOC %	TOC %	OM %
Initial	FS	7.37 ± 0.01^{e}	80.33 ± 0.58^{o}	101.67 ± 0.58^{o}	6.72 ± 0.05^{m}	598.66 ± 0.35^{k}	0.33 ± 0.04^{k}	0.44 ± 0.05^{k}	0.57 ± 0.07^{k}
	SS	6.99 ± 0.02^{h}	395.67 ± 0.58^{d}	301.00 ± 1.00^{b}	3.43 ± 0.01^{p}	565.68 ± 1.75^{l}	0.55 ± 0.03^{j}	0.73 ± 0.04^{j}	0.94 ± 0.05^{j}
Final	FS+P	7.33 ± 0.03^{ef}	128.33 ± 0.58^{m}	116.67 ± 1.53^{n}	11.26 ± 0.02^{i}	703.22 ± 0.35^{i}	1.09 ± 0.07^{g}	1.45 ± 0.10^{g}	1.88 ± 0.13^{g}
	SS	6.85 ± 0.04^{i}	486.00 ± 1.00^{a}	309.33 ± 1.15^{a}	4.55 ± 0.01^{o}	439.18 ± 0.18^{o}	0.36 ± 0.04^{k}	0.48 ± 0.05^{k}	0.62 ± 0.07^{k}
	SS+P	6.94 ± 0.03^{h}	421.67 ± 1.53^{b}	300.33 ± 1.53^{b}	5.53 ± 0.01^{n}	461.46 ± 0.70^{n}	0.49 ± 0.02^{j}	0.65 ± 0.03^{j}	0.84 ± 0.04^{j}
	SS+P+B1	7.27 ± 0.02^{f}	374.67 ± 0.58^{e}	281.33 ± 1.53^{d}	10.76 ± 0.01^{k}	597.96 ± 0.35^{k}	0.77 ± 0.03^{i}	1.02 ± 0.04^{i}	1.31 ± 0.05^{i}
	SS+P+B2	7.34 ± 0.01^{ef}	329.00 ± 1.00^{f}	251.67 ± 1.53^{e}	10.84 ± 0.03^{j}	627.61 ± 0.53^{j}	0.92 ± 0.04^{h}	1.23 ± 0.05^{h}	1.59 ± 0.07^{h}
	SS+P+B1+B2	7.08 ± 0.02^{g}	409.67 ± 1.53^{c}	286.67 ± 1.53^{c}	8.08 ± 0.02^{l}	476.73 ± 0.18^{m}	0.75 ± 0.04^{i}	1.00 ± 0.05^{i}	1.29 ± 0.07^{i}
	SS+C+P	7.77 ± 0.03^{bc}	291.00 ± 1.00^{g}	214.33 ± 1.15^{f}	13.26 ± 0.01^{h}	721.64 ± 0.53^{h}	1.84 ± 0.03^{f}	2.45 ± 0.04^{f}	3.16 ± 0.05^{f}
	SS+C+P+B1	7.85 ± 0.04^{a}	280.33 ± 0.58^{h}	191.00 ± 1.00^{h}	15.54 ± 0.02^{f}	747.96 ± 0.18^{f}	1.95 ± 0.03^{e}	2.60 ± 0.04^{e}	3.36 ± 0.05^{e}
	SS+C+P+B2	7.87 ± 0.15^{a}	278.67 ± 1.53^{i}	175.00 ± 1.73^{i}	15.68 ± 0.02^{e}	751.82 ± 0.18^{e}	2.14 ± 0.05^{d}	2.85 ± 0.07^{d}	3.68 ± 0.09^{d}
	SS+C+P+B1+B2	7.83 ± 0.03^{ab}	281.33 ± 1.53^{h}	199.00 ± 1.00^{g}	14.67 ± 0.01^{g}	730.06 ± 0.53^{g}	1.85 ± 0.03^{f}	2.46 ± 0.04^{f}	3.18 ± 0.05^{f}
	SS+B+P	7.35 ± 0.05^{ef}	208.00 ± 1.00^{j}	172.67 ± 1.53^{j}	17.17 ± 0.02^{d}	759.01 ± 0.35^{d}	2.45 ± 0.05^{c}	3.26 ± 0.06^{c}	4.21 ± 0.08^{c}
	SS+B+P+B1	7.49 ± 0.02^{d}	145.67 ± 0.58^{l}	132.67 ± 1.15^{l}	18.36 ± 0.01^{b}	870.59 ± 0.35^{b}	2.89 ± 0.02^{a}	3.86 ± 0.03^{a}	4.98 ± 0.04^{a}
	SS+B+P+B2	7.75 ± 0.02^{c}	125.00 ± 1.00^{n}	120.67 ± 0.58^{m}	19.46 ± 0.03^{a}	911.12 ± 0.18^{a}	2.90 ± 0.03^{a}	3.86 ± 0.04^{a}	4.99 ± 0.05^{a}
	SS+B+P+B1+B2	7.40 ± 0.02^{e}	197.00 ± 1.00^{k}	154.33 ± 1.15^{k}	18.09 ± 0.01^{c}	795.33 ± 0.88^{c}	2.72 ± 0.05^{b}	3.62 ± 0.07^{b}	4.68 ± 0.09^{b}

FS= Fresh soil, SS=CPF spiked soil, FS+P=Fresh soil + Plant, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

EC= Electrical conductance, TDS= Total dissolved solids, OOC= Oxidizable organic carbon, TOC= Total organic carbon, and OM= Organic matter, Data is presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means.

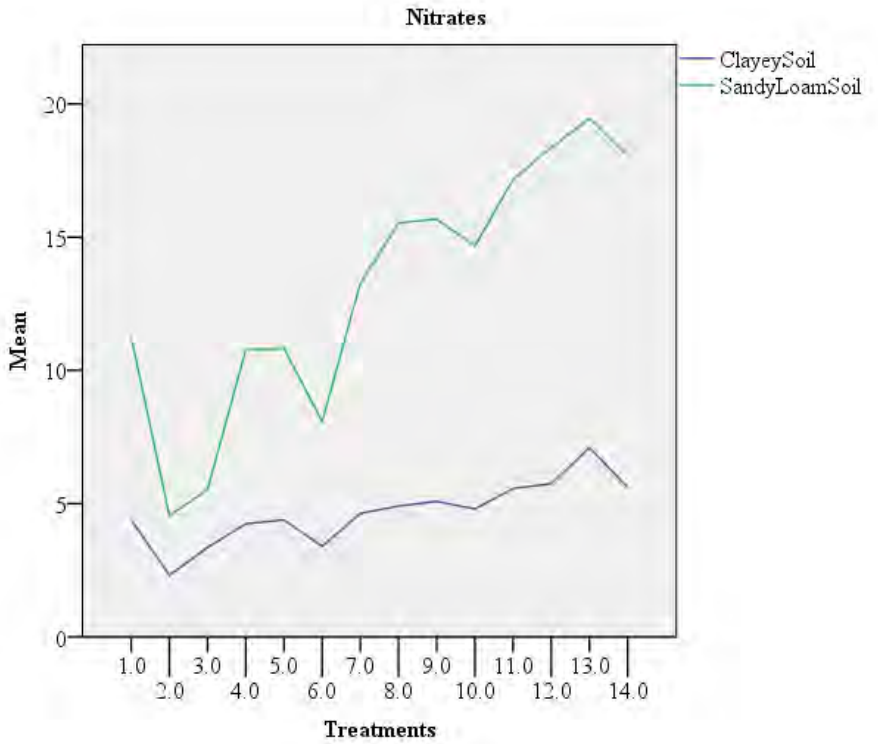

Figure 3.3: Correlation between Nitrate Content in Clayey and Sandy Loam Soil

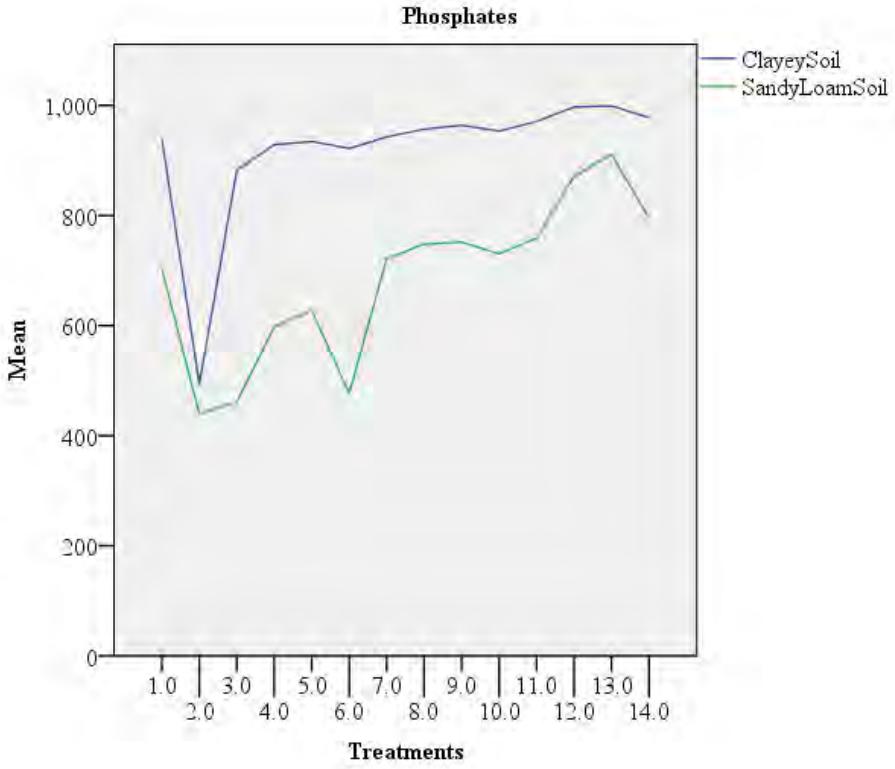

Figure 3.4: Correlation between Phosphate Content in Clayey and Sandy Loam Soil

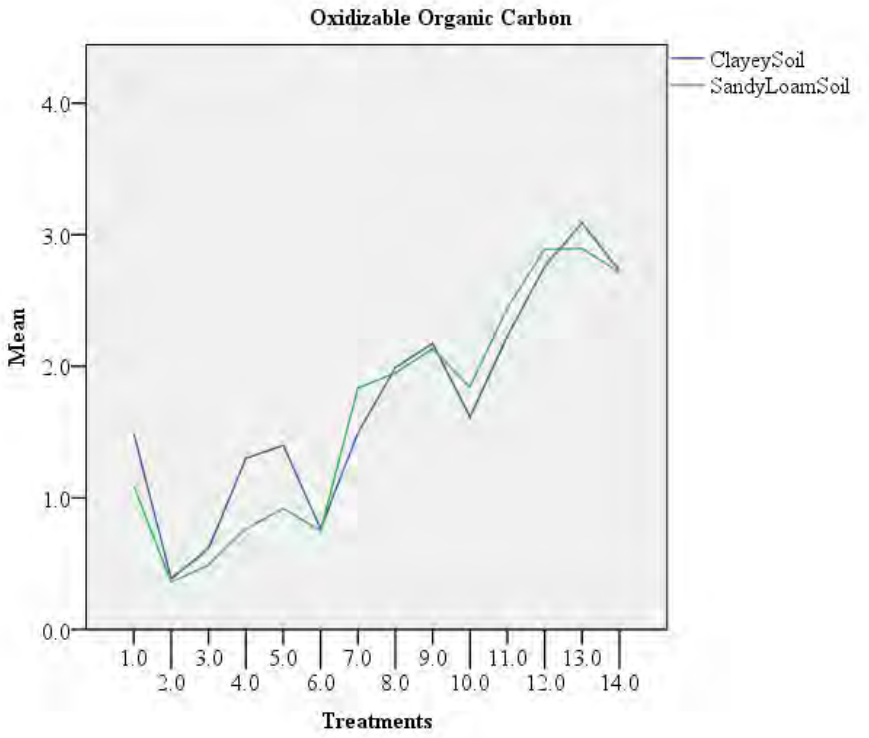

Figure 3.5: Correlation between OOC in Clayey and Sandy Loam Soil

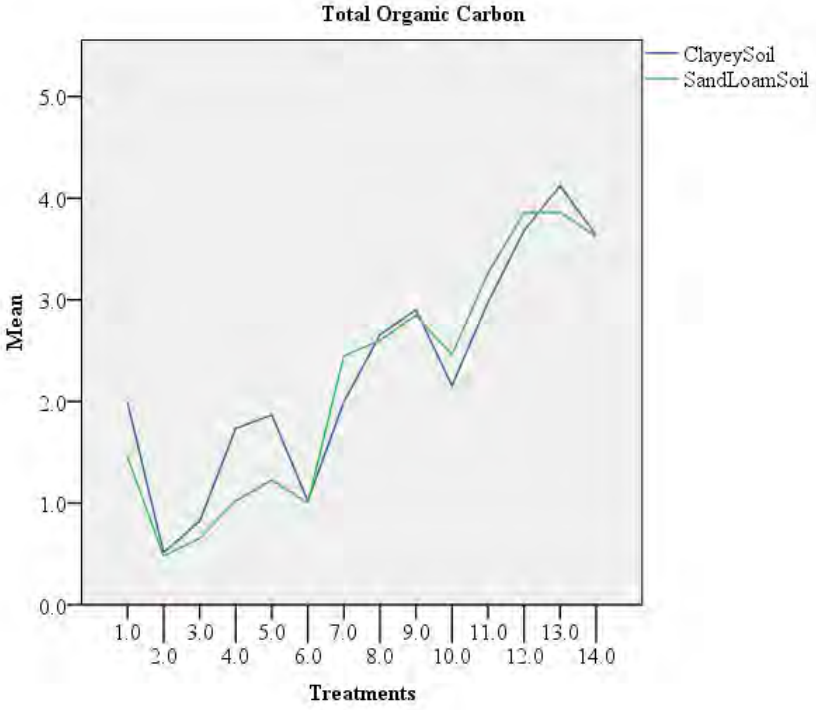

Figure 3.6: Correlation between TOC in Clayey and Sandy Loam Soil

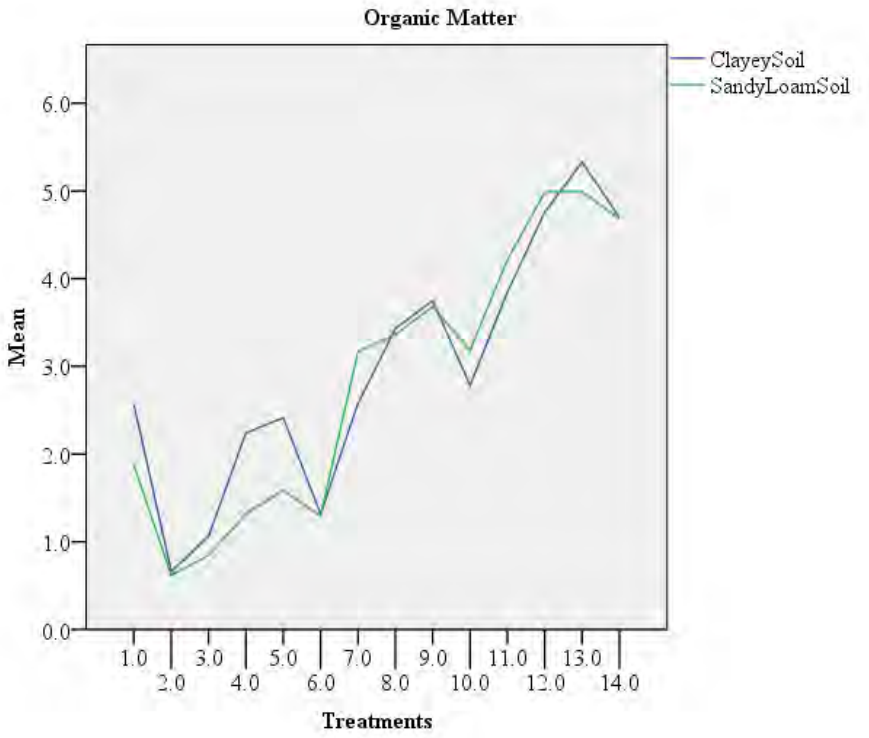

Figure 3.7: Correlation between Organic Matter in Clayey and Sandy Loam Soil

3.2. Effects of the Applied Treatments on Soil Enzymatic Profile in Different Soil Textures

The impact of different treatments on the studied soil enzymatic activities, including dehydrogenase, urease, phosphatase, and catalase for clayey and sandy loam soil is presented in Table 3.3 and 3.4 respectively. Among all studied soil enzymatic activity statistically significant differences were noted, between the applied treatments.

