Comparative Efficiency of Biochar and Vermicompost to Minimize Chromium Toxicity in Tomato *(Solanum lycopersicum)*

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Environmental Science

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DEDICATION

“This thesis is devoted to my family whose continuous support and prayers are always with me whenever and wherever required.”

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Jamaluddin Kabir

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
APX	Ascorbate Peroxidase
BET	Brunauer–Emmett–Teller
CAT	Catalase
CEC	Cation Exchange Capacity
Cr	Chromium
AAS	Atomic Absorption Spectroscopy
DI	Deionized
DIM	Daily Intake Metal
DNA	Deoxynucleic Acid
DW	Dry Weight
EC	Electrical Conductivity
ECM	Electric Conductivity Meter
EDTA	Ethylenediaminetetraacetic Acid
EDX	Energy Dispersive X-Ray
EL	Electrolyte Leakage
Fe-BC	Iron-Enriched Biochar
FIR	Far-Infrared
DI	Deionized
DIM	Daily Intake Metal
DNA	Deoxynucleic Acid
FTIR	Fourier Transform Infrared Radiation
FW	Fresh Weight
FYM	Farmyard Manure
GEC	Guidance and Examination Committee
HAP	Hazardous Air Pollutants
HM	Heavy Metal
HRI	Human Risk Index
MDA	Malondialdehyde

MIR	Mid-Infrared
MSI	Membrane Stability Index
NIR	Near-Infrared
FTIR	Fourier Transform Infrared Radiation
NMR	Nuclear Magnetic Resonance
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
POD	Peroxidase
PTE	Potentially Toxic Elements
ROS	Reactive Oxygen Species
RWC	Relative Water Content
SEM	Scanning Electronic Microscopy
SL	Shoot Length
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Sciences
TCA	Tricarboxylic Acid Cycle
TCC	Total Chlorophyll Contents
TCLP	Toxicity Characteristics Leaching Procedure
TGA	Thermogravimetric Analysis

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ABSTRACT

This study aimed to evaluate the impact of chromium (Cr) toxicity on physiological and biochemical parameters, as well as the growth performance of tomato plants. The experiment involved a pot experiment with different organic amendments, including biochar (BC). iron-enriched biochar (Fe-BC), farmyard manure (FYM) and vermicompost (VC). The organic amendments were fine-sieved and characterized using scanning electronic microscopy (SEM), energy dispersive x-ray (EDX), fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). Tomato plants were harvested after 75 days of growth in Cr contaminated soil, and their growth, physiological and biochemical parameters were analyzed. Results showed that shoot growth, biomass, relative water contents, membrane stability index, and chlorophyll contents decreased with increasing Cr concentration, however, plants were unable to survive at 100 ppm Cr. Compared to control, significant decreases in shoot length (SL) (40 & 61%), fresh weight (FW) (28 & 65%), membrane stability (MSI) (22 & 52%), and relative water content (RWC) (13 & 31%) were observed at 25 & 50 ppm Cr, respectively. The toxic effect of Cr was attributed to Cr-induced oxidative damage by 1.9 and 2.7 folds under 25 and 50 ppm Cr, respectively, as compared to non-stressed plants. Application of four amendments was effective in reducing Cr stress in tomato plants and among them, VC significantly reduced Cr stress as compared to other amendments and showed the highest growth. Interestingly, the addition of organic amendments helped the plants to withstand the toxic level of 100 ppm Cr. The positive impact was majorly attributed to reduced uptake of Cr, increased antioxidative enzyme activities (SOD=3.11 folds, CAT=1.14 folds, POD=2.95, and APX=1.76 folds), and ultimately reduced damage caused by oxidative stress. Overall, the results of the present study indicate the potential use of different organic amendments for mitigating abiotic stresses in plants, but VC had better performance than other amendments e.g., Cr toxicity, however, field tests are recommended to ascertain the response under natural conditions.