Soil catalase activity (0.02 M KMnO4 g^{-1} h^{-1}) was not recorded in clayey soil while in sandy loam soil, the significantly lowest soil catalase activity was noted for SS and SS+P treatments, 0.62 and 0.62, respectively. The statistically higher dehydrogenase (µgtriphenylformazan g^{-1} h^{-1}) was noted in SS+C+P+B2 in both clayey and sandy loam soil, which was 2.51 and 3.64 respectively. Phosphatase activity (µg p-nitrophenol g^{-1} h^{-1}) was the highest in the same treatment, i.e. SS+C+P+B2 that was 123.82, and 179.70 for clayey and sandy loam soil, respectively. For the urease activity (µg urea hydrolysed g^{-1} h^{-1}) the significantly higher activity was noted for SS+C+P+B2 treatment, having value

of 505.36, and 1201.14 for clayey and sandy loam soil, respectively. Thus, clayey soil did not support catalase activity while sandy loam soil did. On the other hand, dehydrogenase, urease and phosphatase activities were more in sandy loam soil as compared to clayey soil. There is difference between the treatments of same soil type, but significant difference was observed in the values of both soil types for the enzymatic activity, though the trend within the treatments remained the same.

Table 3.3: Impact of Different Treatments on Soil Enzymatic Activities in Clayey Soil

Treatments	Dehydrogenase µg triphenylformanzan g^{-1} h^{-1}	Urease µg urea hydrolysed g^{-1} h^{-1}	Phosphatase µg p-nitrophenol g^{-1} h^{-1}
FS+P	0.92 ± 0.02^{fg}	444.67 ± 0.66^{i}	73.09 ± 0.18^{j}
SS	0.89 ± 0.01^{g}	437.78 ± 1.64^{j}	69.37 ± 0.05^{l}
SS+P	0.91 ± 0.01^{fg}	439.42 ± 0.66^{j}	70.57 ± 0.23^{k}
SS+P+B1	0.96 ± 0.03^{fg}	451.88 ± 0.66^{h}	76.63 ± 0.05^{h}
SS+P+B2	0.97 ± 0.07^{fg}	453.85 ± 1.31^{g}	80.53 ± 0.37^{g}
SS+P+B1+B2	0.94 ± 0.01^{fg}	446.31 ± 0.98^{i}	75.30 ± 0.28^{i}
SS+C+P	1.02 ± 0.02^{efg}	465.01 ± 0.66^{e}	82.92 ± 0.18^{e}
SS+C+P+B1	1.87 ± 0.11^{b}	493.55 ± 0.98^{b}	100.18 ± 0.18^{b}
SS+C+P+B2	2.51 ± 0.04^{a}	505.36 ± 2.30^{a}	123.82 ± 0.32^{a}
SS+C+P+B1+B2	1.31 ± 0.24^{c}	492.56 ± 0.66^{b}	99.95 ± 0.23^{b}
SS+B+P	1.00 ± 0.02^{efg}	456.48 ± 1.31^{f}	81.17 ± 0.18^{f}
SS+B+P+B1	1.12 ± 0.02^{de}	485.67 ± 0.98^{c}	86.50 ± 0.28^{d}
SS+B+P+B2	1.20 ± 0.09^{cd}	487.31 ± 0.66^{c}	92.19 ± 0.09^{c}
SS+B+P+B1+B2	1.04 ± 0.01^{ef}	476.16 ± 1.31^{d}	86.18 ± 0.32^{d}

FS+P=Fresh soil + Plant, SS=CPF spiked soil, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means.

Table 3.4: Impact of Different Treatments on Soil Enzymatic Activities in Sandy Loam Soil

Treatments	**Dehydrogenase**	**Urease**	**Phosphatase**	**Catalase**
	µg triphenylformanzan g^{-1} h^{-1}	**µg urea hydrolysed g^{-1} h^{-1}**	**µg p-nitrophenol g^{-1} h^{-1}**	**0.02 M $KmnO_4$ g^{-1} h^{-1}**
FS+P	1.06 ± 0.02^{ij}	982.66 ± 1.31^{j}	165.38 ± 0.18^{g}	0.71 ± 0.02^{f}
SS	0.90 ± 0.01^{k}	717.60 ± 1.97^{l}	163.45 ± 0.18^{h}	0.62 ± 0.02^{g}
SS+P	1.02 ± 0.03^{j}	933.78 ± 1.64^{k}	165.33 ± 0.96^{g}	0.62 ± 0.02^{g}
SS+P+B1	1.14 ± 0.01^{ghi}	1050.57 ± 0.98^{h}	166.71 ± 0.41^{ef}	0.77 ± 0.04^{f}
SS+P+B2	1.16 ± 0.01^{gh}	1070.25 ± 0.98^{g}	166.71 ± 0.78^{ef}	0.77 ± 0.04^{f}
SS+P+B1+B2	1.10 ± 0.03^{hij}	1010.22 ± 1.31^{i}	166.02 ± 0.46^{fg}	0.75 ± 0.02^{f}
SS+C+P	1.27 ± 0.01^{ef}	1095.84 ± 0.98^{f}	167.67 ± 1.01^{cde}	0.92 ± 0.06^{e}
SS+C+P+B1	3.00 ± 0.11^{b}	1146.68 ± 1.31^{b}	169.09 ± 0.87^{b}	1.54 ± 0.04^{b}
SS+C+P+B2	3.64 ± 0.04^{a}	1201.14 ± 7.87^{a}	179.70 ± 1.47^{a}	2.64 ± 0.06^{a}
SS+C+P+B1+B2	2.33 ± 0.09^{b}	1133.24 ± 0.98^{c}	168.86 ± 0.28^{bc}	1.44 ± 0.02^{c}
SS+B+P	1.20 ± 0.07^{fg}	1094.53 ± 0.33^{f}	167.30 ± 0.18^{de}	0.90 ± 0.04^{e}
SS+B+P+B1	2.15 ± 0.02^{d}	1101.74 ± 0.33^{e}	167.76 ± 0.55^{cde}	0.94 ± 0.04^{e}
SS+B+P+B2	2.16 ± 0.05^{d}	1109.29 ± 0.66^{d}	168.41 ± 0.28^{bcd}	1.03 ± 0.04^{d}
SS+B+P+B1+B2	1.35 ± 0.02^{e}	1102.40 ± 2.30^{e}	167.76 ± 0.18^{cde}	0.92 ± 0.06^{e}

FS+P=Fresh soil + Plant, SS=CPF spiked soil, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was “a” column wise followed by later alphabets for lower means.

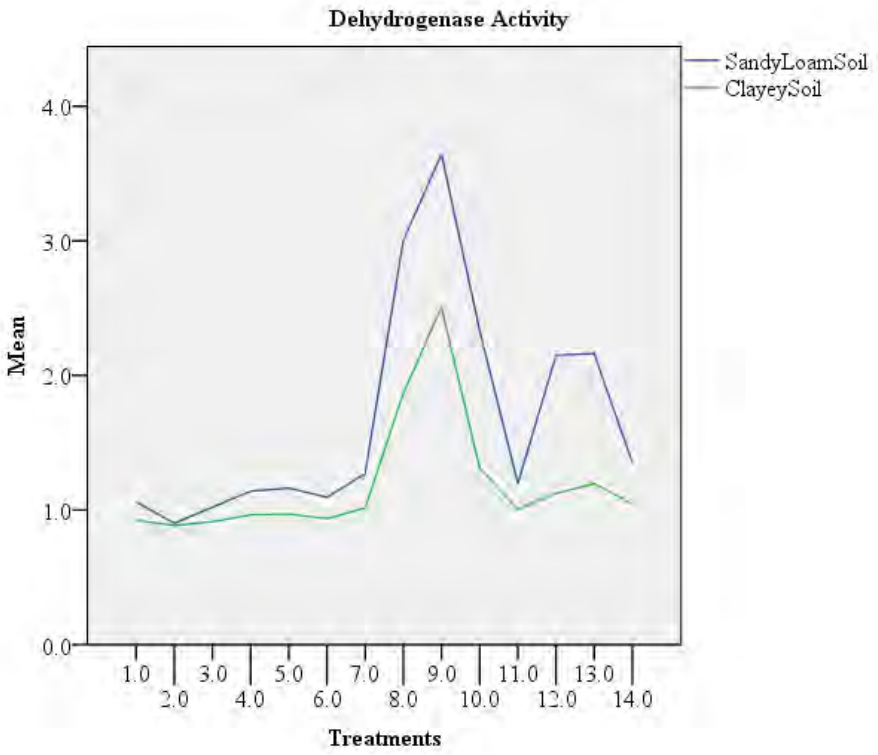

Figure 3.8: Correlation between Dehydrogenase Activity in Clayey and Sandy Loam Soil

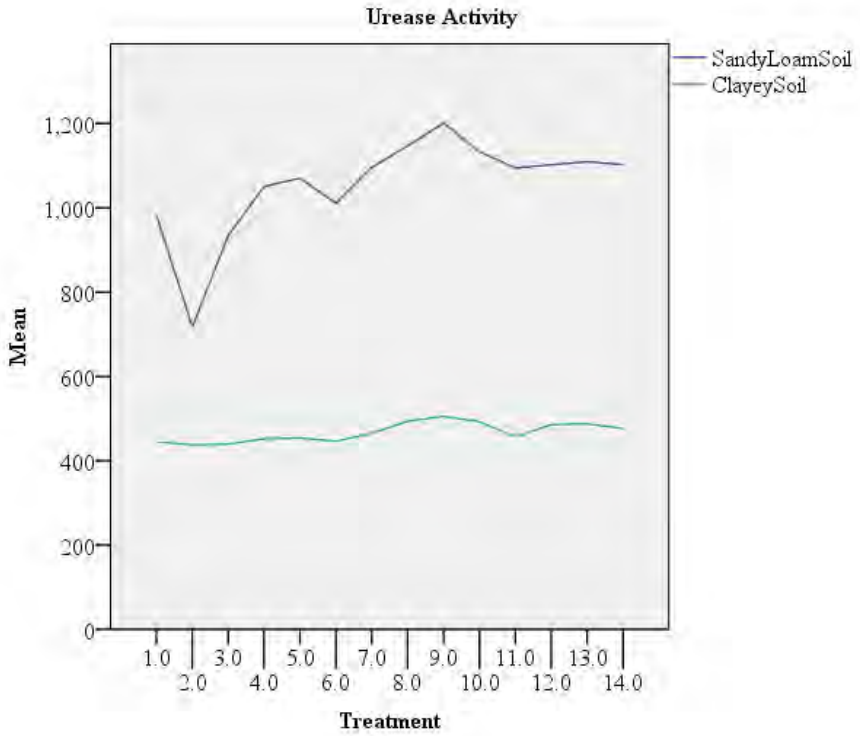

Figure 3.9: Correlation between Urease Activity in Clayey and Sandy Loam Soil

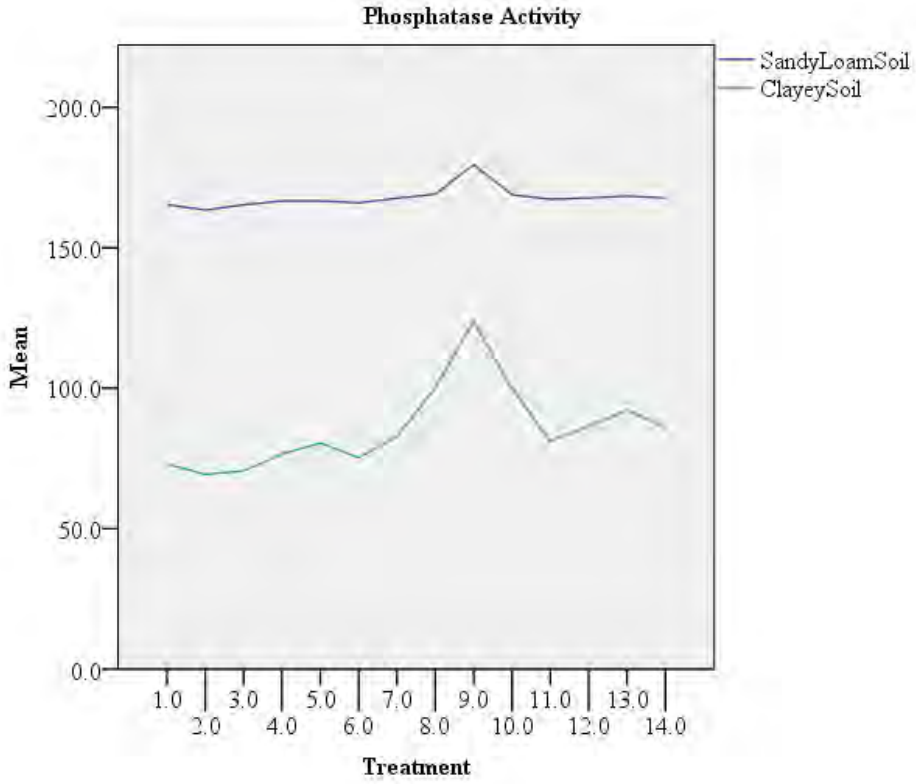

Figure 3.10: Correlation between Phosphatase Activity in Clayey and Sandy Loam Soil

3.3. Effects of the Applied Treatments on the Physiological Parameters of *Triticum aestivum*

The exposure to CPF contamination in different treatments of both soils resulted in statistically significant differences on the growth of *Triticum aestivum*. Plants were grown in FS+P, SS+P, SS+P+B1, SS+P+B2, SS+P+B1+B2, SS+C+P, SS+C+P+B1, SS+C+P+B2, SS+C+P+B1+B1, SS+B+P, SS+B+P+B1, SS+B+P+B2, and SS+B+P+B1+B2 treatments and results of plants grown in clayey soil are represented in Table 3.5, while the results of plants grown in sandy loam soil are represented in Table 3.6. The presences of CPF in soil resulted in reduced plant growth and highest growth was noted for SS+B+P+B2 treatment of both soil textures. The studied parameters of plant include root and shoot length, their fresh weight, and dry weight.

In clayey soil, maximum root length (cm), fresh and dry weight (g) was noted for the treatment SS+B+P+B2 which were 32.3, 9.5 and 6.4, respectively. After this, the treatment SS+B+P+B1 had the higher root length, fresh weight and dry weigh that were

31.7, 8.7 and 5.5, respectively. The shoot length (cm), fresh and dried weights (g) were also found highest in SS+B+P+B2 treatment, which were 38.1, 6.3 and 1.7, respectively, while significant lower values were found with the SS+P treatment, which were 24.3, 02.2 and 0.4, respectively. After SS+B+P+B2, the treatment SS+B+P+B1 resulted in maximum shoot length (cm), fresh and dried weights (g) that were 37.7, 6.0 and 1.4, respectively.