CHAPER 1

1. INTRODUCTION

1.1 Background

Heavy metals are harmful substances that are not decomposing and may build up harmful concentrations in the natural environment. Heavy metals are permanent and hazardous compounds that can build up to unsafe quantities in surroundings. They have existed within the Earth's surface from when it was formed. Heavy metals have been released into both soil and water due to a huge increase in their use. The release of heavy metals is considerably exacerbated by human activities, particularly metal industry, waste disposal, agriculture, and automobile emissions. Volcanic activity, corrosion, and erosion are all processes that occur naturally (Briffa et al., 2020). Given how frequently soils are exposed to these effects, it has been projected that the human-caused component impacts the entire present heavy metals' existence within the ground completely. That has now become a serious concern for the scientific community (Dotaniya et al., 2022). Poisonous metals are delivered and gathered in the biological system because of constant anthropogenic movements like metal handling, non-renewable energy source ignition, uncontrolled pesticide as well and synthetic compound utilization. The high convergence of various contaminants in the climate due to modern exercises leads to significant modifications in the environment and is furthermore responsible for a dangerous atmospheric deviation, which at last affects human and creature well-being (McBride & Laćan, 2018). The annual cycle of temperature is affected by the steady warming of the world's climate, which causes changes in the plants' spatial and transient exercises (Duan et al., 2019). Harmful metals obliterate the earth's biggest food web, and it is almost inconceivable to eliminate them without using organic, synthetic, and physical techniques due to their level of steadiness (Sharma et al., 2021). Excessive accumulation of heavy metals in the soil severely threatens human health through the food chain (Altaf et al., 2021). Industrialization is a crucial practice in socioeconomic growth and development. However, industrial pollutants are often responsible for disrupting the surroundings and grow to be a primary cause of human beings' society. Industrial processes like painting, fabric dying, chromium plating, electrostatic coating, paper production, and pulp industries use chromium (Cr), and the wrong disposal of

Cr-contaminated wastes is accountable for the release of Cr in the surroundings (He & Li, 2020). The ashes that are produced due to the burning of coal or metropolitan wastes agricultural residues increase the more desirable concentration of Cr in the soil (Yang et al. , 2020).

Cr is a shiny white metal that is easily available on the earth shell, has no natural capabilities, exists in various oxidation forms, and is directly accessible for take-up in soil-plant frameworks (Bilal et al., 2018). Cr occurrence in seawater, the earth's crust, and industrial effluents particularly from the tanneries have significantly increased its concentration in the water bodies and soil medium (Kumar etal., 2016). The improved gradual addition of Cr in cultivating lands causes a decrease in the efficiency of financially critical yields (Wakeel et al., 2018). In Pakistan Cr concentration is about 100 to 150 mg/kg soil which is higher than over the world is 60 mg/kg (Waseem et al., 2014). Cr has a few oxidative structures; however trivalent and hexavalent structures are the two most stable ones occurring in the soil (Khatun et al., 2019). Plants can take up Cr effectively by means of the decrease of trivalent structure into a hexavalent structure either through a simplistic route or by complex development with primary nutrients (Bilal et al., 2018). The hexavalent type of Cr is a highly poisonous structure for plants. Hexavalent Cr exhibits harmful impacts on plants due to its strong interaction with cell membranes, leading to rapid penetration into plant cells. Once it enters, it causes the generation of free radicals and reactive oxygen species (ROS) and raises its harmful effects. It enters in the form of chromate or dichromate ions through common anion channels in the cell's membrane (Ahemad, 2015).

The incidence of Cr toxicity in plants can be minimized through immobilization via the addition of various organic amendments and. plant residues, vermicompost, organic manures, and green manure into chromium-contaminated soils (Kumar etal., 2016). Among various organic amendments, the addition of biochar has gained increasing attention due to its multiple positive impacts on soil quality and plant growth. Biochar is carbon-enriched material with some specific properties such as large surface area, high pH, strong adsorption capacity, and high volumes of voids and all these properties make them suitable for the reduction of heavy metals concentration. Organic amendments such as biochar can be made from different materials such as wood chips, livestock residue, plant residue, and municipal solid waste which can be used for the reclamation of heavy metal-contaminated soils (Wei et al., 2019). The application of biochar can enhance soil fertility, soil water holding capacity, plant growth and yield, hold essential nutrients, improve soil physical and biological characteristics, restrain heavy metals in soil and decrease plant uptake (Khan et al., 2020). This is due to biochar creating sites for the adsorption of nutrients from the available organic wastes and playing a role in slow-release fertilizer and where organic wastes enhance soil aggregation and which further increases the retaining of nutrients and water in the soil (Altaf et al., 2021). Furthermore, it is reported that increased soil microbial activity results in the amelioration of soil quality (Paul et al., 2020). These changes generally enhance immobility and decrease the bioavailability of trace elements, thus contributing to the re-establishment of vegetation and thus increasing plant growth. The immobilizing impacts of such amendments are considered that various complicated processes occur such as the formation of stable compounds with organic substances, precipitation, and ion exchange. Iron-enriched biochar has also been applied to restrain chromium toxicity in the soil, iron-enriched biochar can reduce the availability of chromium by 42.63% (Medha et al., 2021).