In treatments that had sandy loam soil, root length (cm), fresh and dry weight (g) the maximum growth was noted for the treatment SS+B+P+B2, which were 48.7, 10.1 and 6.4, respectively. After this, the treatment SS+B+P+B1 had the higher trend for root length, fresh weight and dried weight that were 36.5, 10.0 and 4.9, respectively. The shoot length (cm), fresh and dried weights (g) were also found highest in SS+B+P+B2 treatment, which were 41.1, 7.9 and 3.6, respectively, while significant lower value was found with the SS+P treatment which were 33.2, 3.8 and 1.6, respectively. It was followed by SS+B+P+B1 treatment, its shoot length (cm); fresh weight and dried weight (g) were 41.0, 7.2 and 3.4, respectively.

It was noted that in terms of length, fresh weight and dry weight of roots and shoots, sandy loam soils supported the plant better than clayey soil by increasing plant length and weight in both roots and shoots.

Table 3.5: Physiological Parameters of *Triticum aestivum* in Clayey Soil

Treatments	**Roots**			**Shoots**		
	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry Weight (g)
FS+P	18.3 ± 0.2^{f}	4.3 ± 0.3^{f}	2.4 ± 0.3^{def}	30.5 ± 0.3^{d}	4.0 ± 0.3^{ef}	1.0 ± 0.6^{abcd}
SS+P	10.0 ± 0.7^{i}	3.3 ± 0.3^{h}	1.5 ± 0.4^{g}	24.3 ± 0.2^{g}	2.2 ± 0.2^{g}	0.4 ± 0.0^{d}
SS+P+B1	13.3 ± 0.5^{g}	4.0 ± 0.2^{fg}	2.0 ± 0.3^{efg}	29.1 ± 0.6^{e}	3.6 ± 0.3^{f}	0.5 ± 0.2^{cd}
SS+P+B2	13.4 ± 0.5^{g}	4.2 ± 0.1^{f}	2.3 ± 0.3^{def}	29.2 ± 0.6^{e}	3.8 ± 0.4^{ef}	1.0 ± 0.3^{bcd}
SS+P+B1+B2	11.5 ± 0.7^{h}	3.7 ± 0.2^{gh}	1.8 ± 0.2^{fg}	27.6 ± 0.1^{f}	2.8 ± 0.7^{g}	0.4 ± 0.1^{d}
SS+C+P	21.2 ± 0.6^{e}	4.3 ± 0.3^{f}	2.7 ± 0.2^{de}	31.3 ± 0.2^{d}	4.3 ± 0.2^{de}	1.0 ± 0.4^{abcd}
SS+C+P+B1	23.1 ± 0.4^{d}	5.1 ± 0.3^{e}	2.8 ± 0.4^{d}	36.2 ± 0.6^{c}	4.5 ± 0.1^{de}	1.1 ± 0.4^{abc}
SS+C+P+B2	23.5 ± 0.8^{d}	5.7 ± 0.2^{d}	2.9 ± 0.4^{d}	37.1 ± 0.6^{b}	4.9 ± 0.7^{cd}	1.2 ± 0.5^{abc}
SS+C+P+B1+B2	21.9 ± 0.7^{e}	4.8 ± 0.3^{e}	2.7 ± 0.3^{d}	36.0 ± 0.7^{c}	4.4 ± 0.2^{de}	1.1 ± 0.5^{abc}
SS+B+P	25.5 ± 0.5^{c}	5.8 ± 0.1^{d}	2.9 ± 0.5^{d}	37.0 ± 0.7^{b}	5.4 ± 0.3^{bc}	1.3 ± 0.6^{abc}
SS+B+P+B1	31.7 ± 0.5^{a}	8.7 ± 0.2^{b}	5.5 ± 0.3^{b}	37.7 ± 0.6^{ab}	6.0 ± 0.3^{ab}	1.4 ± 0.3^{ab}
SS+B+P+B2	32.3 ± 0.5^{a}	9.5 ± 0.3^{a}	6.4 ± 0.4^{a}	38.1 ± 0.1^{a}	6.3 ± 0.2^{a}	1.7 ± 0.2^{a}
SS+B+P+B1+B2	27.2 ± 0.5^{b}	6.3 ± 0.6^{c}	4.2 ± 0.5^{c}	37.5 ± 0.3^{ab}	5.8 ± 0.6^{ab}	1.4 ± 0.4^{ab}

FS+P=Fresh soil + Plant, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means.

Table 3.6: Physiological Parameters of *Triticum aestivum* in Sandy Loam Soil

Treatments	**Roots**			**Shoots**		
	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry Weight (g)
FS+P	28.8 ± 0.5^{g}	5.8 ± 0.3^{e}	2.6 ± 0.5^{d}	36.6 ± 0.4^{ef}	5.6 ± 0.3^{de}	2.3 ± 0.2^{ef}
SS+P	18.5 ± 0.4^{k}	4.6 ± 0.3^{f}	2.4 ± 0.4^{d}	33.2 ± 0.1^{h}	3.8 ± 0.2^{g}	1.6 ± 0.4^{g}
SS+P+B1	22.7 ± 0.5^{i}	5.6 ± 0.5^{e}	2.5 ± 0.5^{d}	34.9 ± 0.3^{g}	4.6 ± 0.5^{f}	1.9 ± 0.2^{fg}
SS+P+B2	25.1 ± 0.1^{h}	5.7 ± 0.5^{e}	2.6 ± 0.4^{d}	36.2 ± 0.1^{f}	5.3 ± 0.4^{e}	2.1 ± 0.1^{fg}
SS+P+B1+B2	20.0 ± 0.4^{j}	4.8 ± 0.3^{f}	2.4 ± 0.4^{d}	34.7 ± 0.3^{g}	4.6 ± 0.4^{f}	1.8 ± 0.5^{fg}
SS+C+P	30.1 ± 0.3^{f}	6.0 ± 0.2^{de}	2.7 ± 0.4^{d}	36.9 ± 0.3^{e}	6.0 ± 0.2^{d}	2.3 ± 0.2^{def}
SS+C+P+B1	33.5 ± 0.6^{d}	6.6 ± 0.4^{d}	3.7 ± 0.0^{c}	37.9 ± 0.1^{d}	6.7 ± 0.3^{bc}	2.9 ± 0.4^{bcd}
SS+C+P+B2	34.3 ± 0.1^{c}	6.7 ± 0.4^{d}	3.9 ± 0.1^{c}	37.9 ± 0.4^{d}	7.0 ± 0.3^{bc}	2.9 ± 0.2^{bc}
SS+C+P+B1+B2	32.7 ± 0.3^{e}	6.1 ± 0.3^{de}	2.8 ± 0.2^{d}	36.9 ± 0.4^{e}	6.5 ± 0.3^{c}	2.8 ± 0.4^{cde}
SS+B+P	34.4 ± 0.1^{c}	7.9 ± 0.4^{c}	4.0 ± 0.1^{c}	38.7 ± 0.3^{c}	7.2 ± 0.1^{b}	3.0 ± 0.4^{abc}
SS+B+P+B1	36.5 ± 0.4^{b}	10.0 ± 0.5^{a}	4.9 ± 0.4^{b}	41.0 ± 0.4^{a}	7.2 ± 0.4^{b}	3.4 ± 0.3^{ab}
SS+B+P+B2	48.7 ± 0.3^{a}	10.1 ± 0.5^{a}	6.4 ± 0.4^{a}	41.1 ± 0.3^{a}	7.9 ± 0.3^{a}	3.6 ± 0.2^{a}
SS+B+P+B1+B2	36.0 ± 0.3^{b}	8.7 ± 0.4^{b}	4.8 ± 0.3^{b}	39.6 ± 0.4^{b}	7.2 ± 0.2^{b}	3.1 ± 0.4^{abc}

FS+P=Fresh soil + Plant, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was “a” column wise followed by later alphabets for lower means.

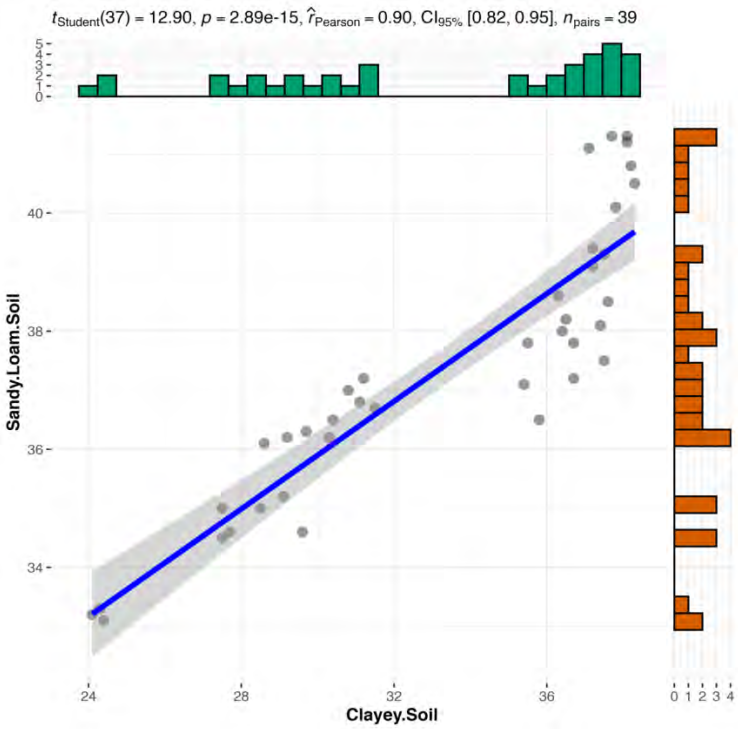

Figure 3.11: Correlation between Shoot length of Wheat in Clayey and Sandy Loam Soil

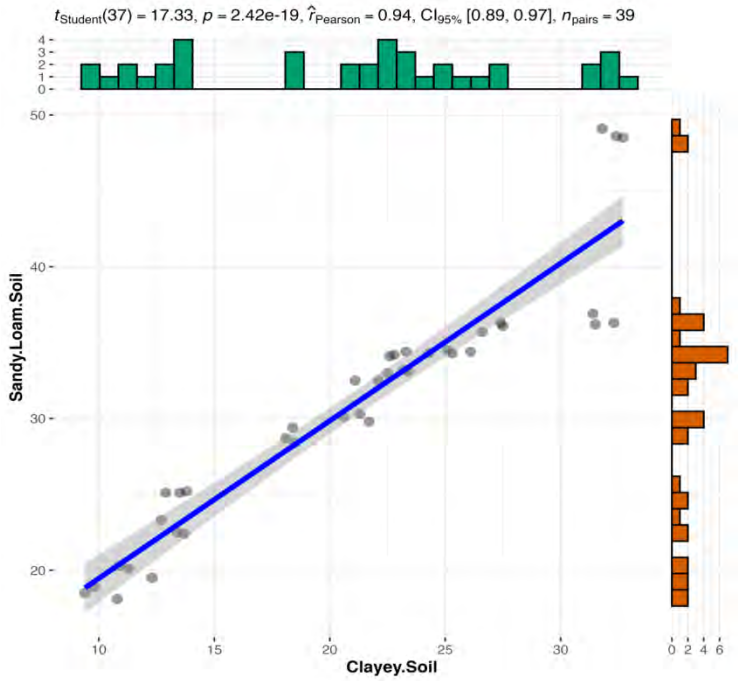

Figure 3.12: Corrleation between Root Length of Wheat in Clayey and Sandy Loam Soil

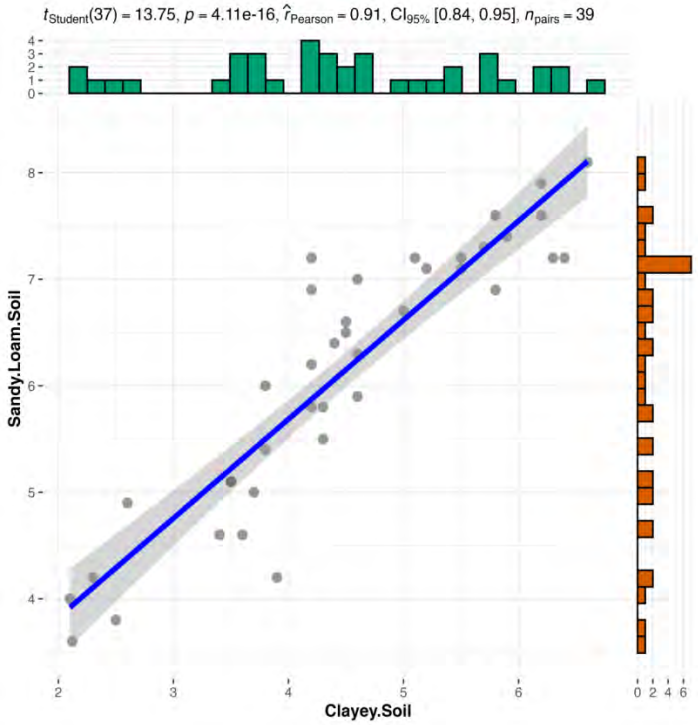

Figure 3.13: Corrleation between Shoots' Fresh Weight of Wheat in Clayey and Sandy Loam Soil

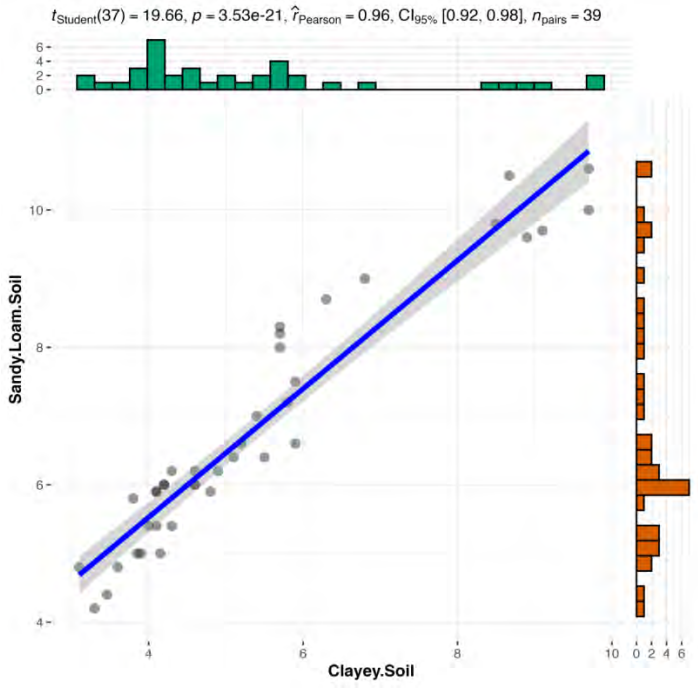

Figure 3.14: Correlation between Roots' Fresh Weight of Wheat in Clayey and Sandy Loam Soil