Moreover, Dad et al., (2020) studied that engineered biochar i.e., enrichment of biochar with other beneficial elements such as iron (Fe) nanoparticles doping on BC surface can further enhance crop growth and its productivity. Previous studies also showed that Fe-BC reduced the bioavailability of heavy metal ions in soil and reduced the translocation of Cd, Cr, Pb, and Cu by 4.23–109.33% in heavy-metal-contaminated soils.

To this end, the immobility of metals by the application of highly adsorptive organic matter is a new economically sound remediation technique. In recent years, VC - a humus-type organic material obtained from earthworm excreta has been reported to impart multiple benefits to agricultural soils. It is the best option for soil fertility owing to beneficial properties like high nitrogen substances, high cation exchange, good porosity, proper aeration, drainage, water holding capacity, and microbial activities, thus, VC is used as an eco-friendly and cheap source of remediation methods (Khosropour et al., 2021).

The impact of organic amendments on the mobility and bioavailability of metals depends on the type of organic materials, their biodegradation capacity, their impact on soil chemical and physical properties, and the soil type and metal under consideration. The movement and very few relative studies have been conducted so far and the choice of a particular organic amendment in assisted Phyto stabilization strategies often remains empirical (Kumar etal., 2016). As a result, in the current study, Fe-BC will be utilized in conjunction with vermicomposting amendments to immobilize Cr metal in the soil to reduce its absorption by the tomato plant.

1.2 Problem Statement

Many research studies showed that the concentration of Cr in the soil has increased continuously due to anthropogenic activities. Land that is near industries and urban sites is mostly contaminated with heavy metals. Some of the heavy metals are leaching down and contaminating the groundwater and soil which becomes a major problem for human health when that soil is used for agricultural purposes to produce foods for consumption. The beneficial effects of different organic amendments including BC and vermicomposting have been reported to minimize metal uptake by different plant species. However, a comprehensive study evaluating the comparative effect of BC (pristine vs. engineered) and VC on the growth of tomato plants grown under Cr-contaminated soil is still lacking. The results of this study will provide a deep understanding of the processes involved in organic amendment-mediated metal immobilization and thus provide a cost-effective and eco-friendly solution.

1.3 Objectives

This research study includes the following key objectives:

1. Investigating the consequences of, BC, Fe-BC, FYM and VC amendments on tomato growth under chromium toxicity.
2. To understand tomato plants' physiological and biochemical responses against Cr toxicity under different organic amendments.
3. To ascertain the comparative efficiency of BC and VC for reducing Cr toxicity in tomato plants.

1.4 Significance of the study

Anthropogenic activities are contributing multiple contaminants to the environment, particularly in Pakistan where there is no strict compliance with the regulations set by the government authorities. There is a need to highlight potentially harmful impacts and related risks to develop policies or guidelines to protect the local populations.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Soil Contamination

Contamination of soil may be both intentional and unintentional. Wastewater irrigation, insecticides, livestock manure, fertilizers, leaded paint, mine raw material waste (mine tailing), wastewater sludge, leakage of crude oil, combustion of coal residues, and garbage burial are all occurrences of deliberate soil contamination. Because heavy metals are getting into the food chain, the ecosystem is being devastated. Furthermore, heavy metals reduce the degradability of organic contaminants, which has the effect of poisoning the environment twice. These metals are present in the soil and impose risks on the entire biosphere. They are ingested directly by organisms and absorbed by plants, where they can be harmful to both the plant and the food chain by consumption. They also change the soil's natural chemistry and properties like pH and color, which lowers its quality and contaminates the groundwater (Briffa et al., 2020).

2.2 Heavy Metal

The density of heavy metals, which can be up to five times greater than that of water, is a result of their larger atomic mass. They are persistent in the environment of the earth and naturally occur, but they are classified as pollution when their concentrations exceed unnatural levels and cause problems with dangerous exposure to living things. Therefore, natural, and anthropogenic sources both contribute to heavy metal pollution (Dickinson, et al., 2019).