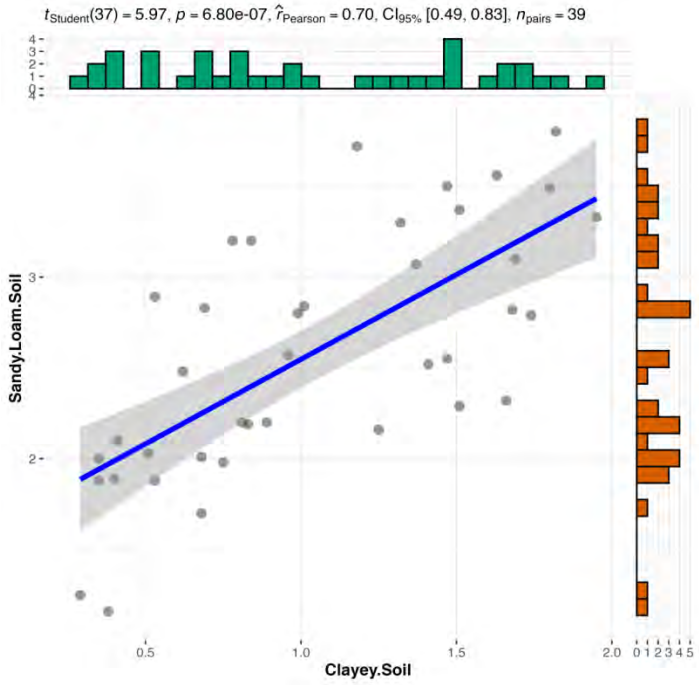

Figure 3.15: Correlation between Shoots' Dry Weight of Wheat in Clayey and Sandy Loam Soil

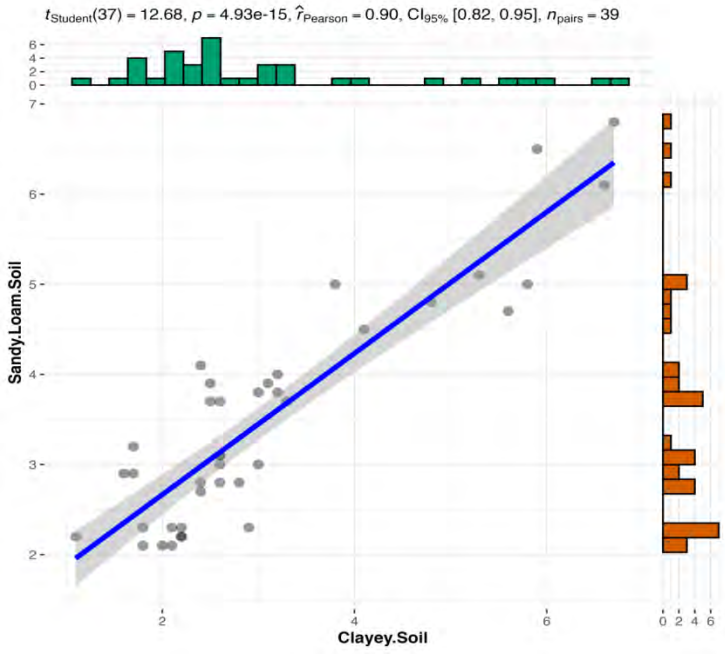

Figure 3.16: Correlation between Roots' Dry Weight of Wheat in Clayey and Sandy Loam Soil

3.4. Stress and Damage to *Triticum aestivum* due to CPF Exposure

CPF showed impact on *Triticum aestivum* by influencing chlorophyll level and stress indicators in different treatments of both soil textures; the results for leaf pigments (Chlorophyll a, Chlorophyll b, Total Chlorophyll content) are presented in Figure 3.3 and Figure 3.4 and Carotenoid content is present in Figure 3.5, while Figure 3.6 is for H_2O_2, and Figure 3.7 is for MDA contents. The levels of chlorophyll and carotenoid (pigment content mg g^{-1} plant FW) in different treatments of both soils were found to have statistically significant variation among different applied soil treatments.

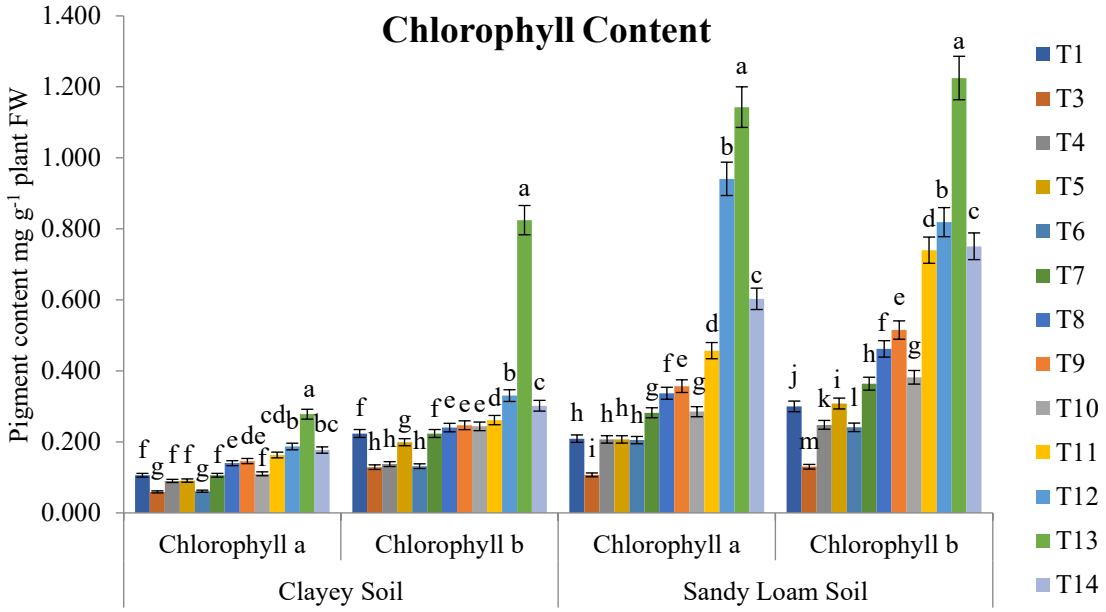

Figure 3.17: Chlorophyll a and b Levels of *Triticum aestivum* in Different Treatments

In terms of plants grown on clayey soil, the highest level of Chlorophyll a, b and Total Chlorophyll were noted for the treatment SS+B+P+B2 and the values were 0.278, 0.825 and 1.102, respectively. These values varied significantly from all other applied treatments. The lower values for Chlorophyll a, b and Total Chlorophyll were found in the treatment SS+P that were 0.059, 0.129 and 0.188, respectively. In case of carotenoid content, the significantly higher values 174.47 were also observed for the treatment SS+B+P+B2 while significantly lower values 126.04 were noted for the treatment SS+P.

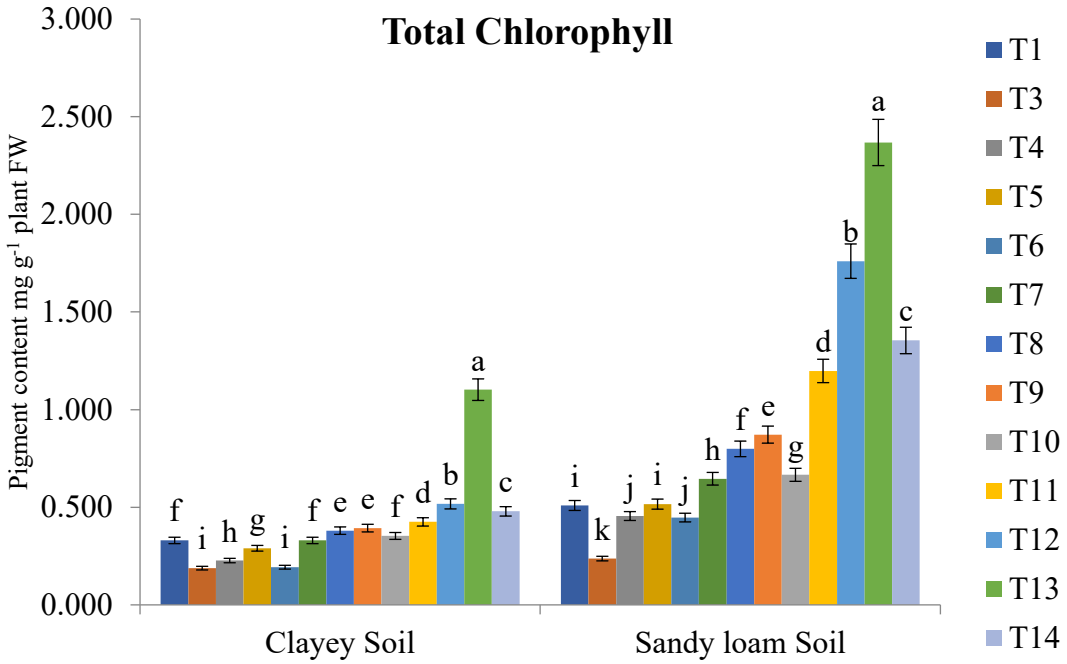

Figure 3.18: Total Chlorophyll Levels of *Triticum aestivum* in Different Treatments

For the plants grown on sandy loam soil, the highest level of Chlorophyll a, b and Total Chlorophyll were noted for the treatment SS+B+P+B2 and the values were 1.143, 1.225 and 2.368, respectively. These values also varied significantly from all other applied treatments. The lowest values for Chlorophyll a, b and Total Chlorophyll were found in the treatment SS+P that were 0.107, 0.130 and 0.237, respectively. In case of carotenoid content, the significantly higher values 288.28 were observed for the treatment SS+B+P+B1+B2 while significantly lower values 48.68 were noted for the treatment SS+P.

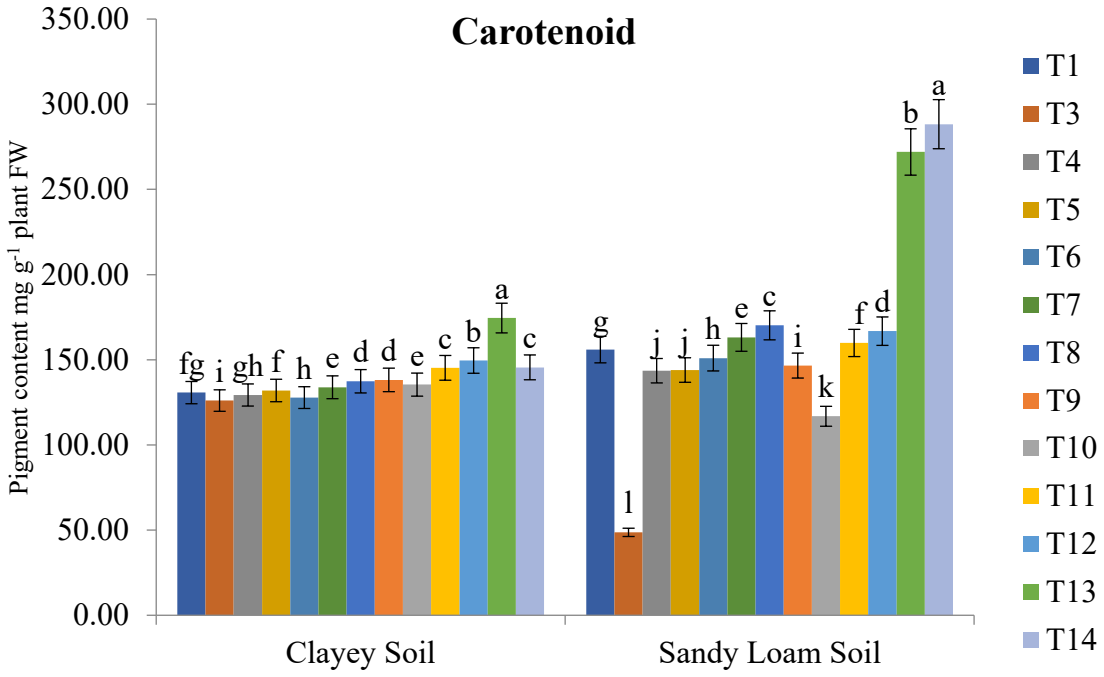

Figure 3.19: Carotenoid Levels of *Triticum aestivum* in Different Treatments

Collectively, plants grown on sandy loam soil had more Chlorophyll a, b, Total Chlorophyll and Carotenoid content in comparison to the pigment present in plants grown on clayey soil.

The H_2O_2 (µM of H_2O_2 g^{-1} of FW) and MDA (µM of MDA g^{-1} of FW) levels among different applied treatments of both soil textures were found to vary significantly (Figure 3.4 and Figure 3.5). The lower levels of both stress indicators represent the residual level of H_2O_2 and MDA contents in *Triticum aestivum* and was noted for plants grown in the absence of CPF in treatment FS+P of both soils.

For the plants grown in clayey soil, the highest level for H_2O_2 content was observed for the treatment SS+P and the observed value was 1.52. The reduction in H_2O_2 content was observed for treatment FS+P and SS+B+P+B2, which were 1.10 and 1.12. The reduction in H_2O_2 content of the treatment SS+B+P+B2 indicates that the combination of this applied method may have the ability to cope with the stress of CPF. The MDA level was also found lower in treatments FS+P and SS+B+P+B2 that were 2.55 and 6.39 respectively while the higher MDA level was noted in SS+P with the value of 8.71. The

reduction in MDA level of the treatment SS+B+P+B2 also indicates that if the combination of this applied treatment is given to the plant, it will reduce the CPF stress.

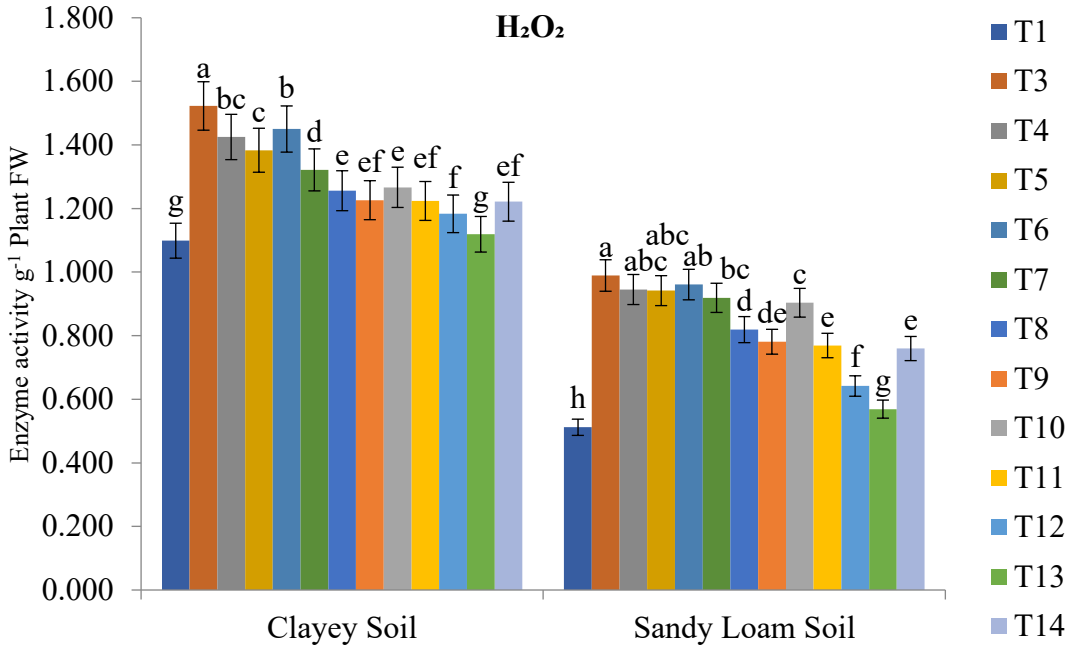

Figure 3.20: H_2O_2 Content in *Triticum aestivum* due to CPF Exposure

For the plants grown on sandy loam soil, the highest level for H_2O_2 content was observed for the treatment SS+P and the observed value was 0.99. The reduction in H_2O_2 content was observed for treatment FS+P and SS+B+P+B2, which were 0.51 and 0.57. The reduction in H_2O_2 content of the treatment SS+B+P+B2 particularly in this soil indicates that the combination of this applied method may have the ability to cope with the stress of CPF better in sandy loam soil. The MDA level was also found lower 0.45 and 0.56 in treatments FS+P and SS+B+P+B2 while the higher MDA level was noted in SS+P with the value of 7.49. The reduction in MDA level of the treatment SS+B+P+B2 also indicates that it will reduce the CPF stress if the combination of this applied treatment is given to the plant.

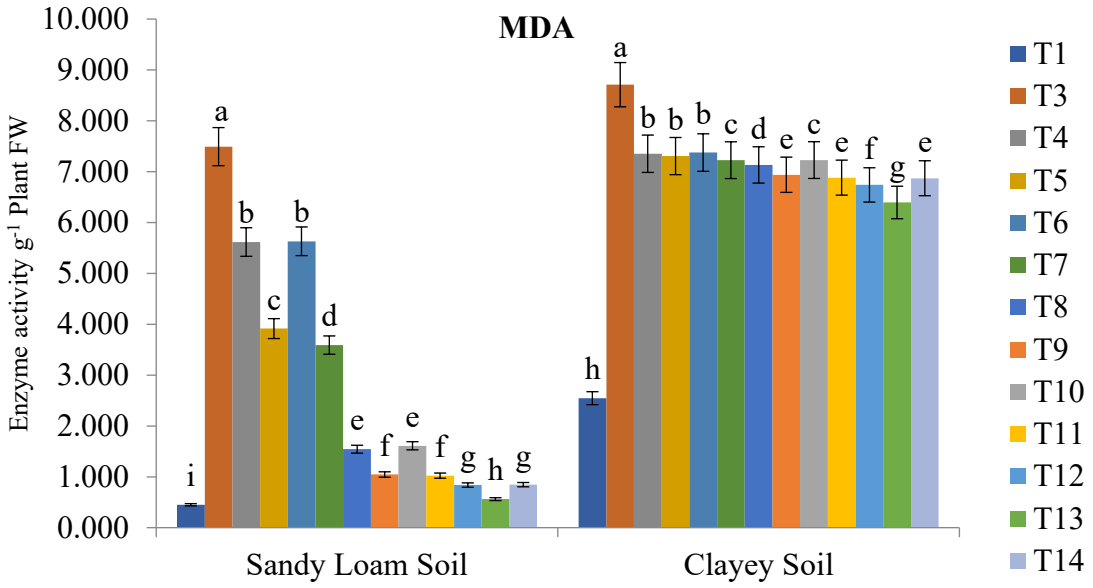

Figure 3.21: MDA Content in *Triticum aestivum* due to CPF Exposure

Overall, it is observed that clayey soils have more stressed environment for plants as the values of H_2O_2 and MDA Are higher in comparison to the plants in sandy loam soil type. Furthermore, sandy loam soils provide the treatments a better environment to overcome CPF stress as the treatments in this soil texture have performed better than clayey soils.

3.5. *Triticum aestivum* Enzymatic Status due to CPF Exposure

Enzyme activities including APX, GPX, CAT, and SOD in *Triticum aestivum* upon exposure to CPF in clayey as well as sandy loam soils are presented in Figure 3.8, 3.9, 3.10 and 3.11 respectively. Higher values of APX, GPX, CAT, and SOD were observed in SS+P treatment of both soils with difference in values.

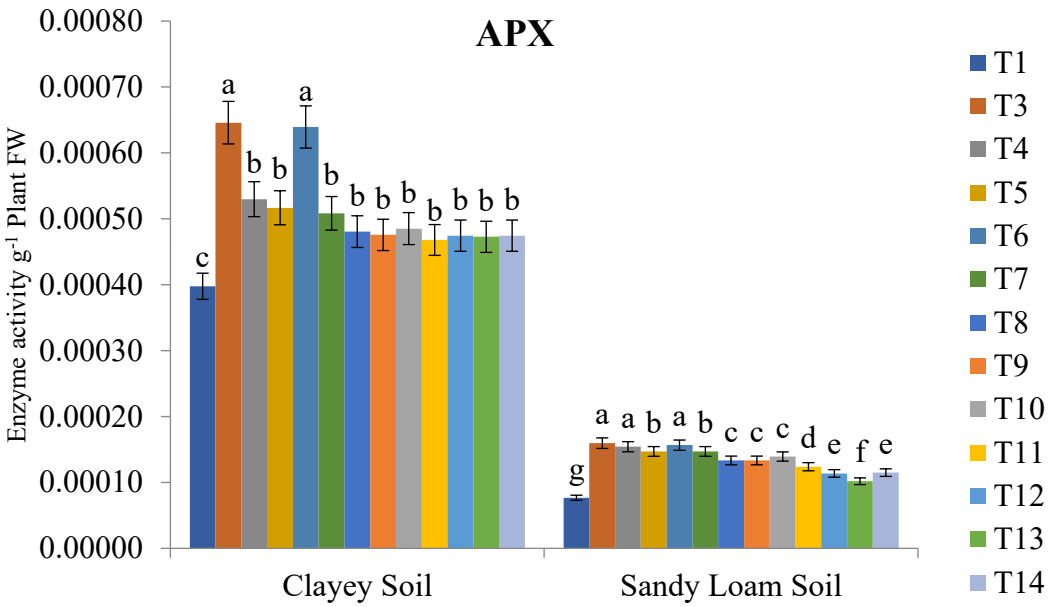

Figure 3.22: APX Activity in *Triticum aestivum*

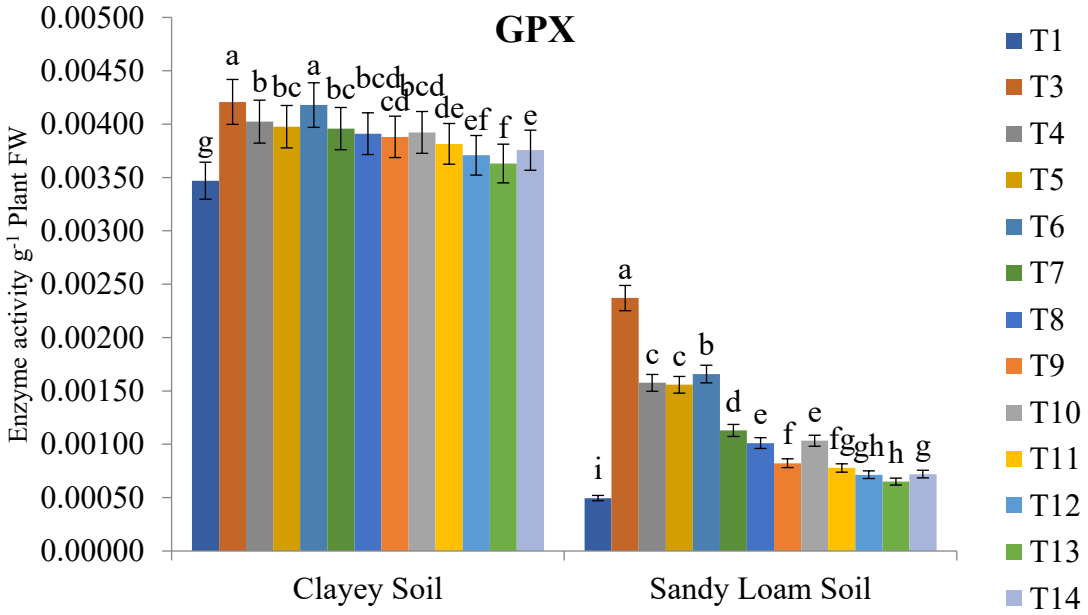

Figure 3.23: GPX Activity in *Triticum aestivum* with Different Treatments

For the plants present in clayey soil, APX, GPX, CAT, and SOD had highest values in SS+P treatment that were 0.00065, 0.00421, 0.000065, and 0.00818, respectively, suggesting that higher concentration of CPF resulted this effect. While the lower values of APX, GPX, CAT, and SOD with higher reduction were noted for the treatment FS+P

and SS+B+P+B2. The values of APX, GPX, CAT, and SOD for FS+P were 0.00040, 0.00347, 0.000033, and 0.00649, respectively, while the values of APX, GPX, CAT, and SOD for treatment SS+B+P+B2 were 0.00047, 0.00363, 0.000030, and 0.00771, respectively.

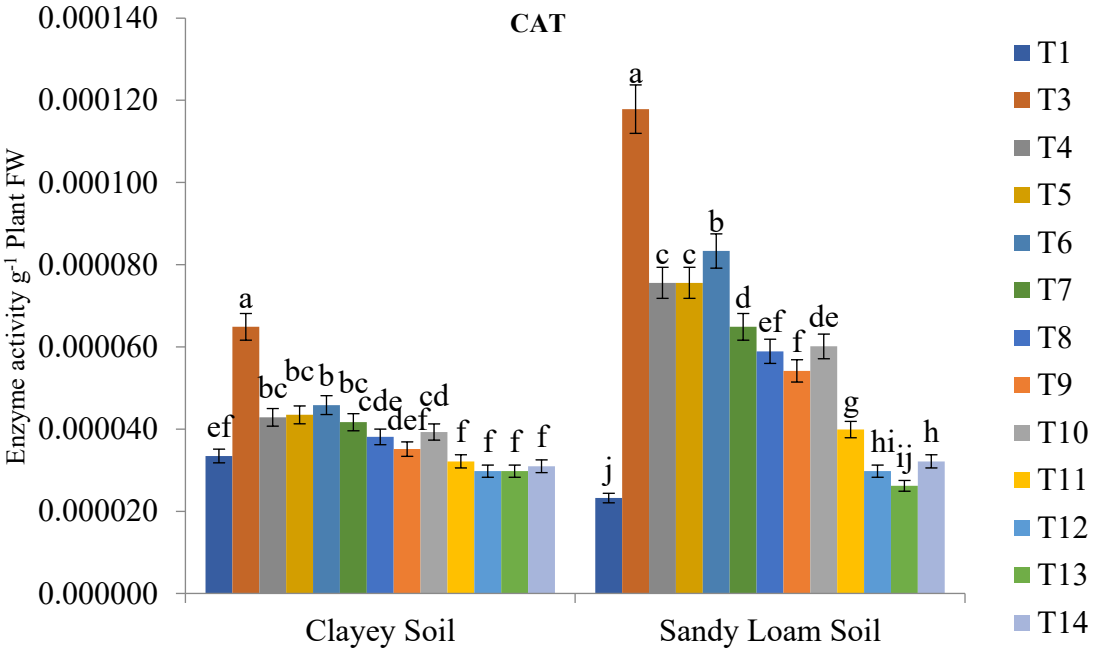

Figure 3.24: Catalase Activity in *Triticum aestivum* with Different Treatments

For the plants present in sandy loam soil, APX, GPX, CAT, and SOD also had highest values in SS+P treatment that were 0.00016, 0.00237, 0.000118 , and 0.00794, respectively, suggesting that higher concentration of CPF resulted this effect. While the lower values of APX, GPX, CAT, and SOD with higher reduction were noted for the treatment FS+P and SS+B+P+B2. The values of APX, GPX, CAT, and SOD for FS+P were 0.00008, 0.00050, 0.000023, and 0.00718, respectively, while the values of APX, GPX, CAT, and SOD for treatment SS+B+P+B2 were 0.00010, 0.00065, 0.000026, and 0.00737, respectively.

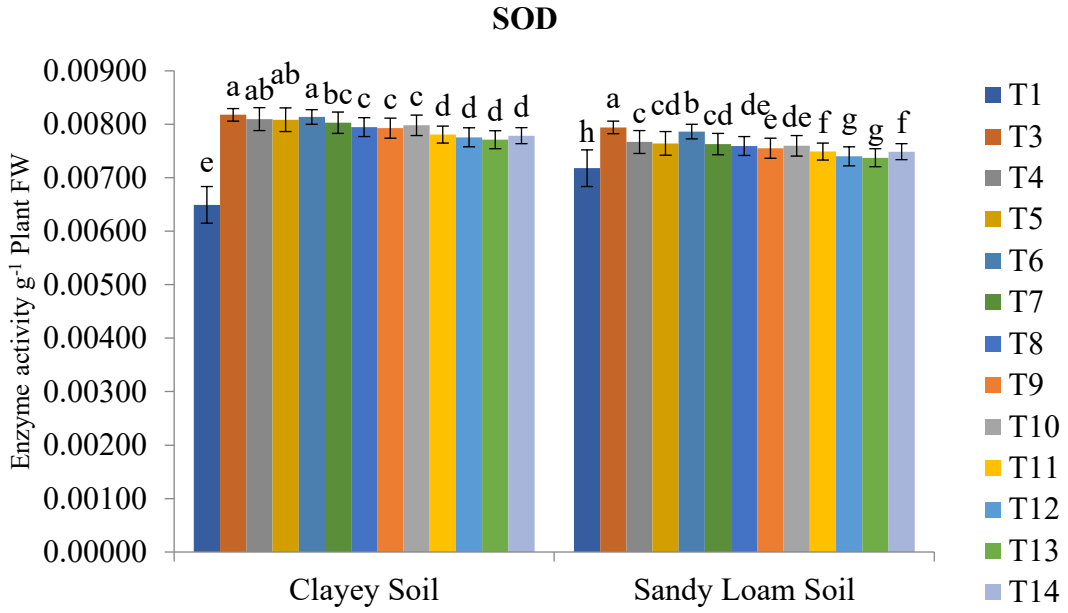

Figure 3.25: SOD Activity in *Triticum aestivum* with Different Treatments

Collectively, more enzymes were produced in the plants grown on clayey soil in comparison to the plants in sandy loam soil as they were facing more stressed environment in clayey soil.

3.6. Soil Microbial Count

The results of microbial count in the plant rhizosphere of different treatments in different soils are presented in Table 3.7. The spiked soil samples amended with compost showed higher level of microbial count in both soils. In particular, SS+C+P+B2 showed highest level of microbial count in both soils, though the number varied. SS treatments in which no bacteria were added showed significantly lower CPF degradation rate. Significantly higher level of rhizospheric CFUs were noted in treatment where CPF degrading bacterial strain *Pseudomonas aeruginosa* was added, which was SS+C+P+B2. In clayey soil, the CFU count for SS+C+P+B2 was $4.10*10^5$, while in sandy loam soil, the CFU count was $8.47*10^7$. The lowest microbial count was noted in the treatment SS+P+B1+B2, which was $2.40*10^3$ for clayey soil and $5.3*10^5$ for sandy loam soil. Thus, the microbial count and survival of bacterial strains was more in sandy loam soil.

Table 3.7: Bacterial Count in Rhizosphere of Different Treatments

Treatments	**CFUs (cells g^{-1} of soil) Clayey Soil**	**CFUs (cells g^{-1} of soil) Sandy Loam Soil**
SS+P+B1	$2.85*10^3 \pm 5.00*10^{2\ h}$	$5.85*10^5 \pm 5.00*10^{3\ h}$
SS+P+B2	$2.96*10^3 \pm 5.00*10^{2\ g}$	$6.40*10^5 \pm 2.00*10^{3\ g}$
SS+P+B1+B2	$2.40*10^3 \pm 1.00*10^{2\ i}$	$5.30*10^5 \pm 3.00*10^{3\ i}$
SS+C+P+B1	$3.80*10^5 \pm 1.00*10^{2\ b}$	$8.27*10^7 \pm 1.50*10^{3\ b}$
SS+C+P+B2	$4.10*10^5 \pm 1.00*10^{2\ a}$	$8.47*10^7 \pm 5.00*10^{3\ a}$
SS+C+P+B1+B2	$3.65*10^5 \pm 5.00*10^{3\ c}$	$3.84*10^7 \pm 5.00*10^{4\ c}$
SS+B+P+B1	$3.10*10^4 \pm 1.00*10^{3\ e}$	$6.05*10^6 \pm 3.00*10^{3\ e}$
SS+B+P+B2	$3.50*10^4 \pm 1.00*10^{3\ d}$	$6.06*10^6 \pm 2.50*10^{4\ d}$
SS+B+P+B1+B2	$2.95*10^4 \pm 5.00*10^{3\ f}$	$2.50*10^6 \pm 1.00*10^{3\ f}$

SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was "a" column wise followed by later alphabets for lower means.