So even though usual circumstances do not significantly contaminate the environment with heavy metals, certain processes, and events, such as the weathering of rocks and sediments, volcanic eruptions, forest fires, sea salt sprays, and other such biogenic sources, have been reported to be the cause of the release of unwanted amounts of heavy metals into the natural environment. According to reports, these sources have contributed to pollution with substances such as As, Cu, Cr, Cd, Pb, Hg, Ni, and Zn, which can be toxic even at low amounts (Masindi & Muedi, 2018).

2.3 Heavy metal toxicity

For environmental evolutionary, nutritious, and ecological reasons, heavy metals are significant environmental contaminants, and their toxicity is an issue that is becoming more and more essential (Saleem et al., 2020). The main causes of the heavy metal increase in soil are humanistic activities such as the application of fertilizers and pesticides, mining, and the discharge of industrial effluents and sewage sludge (Zaheer et al., 2020). As a result, several fodders and agricultural crops cultivated on soil polluted with metals have the potential to collect large concentrations of metals, which is extremely dangerous for both human and animal health (Alatawi et al., 2022). High concentrations of potentially harmful materials in cultivated soil increase the risk that people may be exposed to such substances, while also contributing to the contamination of both aquatic and terrestrial habitats (Ahmad, et al., 2022). Due to the bioaccumulation and biomagnification of heavy metals in living organisms, the accumulation of toxic metals in a variety of environmental compartments poses a special risk to biotic health, including human health. Although it serves no vital metabolic purpose in plants, the potentially hazardous metal Cr can have negative impacts on plant growth, photosynthesis, mineral nutrient uptake, and crop quality due to soil concentrations that are too high (Ashraf, et al., 2017).

The three heavy metals that are highly concerned are Cd, Cr, and lead (Pb) since they have a negative influence on hunting health even with minute concentrations. According to (Wang, et al., 2021), adding nitrogenous fertilizers containing ammonium-N may increase the amount of Cd in plants, even if heavy metals are not at significant levels in the fertilizers. Furthermore, Zhang et al., (2014) found that some synthetic fertilizers can raise the levels of Cd, Cr, and Pb in plants. Being a quickly rising vegetable crop, the tomato was known early on to have a high absorption of Cr, Cd, and other metals in its shoots and roots (Trebolazabala et al., 2017). When utilized as a phytoremediation plant, the fruits of vegetables, which are typical consumable components, could not be utilized as foodstuff. In a previous report, Trebolazabala et al., (2017) discovered that tomato fruits had the least amount of metal buildup (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Sr, Ti, V, and Zn) comparing over any other sections examined. The amounts of the three most harmful elements (Pb, Cd, and Cr) in tomato's edible portions were less than the highest allowable limits set by the European Commission. Nonetheless, it is generally accepted that just a few tomato qualities, such as mature culture techniques, quick growth character, large biomass, and ease of harvesting and handling, but not with the goal of crop yield, might be used in phytoremediation research and application. Indeed, certain study findings on cellular and molecular reactions, along with toxic metal accumulating in tomato roots and shoot under heavy metal stress, have already been published (Borges et al., 2018).

Tomatoes *(Lycopersicon esculentum Mill)* are a member of the Solanaceae family, and they are a highly priceless vegetable in terms of nutrition. Because of their properties, they are eaten globally, even within areas in which they are not grown. Tomato demand is increasing since these fruits have a very nice flavor and a broad variety of applications (fresh, in salads, or treated). The piece of evidence that tomatoes are often eaten raw means that their vitamins and mineral salts are totally utilized by the body, which contributes to their nutritious value. Tomatoes provide essential health advantages: lowering blood sugar levels, enhancing vision, avoiding renal and biliary illnesses, preserving skin health, building bones with K and Ca, and lowering the risk of developing cancer due to antioxidant chemicals (Mallick, 2021).

As stated in the previous research, several current findings were decided based on heavy metal concentrations in tomatoes cultivated in contaminated soils, such as mine sites, tomato plants watered through wastewater, including those produced in urban and suburban areas of major cities (Bayissa & Gebeyehu, 2021). At the present time, there are few publications on toxic metals that are absorbed by vegetable crops in farming lands. Even though several studies have revealed the influence of metal poisoning within urban soils and industry on public health (Sur et al., 2022) along with eating infected plant foods.