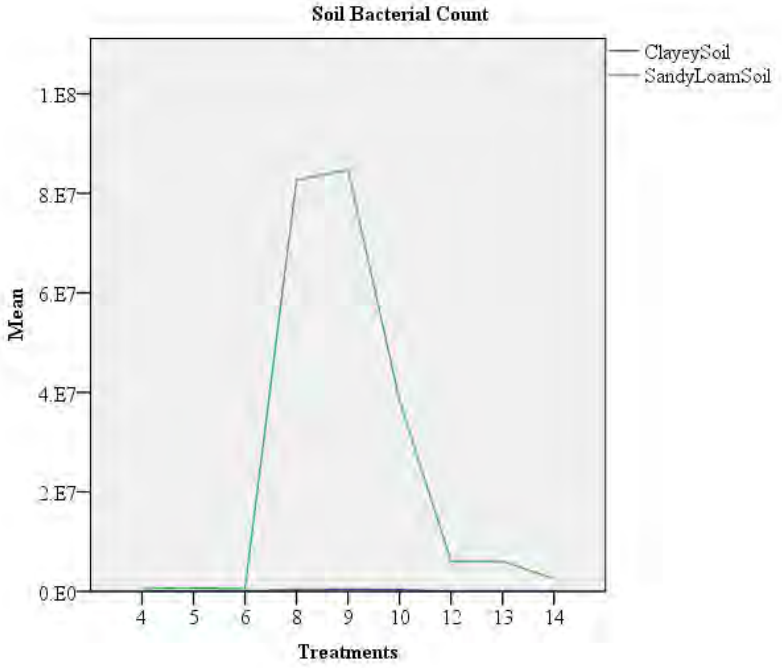

Figure 3.26: CFU Count in Rhizosphere of Clayey and Sandy Loam Soil Type

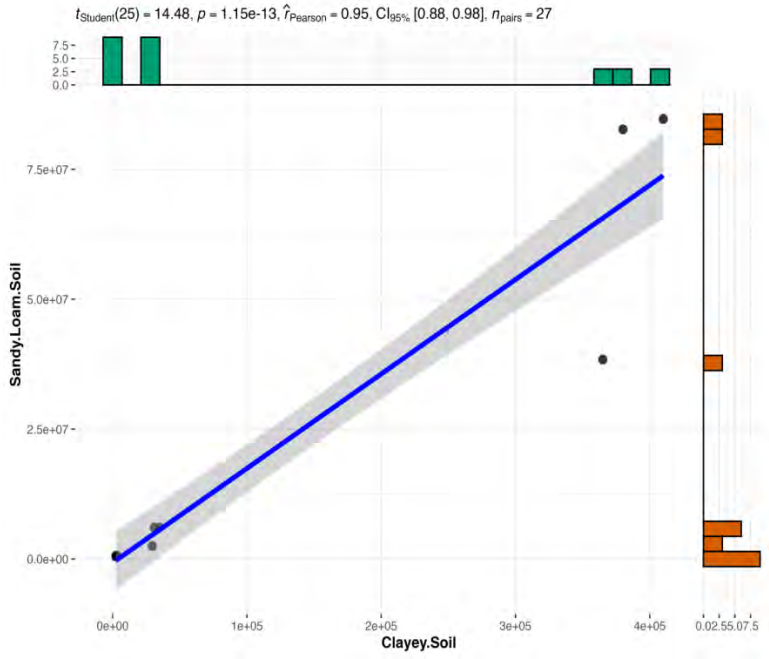

Figure 3.27: Correlation between CFU of Different Soil Types

3.7. Chlorpyrifos Uptake by *Triticum aestivum* and Degradation in Soil

Presence of CPF in soil and its uptake by *Triticum aestivum* by clayey and sandy loam soil is presented in Table 3.8 and 3.9 respectively.

It is observed that, in clayey soil, highest value of CPF (148.29 mg/kg) was noted in SS treatment where no plant or additional amendment was applied. The addition of plant in treatment SS+P reduced the level to 89.80mg/kg, depicting the influence of plant on degradation. The lowest value of 54.62mg/kg was recorded in the treatment SS+C+P+B2, showing that the combined use of plant, *Pseudomonas aeruginosa*, and compost can degrade CPF to the maximum level, i.e. 63.5% CPF was removed from the soil. In treatments where biochar was added, SS+B+P+B2 showed the minimum CPF in soil, i.e. 56.39mg/kg, depicting that it has bound CPF and restricted its presence in soil. Further, the uptake by roots and shoots was maximum in SS+P, as it was noted 45.06, and 10.55mg/kg, respectively. On the other hand, the trend was different in case of treatments amended with biochar as SS+B+P+B2 showed least uptake of CPF in both roots and shoots with the values of 5.93, and 4.56, respectively. This depicts that biochar made the CPF in soil unavailable for the plant to uptake by binding it.

In sandy loam soil, it is observed that the highest value of CPF (144.92 mg/kg) was noted in SS treatment where no plant or additional amendment was applied. The addition of plant in treatment SS+P reduced the level to 56.29mg/kg, depicting the influence of plant on degradation. The lowest value of 5.20mg/kg was recorded in the treatment SS+C+P+B2, showing that the combined use of plant, *Pseudomonas aeruginosa*, and compost degraded CPF to the maximum level. In treatments where biochar was added, SS+B+P+B2 showed the minimum presence of CPF in soil, i.e.,11.97mg/kg, depicting that it has bound CPF and restricted its presence in soil. Further, the uptake by roots and shoots was maximum in SS+P, as it was noted 69.33, and 10.58mg/kg, respectively. On the other hand, the trend was different in case of amended treatments as SS+B+P+B2 showed least uptake of CPF in both roots and shoots with the values of 20.30, and 4.15mg/kg respectively. This depicts that biochar made the CPF in soil unavailable for the plant to uptake by binding it.

Table 3.8: Concentration of CPF in Soil and Uptake by *Triticum aestivum* in Clayey Soil

Treatments	Soil mg/kg	Roots mg/kg	Shoots mg/kg
FS+P	0.44 ± 0.10^{h}	0.27 ± 0.02^{h}	0.06 ± 0.03^{f}
SS	147.62 ± 2.76^{a}		
SS+P	89.80 ± 1.39^{b}	45.06 ± 1.75^{a}	10.55 ± 1.18^{a}
SS+P+B1	83.15 ± 1.37^{c}	32.93 ± 1.05^{b}	9.52 ± 0.53^{ab}
SS+P+B2	71.28 ± 2.34^{d}	26.24 ± 0.82^{c}	9.33 ± 0.50^{ab}
SS+P+B1+B2	80.40 ± 2.96^{c}	42.46 ± 0.61^{a}	10.34 ± 1.01^{a}
SS+C+P	74.53 ± 2.21^{d}	30.07 ± 1.84^{b}	8.92 ± 1.05^{b}
SS+C+P+B1	55.35 ± 1.90^{g}	24.12 ± 2.25^{cd}	6.72 ± 0.49^{c}
SS+C+P+B2	54.62 ± 1.66^{g}	23.15 ± 2.14^{de}	6.57 ± 0.68^{c}
SS+C+P+B1+B2	57.18 ± 1.13^{g}	25.84 ± 1.77^{cd}	7.43 ± 0.99^{c}
SS+B+P	78.74 ± 1.37^{cd}	23.96 ± 1.76^{de}	7.25 ± 0.75^{c}
SS+B+P+B1	59.34 ± 0.89^{g}	12.93 ± 2.09^{f}	5.06 ± 1.06^{de}
SS+B+P+B2	56.39 ± 0.83^{g}	5.93 ± 1.14^{g}	4.56 ± 0.67^{e}
SS+B+P+B1+B2	61.52 ± 1.45^{f}	13.31 ± 2.11^{f}	5.14 ± 0.33^{de}

FS+P=Fresh soil + Plant, SS=CPF spiked soil, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was “a” column wise followed by later alphabets for lower means.

Table 3.9: Concentration of CPF in Soil and Uptake by *Triticum aestivum* in Sandy Loam Soil

Treatments	Soil mg/kg	Roots mg/kg	Shoots mg/kg
FS+P	0.19 ± 0.02^{l}	0.33 ± 0.18^{k}	0.06 ± 0.02^{f}
SS	144.92 ± 0.71^{a}		
SS+P	56.29 ± 3.29^{b}	69.33 ± 0.41^{a}	10.58 ± 1.30^{a}
SS+P+B1	28.19 ± 1.21^{f}	45.54 ± 1.87^{c}	8.22 ± 0.40^{b}
SS+P+B2	23.40 ± 1.93^{g}	43.69 ± 1.84^{c}	8.15 ± 0.91^{b}
SS+P+B1+B2	33.36 ± 1.54^{e}	55.88 ± 1.10^{b}	8.70 ± 0.85^{b}
SS+C+P	30.27 ± 0.90^{e}	36.68 ± 1.50^{c}	7.53 ± 1.40^{bc}
SS+C+P+B1	8.95 ± 0.81^{j}	28.32 ± 0.63^{g}	7.26 ± 0.87^{bc}
SS+C+P+B2	5.20 ± 0.92^{k}	27.09 ± 1.17^{g}	6.30 ± 0.88^{cd}
SS+C+P+B1+B2	11.02 ± 0.90^{ij}	30.67 ± 0.68^{f}	7.62 ± 0.80^{bc}
SS+B+P	32.29 ± 0.50^{e}	28.42 ± 0.88^{g}	5.17 ± 0.94^{d}
SS+B+P+B1	13.03 ± 0.85^{i}	23.13 ± 1.10^{i}	4.59 ± 0.74^{e}
SS+B+P+B2	11.97 ± 1.63^{i}	20.30 ± 1.20^{j}	4.15 ± 0.34^{e}
SS+B+P+B1+B2	17.02 ± 0.10^{h}	25.12 ± 0.26^{h}	4.77 ± 0.90^{de}

FS+P=Fresh soil + Plant, SS=CPF spiked soil, SS+P= Spiked soil + Plant, SS+P+B1= Spiked soil + Plant + Bacterial strain 1, SS+P+B2= Spiked soil + Plant + Bacterial strain 2, SS+P+B1+B2= Spiked soil + Plant + Bacterial strain 1 + Bacterial strain 2, SS+C+P= Spiked soil + Compost + Plant, SS+C+P+B1= Spiked soil + Compost + Plant + Bacterial strain 1, SS+C+P+B2= Spiked soil + Compost + Plant + Bacterial strain 2, SS+C+P+B1+B2= Spiked soil + Compost + Plant + Bacterial strain 1+ Bacterial strain 2, SS+B+P= Spiked soil + Biochar + Plant, SS+B+P+B1= Spiked soil + Biochar + Plant + Bacterial strain 1, SS+B+P+B2= Spiked soil + Biochar + Plant + Bacterial strain 2, SS+B+P+B1+B2= Spiked soil + Biochar + Plant + Bacterial strain 1+ Bacterial strain 2

Data is presented in means (n = 3 ± SD). Significantly highest mean was “a” column wise followed by later alphabets for lower means.

Degradation (%) of CPF in rhizospheric soil samples of both soil textures are presented in Figure 3.10. The significantly higher rate of CPF remediation was found in treatment SS+B+P+B2, i.e. 55.51% in clayey soil and 75.77% in sandy loam soil. Significantly lower degradation rate was observed in SS; where there was no additional treatment was applied. The rate of degradation in SS was 3.05% in clayey soil and 9.41% in sandy loam.

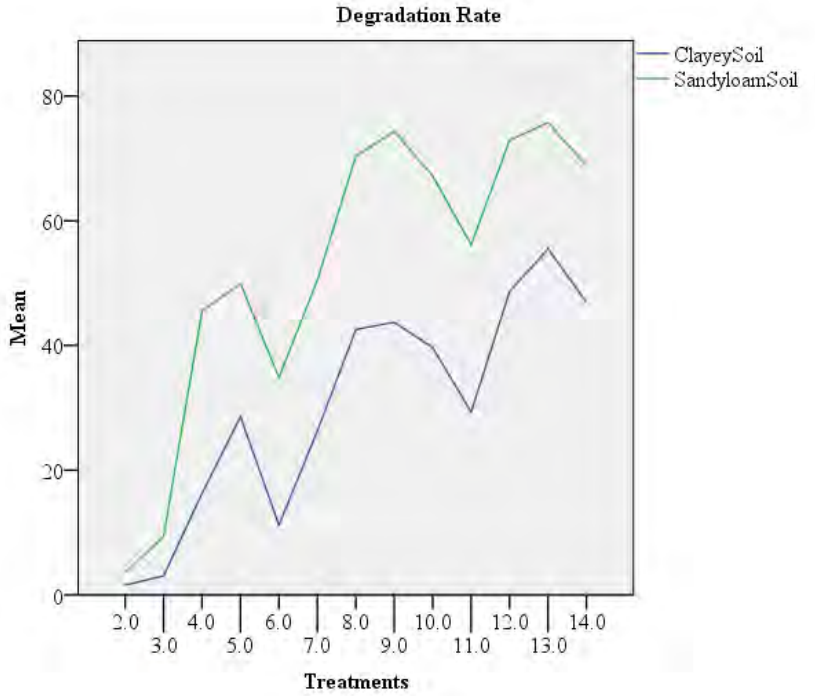

Figure 3.28: Degradation of CPF in Different Treatments of Clayey and Sandy Loam Soil

Chapter 4

Discussion

A current investigation was conducted for the remediation of CPF using novel integrated methods in soils of two different textures. This experimental study evaluated the efficiency of the individual, as well as a combination of various remediation processes i.e. bioremediation using microbial strain, phytoremediation using *Triticum aestivum*, and bioaugmentation by use of compost and biochar as amendments to enhance the rhizoremediation of CPF contaminated soils. The potential application of bioremediation and phytoremediation along with bioaugmentation was also investigated in both soil textures. By far there are several problems associated with the integration that include the availability of resources, the presence of organic matter limiting bacterial activity, the duration of time, the environmental effect, and the level of sustainability.

The physicochemical status of soil contaminated with CPF alters to a certain extent that in turn influences soil microbial and enzymatic activity. Further, the addition of organic amendments, particularly compost and biochar increases soil pH by 0.28–2.29 pH units compared to the unamended soil (Frimpong et al., 2020). Soil pH is considered to be a key soil parameter as it influences various activities in soil. In this study, the pH of fresh soil, as well as spiked soil, was observed almost in the same range. A slight increase was observed in treatments amended with compost and biochar. Sandy loam soil showed a little more variation in soil pH as the pH of all initial and final treatments was under the range of 7.19 to 7.44 in clayey soil, while it was under the range of 6.85 to 7.87 in sandy loam soil. Singh et al., (2003) reported that CPF degradation was more rapid in neutral-pH, i.e. pH above 6.7, and in alkaline soils.

Electrical conductivity and total dissolved solids in soil are indicators of salinity which states the quantification of total salts present in the soil sample. The soil EC tells us about the health of the soil and the EC value ranging between 0 to 2000 μSm^{-1} is usually suitable for plant growth. Additionally, the EC of the soil increases with the addition of CPF but reduces when the contaminated soil is amended with bio-stimulating agents. In this study, the EC of the soil varied significantly between the

two different soil textures. Clayey soil had more EC, ranging from 200 μSm^{-1} to 641 μSm^{-1},in comparison to sandy loam soil where the EC ranged from 80 μSm^{-1} to 486 μSm^{-1}. Furthermore, the EC of the contaminated soil was higher in both soils than the EC of the fresh soil because the CPF was added to it. EC of the CPF contaminated soil was much higher than the EC of soil having the plant, bacterial strains, and organic amendments because, in combined treatments, the combined effect of the plant, bacteria, and organic amendments has efficiently eluted the charged species from the soil by absorption of ionic species in plant roots. Hence, the plant's soil-water balance is improved and making the soil suitable for plant growth.

In this study, the TDS values of the CPF-contaminated soil were higher than the fresh soil in the case of both soil textures. Furthermore, the treatment using an integrated approach of plant, bacterial strain, and organic amendments in contaminated soil has a much lower value than treatment having contaminated soil and plant only. The TDS values of the soil varied significantly between the two different soil textures. Clayey soil had more TDS initially while sandy loam soil has less TDS at the start. After spiking, the trend shifted as clayey soil had TDS values less in comparison to sandy loam soil. Due to the addition of CPF in the soil, the soil EC was increased because the charged species were added to it. It was also found that the EC and TDS of the fresh soil were less than the EC and TDS of the CPF-contaminated soil.

The nutrient status of the contaminated soil indicates the degradation efficiency. Nitrogen and phosphorus are considered primary limiting nutrients (Sarkar et al., 2005). In this study, available nitrates and extractable phosphorous values varied significantly in all the applied treatments of both soils with different textures. Available nitrates and extractable phosphorous content of the spiked soil were observed much lower than of the fresh soil in both soils, though the values differ in each type of soil. Among all the treatments, the treatment SS+B+P+B2 shows the higher values of nutrients in both types of soils, which means that the availability of high nutrients will lead to the degradation of CPF in soil. Moreover, clayey soil had more extractable phosphorous in comparison to sandy loam soil. Liu et al., (2012) reported that soils with higher clay content have high phosphorus retention capacity because clay particles have a very large surface area per unit volume, which can adsorb phosphorus easily. On contrary to this, available nitrates were more in sandy loam soil in comparison to clayey soil.

The extractable phosphorous values were also found lower in the treatments treated with only bacterial strains in comparison to the ones treated with organic amendments because the microbes may consume the phosphorous for the degradation of CPF. The increased availability of nutrients by the addition of organic amendments lead to improved rhizoremediation of CPF and also supported the microbial community growth. Hannet et al., (2021) reported that the application of both biochar and compost improved nutrient concentrations of both soils. Biochar significantly increased soil extractable phosphorous, and the compost application proved to be a sustainable practice to improve available nitrates and extractable phosphorous content in the soil.

Soil organic matter varies in composition and is representative of the residue of roots of plants, soil organisms, and plant material in different stages of decay. It is considered a nutrient reservoir in the soil thus improving soil fertility. It also enhances soil porosity and aeration improves water holding capacity and reduces nutrient leaching (Hussain et al., 2018). Total organic carbon TOC is the carbon stored in organic matter. Usually, oxidizable organic carbon OOC content in the soil is measured and converted into TOC and OM using a constant factor. Furthermore, clayey soils have more OM than sandy loam soils (Moody & Phan, 2008). In this study, the significantly highest values for OOC, TOC, and OM were observed for the treatment SS+B+P+B2 as biochar provides more nutrients to the soil as it prevents nutrients to leach down, acts as an indicator for enhanced rhizospheric microbial activity and plant growth. Agegnehu et al., (2015) reported that the addition of biochar significantly reduces the leaching of nutrients and helps in improving soil fertility, thus promoting plant growth. It also improves the retention of nutrients and water by the soil. It is considered that the CPF-contaminated soil has a limited number of nutrients so lower values of OOC, TOC, and OM were noted for the treatment having contaminated soil, and in this treatment, there was also the absence of plant species and especially there was no addition of organic amendments. The treatments which are amended with the compost and biochar greatly affect the soil's physicochemical properties positively.

Soil enzymes released by plants or microbes are of vital importance in the soil as they determine the nutrient status in soil by governing biochemical transformations taking place in soil (Kumar et al., 2013). Generally, it is reported that soil enzyme activities

are found to be higher in the rhizospheric region compared to the bulk soil. Soil enzymes are classified into different classes including oxidoreductases, isomerases, hydrolases, ligases, transferases, and lyases. Soil enzyme assays are carried out to access soil quality and stress indicators (Gu et al., 2019). Among different soil enzymes studied, one oxidoreductase (dehydrogenase) and two hydrolases (phosphatase and urease) are thoroughly studied enzymes due to their specific importance in transformation processes (Kumar et al., 2017).

Sanchez-Hernandez et al., (2017) reported that short-term chlorpyrifos exposure caused a significant decrease in the activity of carboxylesterase, acid phosphatase, and β-glucosidase, as well as in soil microbial activity as indicated by the reduced catalase and dehydrogenase activities. Urease and phosphatase activities were present in soils as stabilized extracellular enzymes, so changes in their activity were independent of fluctuations of microbial activity and biomass related to chlorpyrifos exposure. Aziz et al., (2021) studied that the activities of soil enzymes were severely affected by the CPF contamination pronouncedly at the initial stages of incubation, and both compost and biochar proved to be effective in alleviating the adverse effects of CPF on the activities of these soil enzymes.

Dehydrogenase belongs to the class of oxidoreductases enzyme and involves in the oxidative degradation of organic compounds by transferring hydrogen and electrons from the substrate to acceptors (Kumar et al., 2013). Dehydrogenase activity was noted significantly higher in treatment SS+C+P+B2 in both types of soils as compared to abiotic control in which comparatively low levels of dehydrogenase activity were observed due to the inhibition effect of CPF. Overall higher dehydrogenase activity was observed for combined approaches having the plant, bacterial strains, and organic amendments compared to individual treatments. The activity was more in sandy loam soil as compared to clayey soil. According to the study conducted by Aziz et al., (2021), the inhibiting effect of CPF on dehydrogenase activity significantly increased with increasing CPF concentration. A significant reduction in enzyme activity suppression was observed with both biochar and compost. Overall, compost-amended contaminated treatments showed significantly high dehydrogenase activities over biochar amended ones. The decrease in soil enzyme activities in CPF spiked soils could be attributed to the increased microbial toxicity of byproducts formed during degradation.

Catalase is another oxidoreductase enzyme present in all aerobic microorganisms, plants, and animals' cells. It plays role in the degradation of hydrogen peroxide thus preventing cells from damage caused by ROS (Stpniewska et al., 2009). All aerobes and facultative anaerobes possess catalase enzyme which functions primarily at the intracellular level but it can also function in the extracellular environment in link with OM or may potentially accumulate clay minerals. Catalase activity was not detected in clayey soil while in sandy loam soil, the highest activity of catalase was observed for treatment SS+C+P+B2. Tunç et al., (2017) reported that the catalase enzyme activity changes under microbial and biochemical factors such as heat, oxygen, moisture, and nutrients. The activity is not directly related to the number of microorganisms in the soil but is related to the amount of organic matter present. Franco-Otero et al., (2012) also reported that the presence of excess phosphorus in the soil harmed the catalase enzyme activity, which possibly is the reason why catalase enzyme was not detected in a clayey soil as it had more phosphorus content.

Overall higher catalase activity was observed for combined approaches having the plant, bacterial strains, and organic amendments compared to individual treatments, whereas in contaminated soil before any applied treatment, lower catalase activity was observed than in soil with applied treatments which indicates that activity of catalase was improved after remediation. Soil catalase activity represents the total activity of the current viable microbial population and the activity of stabilized enzymes in the soil matrix. As per the study by Sanchez-Hernandez et al., (2017), soil microbial activity was reduced in CPF-sprayed soils as indicated by the decreased catalase activities compared with control soils.

Phosphatase belongs to the hydrolase class of enzymes and plays a significant role in the phosphorus cycle which is considered an essential element for sustaining life. Phosphatase plays role in the release of organically bound phosphorus by cleavage of inorganic phosphate groups from organic phosphorus compounds as organisms can only assimilate dissolved P (Margalef et al., 2017). The strains of bacteria from genera *Pseudomonas*, *Bacillus*, and *Rhizobium* are reported to be dominant phosphate solubilizers (De Bolle et al., 2013). Inhibition of phosphatase enzymes can occur as a result of a feedback mechanism due to the presence of inorganic phosphorus. Based on pH, acid and alkaline phosphatase showed a marked difference in their activity i.e.

acid phosphatase was predominantly found in acid soils and alkaline phosphatase in neutral or alkaline soils (Kumar et al., 2011).

The highest phosphatase activity was observed in treatment SS+C+P+B2 of both soils, though the activity was significantly higher in sandy loam soil in comparison to clayey soil. Margalef et al., (2017) have observed that phosphatase production depends on a combination of P demand from plants and microbes, available organic P substrate, and P limitation of the soil. Improvement in phosphatase activity was observed after the application of treatments. Increased levels of phosphatase production were observed for integrated applied treatments compared to individually applied treatments. As per Aziz et al., (2021), the phosphatase activity was significantly reduced by CPF in the unamended soil.

CPF-contaminated soil, while higher phosphatase activities were observed with the application of compost and biochar amendments. However, with compost, these activities were even higher. High activities of phosphatase and urease help plants in the uptake of nitrogen and phosphorus by stimulating their transformations in soil.

Urease is another hydrolytic enzyme that converts urea into carbon dioxide and ammonia (Ma et al., 2014). It requires a water-based medium for its function. Urease enzyme assays give an understanding of the mineralization process of nitrogen. The sensitivity of urease towards pollutants is higher than other extracellular enzymes. Significantly higher urease activity was observed in SS+C+P+B2 of both soils. The activity was less in clayey soil while more in sandy loam soil. It was also observed that urease activity decreased in contaminated soils without applied amendments. As per the study of Aziz et al., (2021), CPF negatively affects soil urease activity. The addition of compost and biochar significantly reduces the negative effects of CPF on soil urease activity. The decrease in urease activity in CPF spiked soils could be attributed to the increased microbial toxicity of byproducts formed during degradation.

In this study, the bacterial strains i.e. *Bacillus vietnamensis* from Environmental Microbiology and Bioremediation lab and *Pseudomonas aeruginosa* from NARC, Islamabad, were selected. These strains were used to remove the CPF contamination from soils of two different textures, i.e. clayey soil and sandy loam soil. In this study, the significantly highest rhizospheric bacterial population was found in treatment

SS+C+P+B2 in both types of soils, but the highest CFUs were observed in sandy loam soil, showing that the soil supported microbial growth. The compost was added as a bio-stimulating agent to enhance the CPF rhizoremediation. It was found that the use of microbial inoculums along with organic amendments enhanced the release of nutrients in the soil and it was also noted that the combined treatment in which the *Pseudomonas aeruginosa*, plant, and compost was used to remove CPF from rhizospheric soil gives the best results than all other applied treatments because the microbes used the CPF as a carbon source for their energy need. In this study, the lowest rhizospheric bacterial population was observed in treatment having contaminated soil, plant, and bacterial consortia of both bacterial strains, which depicts that both bacterial strains did not work well together but performed efficiently when added individually.

Several bacterial and fungal strains have been used to assess the CPF degradation in contaminated soil and wastewater. *Bacillus pumilus* has been previously used for the degradation of chlorpyrifos from the environment (Anwar et al., 2009). The hairy root of *Chenopodium amaranticolor* improved the phytoremediation of chlorpyrifos and metabolized products (Garg et al., 2010). The fungus, *Acremonium* sp. utilized chlorpyrifos as a sole source of nitrogen and carbon (Kulshrestha and Kumari, 2011). Chlorpyrifos resisting bacteria such as, Klebsiella sp., Bacillus *cereus*, and Pseudomonas *aeruginosa* (Lakshmi et al., 2009), *Bacillus pumilus* strain (Anwar et al., 2009), *Lactobacillus brevis* (Islam et al., 2009), and *Ralstonia* sp. (Li et al., 2010) have been used for the removal of chlorpyrifos from the environment as well. Furthermore, Elshikh et al., (2022) reported that *B. cereus* CP6 and *K. pneumonia* CP19 can degrade chlorpyrifos. The degradation of CPF by bacterial consortium could be due to the synergistic property of selected bacterial strains.

Insecticides and other toxic pollutants are more familiar for causing toxicity to the plants (Zhang et al., 2014). In this study, the growth of the *Triticum aestivum* in terms of root and shoot length, fresh weights, dry weights, and plant total biomass was greatly influenced by the CPF contamination in the soil and the soil texture. Clayey soil showed minimum growth and biomass in comparison to sandy loam soil. It is because the clayey soil is too dense for plant roots to spread out and access the nutrients. It also holds too much water and rots roots in place, due to which the plant cannot grow properly. Sandy loam soil, on the other hand, is appropriate for the

growth of plants because it has a high retention capacity. It retains water and nutrients to a considerable extent shedding off the excess due to which plants can access the nutrients in a stable environment and develop properly.

According to (Akhtar et al. 2018) the length of the root is a key factor to check the growth of a healthy plant as it provides water and essential nutrients to the plant. Francisco et al. (2019) stated that the reduction in biomass of the plant is associated with the stress present in the plant. In the case of root and shoot length, fresh weights, dry weights, and plant total biomass, a significantly maximum growth rate was observed for the treatment SS+B+P+B2, where the combination of different strategies may have eliminated the CPF stress. After this combined treatment, the maximum growth of *Triticum aestivum* was shown in treatment SS+B+P+B1, where there was a second bacterial strain present. The minimum growth rate was noted for the treatment SS+P as there was no additional strategy was applied to reduce the CPF stress.

In this study, it was also found that the root length of the plant *Triticum aestivum* was more affected than the shoot length which means that the CPF may have accumulated in root tissues more than in the shoot tissues and caused the reduction in its growth. Similar observations existed in the case of biomass as the root biomass of the treated plants was more reduced than shoot biomass. The trend of growth and biomass was the same in both soils, but the length and weight varied significantly in terms of values. We have seen that the addition of *Pseudomonas aeruginosa* and biochar in combined treatment significantly enhanced the growth of *Triticum aestivum* in terms of root and shoot length, fresh weight, dry weight, and plant total biomass in CPF-polluted soil. It is most probably because biochar has bound CPF and made it unavailable for the plant to uptake due to which, the growth was significantly higher than compost amended treatments. While the growth parameters of *Triticum aestivum* were significantly reduced in CPF polluted soil without any amendment, compost enhanced the activity of indigenous microbes as it slowly adds nutrients to the soil upon degradation, hence increasing the rhizospheric CFUs and leading to higher CPF degradation. The application of organic soil amendments is known to improve soil physicochemical properties (Singh et al., 2009) along with biological functions (Singh and Ghoshal, 2010). The improved soil physicochemical properties perhaps led to enhanced root growth by increasing nutrient uptake.

In a study by Aziz et al., (2021), CPF significantly reduced the shoot and root fresh weights of maize plants. A decrease in growth due to CPF toxicity can be attributed to the inhibition of the activity of 4-hydroxyl phenyl pyruvate dioxygenase (HPPD), which is needed for the growth and development of meristematic tissue (Parween et al., 2011). The suppression of shoot and root biomass of plants by CPF toxicity and significant recovery of this reduction by biochar supplementation in soil has also been reported by Yang et al., (2010). A reduction in plant growth in response to CPF toxicity was also reported by Dubey et al., (2015). The addition of biochar and compost alleviated the damaging effects of CPF on shoot fresh weight and increased the shoot fresh weight compared to those plants where only CPF was applied. Moreover, biochar was found to be more effective in restoring the biomass in all contaminated treatments compared to compost-amended treatments.