2.4 Cr Metal

In the Earth's crust, Cr is the 21st most prevalent metal and the 7th most plentiful element (Zaheer et al., 2020). As one of the top 20 dangerous compounds, according to the Agency for Toxic Substances and Disease Registry, Cr is also one of the 33 urban air toxicants, 188 hazardous air pollutants (HAPs) (US EPA), one of the 18 core HAPs, and one of the 188 HAPs (Rizwan et al., 2019). It poses a major danger to human health due to its ease of entrance into the food cycle and the ease with which it may penetrate groundwater and soil from both natural and manmade sources (Kumar et al., 2016). According to Chebeir et al., (2016) most chromite resources are concentrated in South Africa (84%), Zimbabwe (6%), Kazakhstan (5%), and India (2%), with the remaining 3% found in Brazil, Russia, Finland, the United States, and Canada.

Cr may exist in a variety of oxidation states (from -2 to +6), but the most prevalent and stable forms in the natural world are hexavalent chromate [Cr (VI)] and trivalent chromate [Cr (III)] (Li et al., 2018). The Environmental Protection Agency (EPA) regulates these two kinds independently since they have diverse chemical, epidemiological, and toxicological characteristics. This offers a particular challenge. Higher concentrations of chromium (Cr (VI)) inhibit germination and restrict root and shoot development, resulting in chlorosis, photosynthetic impairment, and chlorophyll decrease. generation and protein synthesis, ultimately leading to plant mortality (Shahid et al., 2017).

Due to the high concentrations of Cr in water and soil resulting from various anthropogenic and natural activities, environmental contamination of Cr has drawn significant attention worldwide. Cr eventually builds up in crops from contaminated soils and poses serious health risks to humans through food chain contamination (Tang, et al., 2019). Plants with higher Cr levels experience ultrastructural changes (Ud-Din et al., 2017). Plants were subjected to oxidative stress, which resulted in increased electrolyte leakage (EL) and malondialdehyde (MDA) concentrations, as well as changes in antioxidant enzyme activity such as SOD, POD, CAT, and APX (Zaheer et al., 2020).

In the past, antioxidative enzymes were crucial in reducing the phytotoxicity of Cr in an aquatic plant called duckweed Lemna minor (Ud-Din et al., 2017), *Spinacia oleracea*, *Vigna radiata*, *Brassica napus*, and *Triticum aestivum* were all cultivated with high Cr concentrations (Gautam et al., 2020); and *Triticum aestivum* (Ashraf et al., 2022). The regulation of stomatal conductance, signal transduction for programmed cell death, alleviation of seed dormancy, senescence, growth regulation, fruit ripening, and initiation of defense metabolism under stress are just a few of the essential metabolisms of plants that ROS are involved in under normal/natural conditions (Imran et al., 2020). Because of the way that ROS interacts with lipids, proteins, enzymes, and DNA, it can induce membrane leakage and enzyme inactivation, which is why increased ROS production causes a variety of biochemical and physiological diseases (Rehman et al., Copper environmental toxicology, recent advances, and future outlook: a review, 2019).

Cell death occurs because of irreversible metabolic dysfunctions that occur inside plant cells. The following toxicity is dependent on the targeted tissue and the kind of ROS produced by Cr-induced ROS interaction with biomolecules. Even more, several studies indicated that exposure to Cr causes a dose-dependent rise in ROS generation (Ranieri et al., 2019). According to Yu et al., (2018), Cr (VI)-mediated increased ROS generation and the consequent oxidative stress were linked to ultrastructural alterations in *Oryza sativa* root cells. Because Cr is known to diminish the Fe, S, and P contents in plants at greater concentrations, extra delivery of these nutrients is frequently proposed to limit Cr deposition inside plant parts because these minerals can compete with Cr for carrier binding (Jobby et al., 2018).

2.5 Biochar (BC)

BC is a solid material with substantial aromaticity and a high carbon content (greater than 60% carbon content) that is generated by the thermochemical transformation of biomass in the presence of restricted oxygen (Biochar, 2012). The carbonized byproduct of organic matter is known as BC, and it may be made from a variety of basic materials. From a theoretical standpoint, any organic material may be utilized as a raw material to create BC (Chen et al., 2020). Agricultural waste, such as rice straw, wheat straw, maize stalks, cotton stalks, and grass stalks, are the major source of inspiration for ongoing research and manufacturing (Ren et al., 2015). Animal waste (such as pig manure, cow dung, etc.) and forest trash (such as pine waste, palm waste, etc.) (Uchimiya et al., 2013).

The physical and chemical characteristics of BC, which are made from various raw materials, vary greatly, and are also influenced by other elements such as the pyrolysis mode, temperature, and duration (Wang et al., 2020). The thermochemical processes that are now in use are microwave pyrolysis, gasification, and pyrolysis (rapid and slow). Hydrothermal carbonization of biomass has also been investigated in China and other countries in recent years (Zhang et al., 2019). Sangani et al., (2020) discovered that pyrolysis may create 12 different types of BC with various qualities from three distinct source materials with four different particle sizes. Furthermore, some studies have found that production temperature significantly impacts the pH, cation exchange capacity, specific surface area, surface functional groups, and mineral concentration of carbonaceous materials.

BC has recently emerged as one of the appealing experimental hotspots due to its unique features as well as its essential importance in climate change, the global biogeochemical cycle, and the environmental system (Lehmann et al., 2006). Crop production, soil quality, nutrient retention, an increase in the soil carbon sink, and a decrease in carbon dioxide emissions are just a few of the properties of soil that BC helps to enhance (Li et al., 2018). Furthermore, it has a wide range of uses in the cleanup of soil quality as well as the removal of heavy metals and organic wastes (Dai et al., 2019). Contrary to traditional soil remediation techniques, BC not only plays a significant role in the immobilization remediation of heavy metal pollutants (Cd, Pb, Zn, etc.) and organic soil pollutants (antibiotics, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc.) but also serves as an effective method of treating organic waste from agricultural and industrial systems (Ahmad et al., 2014).

Several research studies, such as pot and field trials, have found that BC has a great impact on the restoration of polluted with heavy metals soil, whether in agricultural soil, mining area soil, or urban soil (O'Connor et al., 2018). For example, Altaf et al., (2021) investigated the influences of mixtures of FYM (as cow manure)-derived BC with poultry manure or wood-derived (BC) with poultry manure on the growth of tomatoes, the concentration of heavy metals in the edible parts of these crops, quality of the soil that was contaminated with chromite mine tailing-debris as pot-based crop rotation experiment, the tomato was grown in the same soil after harvest of tomato. It showed that the use of such amendments improved the biomass of both plants significantly, increased the amount of Phosphorus availability in the soil, and decreased the heavy metals in the upper part of tomatoes in chromite-contaminated soil. Khan et al., (2020) studied the impacts of BC on mine-contaminated agricultural soil, tomato biomass, bioaccumulation of Cr and Pb in tomatoes, daily intake of metal (DIM), and human health risk index (HRI) related to consumption of tomatoes. Also, this research showed that the application of sugarcane bagasse BC at a 7% rate to mine impacted agricultural soil effectively increased plant biomass, reduced bioaccumulation, and daily intake metal associated Health Risk Index of Cr and Pb as compared to other treatments and the control.

Herath et al., (2015) grew tomato plants & and investigated the total concentration of Cr in tannery waste soil (TWS) which was 12.285 mg per kg per pot. When 5% BC was added to Tannery Waste Soil (TWS), the bioaccumulation of Cr in tomato plants decreased by 97% when compared to without the addition of BC soil. The bioavailability of Cr in the 5% BC amendment was 68% lower than the control, according to the $CaCl_2$ extractability of Cr. In the 5% BC amendment, sequentially extracted Cr in the exchangeable fraction dropped by 98%.