Environmental stresses, whether biotic or abiotic, generate oxidative stress due to enhanced production of ROS i.e., over-flow of ROS that exceeds the defense mechanism. This oxidative stress can cause peroxidation of lipids, protein oxidation, damage to nucleic acid, enzyme inhibition, and activation of programmed cell death (Martí et al., 2009). CPF contamination induces oxidative stress on plants. From the results of this study, it can be seen that CPF contamination positively influenced MDA and H_2O_2 while negatively to photosynthetic pigments and resulted in increased activities by CAT, APX, GPX, and SOD.

Chlorophyll is a vital chemical substance found in autotrophic organisms that play a key role in carrying photosynthesis. Any stress or nutrient injury can adversely affect chlorophyll thus reducing its content. Measurement of chlorophyll content represents the state of the plant and the condition in which it is present thus chlorophyll is considered to be a biomarker of environmental stress (Rastogi et al., 2017).

High chlorophyll levels indicate high availability of nutrients mostly N and P whereas low levels indicate a stressed environment (Hussain et al., 2018). In this study, the highest chlorophyll levels were observed for treatment SS+B+P+B2 followed by treatment SS+B+P+B1, where there was another bacterial strain present; this is an indicator of high nutrient availability. The lowest value was observed for treatment having SS+P, which indicates low nutrient availability due to CPF stress. For the soils of both textures, the trend of highest to lowest chlorophyll values was the same, but

the values were significantly different from each other as plants grown in clayey soil possessed lower values of chlorophyll in comparison to plants grown in sandy loam soil. The reduction of chlorophyll at high concentrations of chlorpyrifos might also be due to the destruction of pigments and the degradation of the pigment-protein complex (Barry et al., 1990). Significant improvement in chlorophyll level was observed in integrated treatments having contaminated soil treated with plant, bacterial strains, and organic amendments. Carotenoids are also plant pigments that facilitate plants by absorbing light from across the color spectrum. The trend for carotenoid content was the same as for chlorophyll content for both soil textures.

Singh et al., (2018) reported that the application of the chlorpyrifos exerted a negative impact on the content of chlorophyll a and chlorophyll b relative to control. The decrease in the content of carotenoids was significant in all the pesticide treatments as compared to the control. However, the combined application of pesticides and soil amendments favored a significantly higher accumulation of chlorophyll a, chlorophyll b, and carotenoid contents with the highest values. The plant growth characteristics increased considerably when CPF was applied in combination with soil amendments and was strongly correlated with the photosynthetic pigments.

Lipid peroxidation is a process where ROS attack lipids especially PUFAS (polyunsaturated fatty acids); a structural component of cell membranes and damage cellular integrity. Lipid peroxidation is comprised of three steps i.e. initiation, propagation, and termination, and produces a variety of oxidation products. Among many different aldehydes which are produced as secondary products; malondialdehyde MDA, is one of them that has been used as a convenient biomarker for lipid peroxidation due to oxidative stress. Hydrogen peroxide is a type of ROS produced inside the plants that act either as a damaging or signaling molecule depending on the delicate balance between its formation and scavenging (Niu and Liao, 2016).

In this study, the treatment having FS+P followed by SS+B+P+B2 exhibits the lowest level of MDA and H_2O_2 among all other treatments applied which indicates a stress-free environment. The highest level of MDA and H_2O_2 were observed for treatment SS+P followed by SS+P+B1+B2 having both bacterial strains and then SS+P+B1.Among all the applied treatments, SS+B+P+B2indicates lower levels of

MDA and H_2O_2 content, which indicates a reduction in CPF stress. For the soils of both textures, the trend of highest to lowest MDA and H_2O_2 content was the same, but the values were significantly different from each other as plants grown in clayey soil possessed higher content of MDA and H_2O_2 in comparison to plant grown in sandy loam soil. Wang et al., (2017) reported that the MDA and H_2O_2 content was increased in wheat contaminated with chlorpyrifos. This indicated that wheat seedlings suffered from moderate or severe oxidative stress caused by chlorpyrifos. Amongst ROS, H_2O_2 is used to illustrate the degree of oxidative injury to cells. Lipid peroxidation may be the first step of cellular membrane damage by organophosphates (Hazarika et al. 2003). Oxidative damage to leaf lipids is estimated by the content of a total of two thiobarbituric acid reactive substances (TBARS), expressed as equivalents of MDA.

The production of antioxidant enzymes is counteracting mechanism of plants in response to oxidative stress and is considered the first line of defense by plants upon stress exposure (Khan et al., 2019). Antioxidants are crucial for our existence otherwise ROS would end up eating up all the cells. Antioxidants are those molecules that can safely donate their electron to free radical specie to stop the chain reaction. They can be enzymatic as well as non-enzymatic. Superoxide dismutase SOD, ascorbate peroxidase APX, guaiacol peroxidase GPX, and catalase CAT are categorized into enzymatic antioxidants whereas ascorbate AsA and glutathione GSH are considered key non-enzymatic antioxidants in a plant cell. Usually, an organelle possesses more than one ROS scavenger (Caverzan et al., 2012). Upon stress exposure, fluctuations in levels of these enzymes can be observed.

Aziz et al., (2021) reported an increase in antioxidant enzyme production while exposed to CPF. Superoxide dismutase is an antioxidant enzyme that converts superoxide radicals into H_2O_2. Catalase and guaiacol peroxidase are defense mechanisms against H_2O_2 which is considered one of the major ROS in the cells. They decompose H_2O_2 into water and oxygen. APX also scavenges H_2O_2 with a much higher affinity than CAT. CAT and APX are highly specific for H_2O_2. CAT has a very fast turnover rate i.e., 1 molecule of CAT catalyzes 40 million molecules of H_2O_2 per second, but they have a much lower affinity for H_2O_2 than APX and peroxides (Mhamdi et al., 2010). Glutathione s-transferase catalyzes the conjugation of GSH with a variety of compounds containing electrophilic centers thus making

compounds more bioavailable and enabling the breakdown of xenobiotics (Martí et al., 2009).

In this study, antioxidant enzyme assays were carried out for APX, GPX, SOD, and CAT. The higher levels of APX, GPX, SOD, and CAT were noted for the treatment with SS+P followed by SS+P+B1+B2 having both bacterial strains and then SS+P+B1which indicates high levels of stress induced by CPF. Upregulation of these enzymes reduces direct cytotoxicity caused by ROS production (Aziz et al., 2021). Significant reduction in the level of these enzymes was observed in treatments where microbial strains and biochar amendment were used along with the plant for remediation so it can be inferred that the use of biological methods along with the organic amendment can be an effective remediation strategy. CAT levels followed the same trend but the values were low in comparison to APX, GPX, and SOD. Higher H_2O_2 production is linked with increased SOD production however lowest APX, GPX, SOD, and CAT levels were observed for treatment SS+B+P+B2 among all treatments applied. The trend of highest to lowest APX, GPX, SOD, and CAT content was the same for the soils of both textures, but the values were significantly different from each other as plants grown in clayey soil possessed higher levels of APX, GPX, SOD, and CAT in comparison to plant grown in sandy loam soil.

SOD is usually regarded as the primary antioxidant enzyme that catalyzes the conversion of superoxide radicals to H_2O_2. Excessive H_2O_2 can be converted to H_2O and O_2 by other antioxidant enzymes such as CAT and APX (Alscher et al., 2002; Wang et al., 2004; Liu et al., 2014). In the study of Wang et al., (2017), the balance of these antioxidant enzymes' activities was disrupted in wheat seedlings subjected to higher concentrations of chlorpyrifos. Toxic organic compounds can give rise to the increased activities of antioxidant enzymes such as SOD, APX, and GPX, which reflect not only the degree of toxicity but the ability to tolerate the stress as well. Enhanced SOD activity was reported under insecticide (Bashir et al. 2007) in Glycine max L., herbicide (Jianga et al. 2010; Wu et al. 2010) in wheat and rice, respectively, suggesting that SOD was stimulated by scavenging H_2O_2 to protect plants from chlorpyrifos toxicity. The SOD activity of maize plants was significantly promoted in CPF-stressed plants compared to untreated control plants. The compost- and biochar-supplemented plants showed lower SOD activities compared with unamended CPF-contaminated plants. However, biochar-amended treatments showed significantly less

SOD activity in all contaminated treatments compared to compost-amended treatments (Aziz et al., 2021).

The CAT activity constitutes the second step in the process of detoxification which is released after the conversion of ROS into H_2O_2 and O_2 by SOD (Aeobi, 1974). The incitement in the activity of CAT due to the effect of pesticides occurred in response to the H_2O_2 accumulation (Song et al., 2006). Similarly, Michałowicz et al. (2009) reported the enhanced activity of CAT to eliminate H_2O_2 in *Triticum aestivum* after treatment with the insecticide pentachlorophenol et 2, 4-dichlorophenol. Chahid et al. (2015) explained the higher activity of CAT on account of the high activity of isoenzymes in tomato leaves after treatment with higher doses of insecticides.

APX uses ascorbate as an electron donor in the first step in the Asc–Glu cycle to remove H_2O_2 (Aziz et al., 2021). APX and GPX protect the cell against oxidative damage by detoxifying the toxic H_2O_2. The role of APX and GPX in the detoxification of H_2O_2 (Morimura et al. 1996) under insecticide-induced oxidative stress is suggested by its marked increase in activity. The same results were observed under the fungicide (Jaleel et al. 2006; Gopi et al. 2007) and herbicide (Jianga et al. 2010) treatments. It is suggested that such increased APX activity might result from the accumulation of H_2O_2 or activation of the ascorbate-glutathione cycle.

This study was conducted to check the degradation rate of CPF in soil by applying different treatments including phytoremediation, bioremediation, and organic amendments. The results of this study depicted that the significantly highest remediation rate of CPF was found in treatment having SS+B+P+B2, which confirms that the plant *Triticum aestivum*along with a CPF degrading bacterial strain, i.e. *Pseudomonas aeruginosa* and biochar has a great potential to remediate CPF in the rhizosphere of the plant by binding CPF. The rhizospheric bacteria and plant enzymes may have mostly taken part in the degradation of CPF in the rhizospheric part of the plant as we have seen that the roots were mostly affected than the shoots of the plant. The significantly lowest degradation rate of CPF was observed in the treatment of SS in which no additional treatment was given.

A study conducted by Aziz et al., (2021) reported a significant reduction in CPF accumulation in maize shoots was recorded with both compost- and biochar-amended treatments compared with unamended treatments. However, biochar-amended soil

exhibited significantly less CPF concentration in shoots compared with compost-amended soil. The incorporation of biochar amendment resulted in a significantly reduced CPF loss in soil, while compost-amended treatments enhanced the CPF residue degradation in soil. The reduced dissipation of CPF in amended soil is due to strong sorption and less desorption from organic amendments (Mahmood et al., 2017) for microbes. A decrease in CPF dissipation in planted soil in response to biochar addition has also been reported (Yu et al., 2009).

Organic matter added to soil provides the most important sorbent surfaces for the nonpolar pesticides having low water solubility because phase partitioning is driven by hydrophobic interactions (Copaja et al., 2014). The mechanism behind the low bioavailability of CPF is the sorption of pesticides on organic amendments. The microporosity and high specific surface area of biochar and a variety of functional groups provided by humic-like molecules and increased specific surface area due to humification of organic macromolecules make them very efficient sorbent materials for CPF, which in turn minimizes the risk of contaminant entrance into the food chain (Moyo et al., 2014), hence reducing their toxicity.

In terms of soil texture, clayey soil was higher in organic matter and extractable phosphorus content, while sandy loam soil was higher in nitrate content (Moody & Phan, 2008). There was a significant difference in CFUs of clayey and sandy loam soil as clayey soil had less microbial count than sandy loam soil. It is because the clayey soil did not support sufficient microbial growth. Clayey soil did not support plant growth and development due to which, the plants grown in this soil had low chlorophyll and carotenoid content. It is because the clayey soil is too dense for plant roots to spread out and access the nutrients. Sandy loam soil, on the other hand, retains water and nutrients to a considerable extent shedding off the excess due to which plants can access the nutrients in a stable environment and develop properly (Dipti et al., 2013).The length and biomass of roots and shoots were also less than the plants grown in sandy loam soil. MDA and H_2O_2 were produced less in sandy loam soil, depicting a less stressed environment in it. As ROS were less in number in sandy loam soil, therefore, APX, GPX, SOD, and CAT were also less in it in comparison to clayey soil. This depicts that clayey soil had a more stressed environment due to which, the rate of degradation was also lower than sandy loam soil. Moreover, the

half-life of CPF is 15 days in sandy loam soil and 58 days in clayey soil (Jaiswal et al., 2017), which is possibly another reason for the slower degradation of CPF in clayey soil in comparison to sandy loam soil.

Chapter 5

Conclusion

The demand for food worldwide is rising along with the global population. Modern agriculture now heavily relies on the usage of pesticides to supply global food demand. With the techniques of intensive agriculture and quick industrialization, soil quality is gradually declining. The excessive and ineffective use of pesticides over the past several decades has led to an accumulation of pesticide residues in soils, which has restricted the soil's ability to purify itself and resulted in severe soil pollution and deteriorated soil quality. Due to rising levels of contamination of soil and groundwater supplies, intensive and frequent use of some pesticides that have low biodegradation rates and high toxicity has raised concerns. Soil contamination is a latent threat to the environment's long-term sustainability. Furthermore, the rate of degradation differs from soil to soil; for these reasons, soil texture is regarded to be a crucial factor, not only for plant growth but also for the degradation of these toxic pesticides. Chlorpyrifos is an insecticide that belongs to the organophosphate class of pesticides; it is the fourth most commonly used pesticide to kill a variety of pests associated with several commercial crops. CPF has a high potential to pollute the environment, and soil in particular. Therefore, the objective of this study was to establish a quick, effective, and ecologically friendly method for removing CPF from the environment by combining phytoremediation, bioremediation, and organic amendments like compost and biochar. The treatment that used sandy loam soil, which was amended with plant, biochar, and *Pseudomonas aeruginosa* demonstrated the highest CPF removal rate. Rhizoremediation of soils contaminated with CPF was found to be effective by the involvement of *Triticum aestivum*. *Pseudomonas aeruginosa* and organic soil amendments demonstrated a significant rise in plant growth parameters and CFUs in rhizospheric soil, and the addition of biochar to the soil showed a significant increase in the content of N, P, OOC, TOC, and OM. Clayey soil was less suitable for plant development and degradation than sandy loam soil. According to the findings of the study, integrated bio-phytoremediation with biochar amendment for CPF in contaminated sandy loam soil can be considered a reliable and effective alternative strategy for removal of CPF rather than a single biological method alone to get around the limitations of one technique. Rhizoremediation rate is

increased together with the enhancement of the physiochemical and biological characteristics of polluted soil when CPF-degrading bacterial strains and plants are used together. Rhizoremediation of CPF-contaminated soils is found to be a good fit for *Triticum aestivum*.

Future Recommendations

For CPF remediation, other plants' potential and suitability should also be considered. In addition, contaminated soil with a sandy loam texture displayed improved plant development characteristics, increased CFUs, and a higher rate of degradation than clayey soil with CPF contamination. Investigations into more integrated methods, such as the application of nano-remediation in conjunction with bio- and phytoremediation as well as other organic amendments for the remediation of CPF-contaminated soil, are still needed.

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