2.6 Iron-enriched biochar (Fe-BC)

The effects of potentially toxic elements (PTEs) on environments have been reduced using some physical, chemical, and biological techniques (Shahzad et al., 2018). Because of its renewability, broad use, and low price, BC is extensively recognized as a highly preferred and efficient ingredient for combating several ecological obstacles, including heavy metal contamination (Liu et al., 2017). BC has a negative charge on its surface and a significant ion exchangeability, it could also help minimize the Phyto availability and translocation of many PTEs, including Ni, Cd, Cu, and Pb, in polluted soils including in plants (Lu et al., 2014). Biochar (BC) treatment promotes heavy metal resistance in plants by activating a variety of biological and physiological processes such as leaf gas exchange, photosynthetic metabolite buildup, and oxidative stress-induced (Rehman et al., Influence of rice straw biochar on growth, antioxidant capacity and copper uptake in ramie (Boehmeria nivea L.) grown as forage in aged copper-contaminated soil, 2019). But according to current studies, BC may be modified with manures, synthetic fertilizers, and compost to greatly boost its potential (Rizwan et al., 2019). For example, adding sulfur to rice husk BC enhanced its potential to adsorb Hg by 73% when compared to BC without alteration (O'Connor et al., 2018). Similarly, adding mineral components to BC (Wu et al., 2019), phosphoric acid (Wang, et al., 2019), improves soil characteristics and increases the oxygen-containing phenolic, carboxylic, and hydroxyl functional groups on the soil surface, converting harmful ions from more accessible to less accessible forms (Wang, et al., 2019). According to Lucena & Hernandez-Apaolaza, (2017), Fe is a critical nutrient, and a scarcity of it causes stunted development and a considerable drop in plant yield. The application of Fe nanoparticles on the surface of BC can boost crop growth and yield (Joseph et al., 2015). According to earlier research, Fe-BC decreased the bioavailability of heavy metal ions in soil and decreased Cd, Cr, Pb, and Cu translocation by 4.23 to 109.33% in heavy metal-contaminated soil (Jia et al., 2020). However, it has not been thoroughly investigated how Fe-BC improves heavy metal tolerance in plants, so recent work was conducted to uncover the probable function of Fe-BC in boosting Cd tolerance in radish. An additional issue to consider when boosting heavy metal tolerance within plants is heavy metal accumulation in the soil. PTEs are inhibited by BC treatment via many described processes (Shen et al., 2018). The immobility of heavy metals is enhanced by improved BC (Igalavithana et al., 2017). For example, toxicity characteristic leaching was found that MgO-coated corncob

BC reduced soil Pb leaching by 50.71% when compared to unmodified corncob BC (Shen et al., 2018). Similarly, rice husk BC treated with non-toxic elemental S decreased Hg contents in toxicity characteristic leaching procedure (TCLP) leachates by 99% (O'Connor et al., 2018).

2.7 Farmyard manure (FYM)

FYM is the primary source of naturally occurring manure for field crops. However, a huge obstacle to its usage as a source of nutrients is its limited availability. FYM has a good effect on crop output and soil qualities, and it may be utilized to reduce heavy metal plant stresses. FYM has a favorable impact on crop yield and has enhanced the physical, chemical, and biological qualities of soil (Alam et al., 2014), and can be employed to minimize the toxicity of heavy metals in plants (Yassen et al., 2007).

Kumar etal., (2016) examined the effect of sewage sludge, and FYM, applied at different levels on mustard *(Brassica juncea)* growth parameters in Cr-contaminated soils such as germination %, chlorophyll content, plant height, number of leaves per plant, and number of branches per plants. Results showed that growth parameters (germination percentage, chlorophyll content (SPAD), plant height, number of leaves per plant, and number of branches per plant) extensively reduced as Cr level increased. This research also found that the application of FYM much improved growth parameters as compared to respective Cr treatments. The addition of Cr to land might be a reliable method of lowering Cr toxicity to crop plants in Cr infected soils. There is relatively little information published about the influence of farmyard manure (FYM) treatment on Cr immobilization and elimination in soil, Cr bioavailability, agricultural output, and Cr absorption (Singh et al., 2007).

2.8 Vermicompost (VC)

VC is the product of both biodegradable matter and earthworms experiencing a significant water retention capacity, microbiological activity, and abundance of nutrients (Makkar et al., 2022). Composting and vermicomposting have traditionally been good recognized as alternate processes for regenerating plant nutrients from biowaste. Green wastes are converted into acceptable, clean, and better-degraded byproducts through such processes. Earthworms may turn biological and domestic trash into greater fertilizer through the process of vermicomposting (Mashur et al., 2021). Although vermicomposting has a longer history than composting, its use has only recently begun (Koka et al., 2019). Composting is a biological technique that involves the oxidative conversion of edible metabolic end into another acceptable natural form that might have been applied to the soil (Chaher et al., 2021), while vermicomposting is a microbiological expansion of composting that combines the natural microbial biodegradation rubbish with the addition of a variety of organisms. It is a distinctive and complicated procedure to get the decayed green finished product (Edwards & Burrows, 1988), its example is VC. Through vermicomposting, the material is treated with microorganisms and enzymes found within the earthworm stomach, which give the manure a distinct structure and properties that set it apart from other compost. Because earthworm microbes produce biochemical changes in nutritious components, they can enhance the nutritional profile and are a stronger organic material when matched with other composts (Soobhany et al., 2017).

Vermicomposting is said to be superior to composting because of:

1) It goes through the mesophilic stage with mesophilic soil-borne pathogens at temperatures of 20-40 °C, as opposed to composting, that moves through the thermophilic phase at a temperature of 40-60 °C and has a quicker rate of bio-oxidation (Makkar et al., 2022).
2) The vermicomposting method improves the nutritional shape of the treated produce and increases the long-term availability of nutrients (Duggan & Jones, 2016). Producing VC is preferable in terms of physical, nutritive, and biochemical properties (Gandhi, 1997).
3) Mineralization and humification are increased with the addition of earthworms and elevated levels of microbes and decaying activities (Doan et al., 2015).
4) Distinctive bioactive compounds found in VC include humic acid, plant growth regulators, and insect deterrents (Edwards & Burrows, 1988).
5) VC has higher permeability and moisture capability than composted material (Edwards & Burrows, 1988).
6) According to Edwards & Burrows, (1988) it has thinner particulate structural characteristics, more pore volume for improved nutritional bioavailability, and higher buffering capacity and preservation of minerals (Shi-Wei & Fu-Zhen, 1991).

The earthworm is still the most effective element in vermicomposting. There is a complicated contact process between earthworms, bacteria, and soil. Mucus, a liquid substance that boosts the topsoil's ability to retain water, is secreted inside this environment by earthworms.

Additionally, it preserves humidity by absorbing it. Because of its splitting characteristics and thus the addition of casts to the neighboring soils, earthworms are essential mediators of nutrient transport in the biogeochemical process (Ahmad, et al., 2021).

Vermicomposting has drawn more attention as an ecologically acceptable alternative to conventional disposal techniques for handling and using organic waste. Through the joint efforts of earthworms and microorganisms, vermicomposting includes the bio-oxidation and stabilization of organic waste under aerobic and mesophilic circumstances (Gong, et al., 2016). An excellent product that can be utilized as a horticultural potting medium and as an organic soil supplement can be produced by vermicomposting.

VC, which is the product of biodegradation during the vermicomposting process, is utilized generally as a soil amendment. Organic fertilizer helps boost crop productivity because of its high humified organic matter content and superior aeration, drainage, and nutrient capabilities that retain water (Tejada et al., 2010), Because of its huge unique surface area, significant cation exchange capacity, and abundance of active functional groups, it can also be considered a suitable amendment for heavy metal stabilization in soil (Wang et al., 2018). Composite materials are typically thought to be better suited for stabilizing heavy metals in soil than a single amendment. For instance, it has been claimed that adding lime and VC to the soil will greatly lessen the toxicity of copper to grape plants (Trentin et al., 2019). According to Wang et al., (2018) VC -shell powder or shell powder composite amendments may simultaneously enhance plant development and stabilize heavy metals in the soil at a cheap cost, nevertheless, their effects on the bioavailability of Cd in soils are yet unknown. Further, in addition to the fact that the bioavailability of heavy metals in the soil and the number of heavy metals in crops may be utilized to assess the impacts of amendments (Hamid et al., 2019), Soil enzymatic activity can also be employed as a proxy for microbial metabolism (Tang et al., 2020), because research has shown that soil supplements can increase soil enzyme activity by minimizing the absorption of heavy metals (Garau et al., 2019), may alter the physicochemical aspects of soil, hence affecting enzyme stability (Yang et al., 2016). VC is highly recommended for growers and farmers as it can stimulate plant growth and development and improve the ability of plants to cope with abiotic stress. Research has shown that VC contains humic and fulvic acids which can reduce stress in plants caused by Cr (VI). Additionally, previous studies have demonstrated that VC can act as a proliferating substrate for applied microbes, thereby enhancing growth and development in plants. These cites several studies to support these claims, including those by (Pande et al., 2007), (Koka et al., 2019), as well as study by (Kožuh et al., 1999). The aforementioned factors have lately sparked researchers' enthusiasm for biodegradation and the usage of VC to examine its effects on soil and plants (Aksakal et al., 2015). Many initiatives are being made to utilize and stabilize various substrates utilizing various earthworm species.

2.9 Characterization techniques

At present, there are several advanced methods available for analyzing soil amendments, including SEM, FTIR, X-ray Diffraction (XRD), TGA, Nuclear Magnetic Resonance Spectroscopy (NMR), Brunauer-Emmett-Teller (BET), proximate and ultimate analysis, and Raman Spectroscopy.

2.9.1 Scanning electron microscopy with Energy dispersive x-ray (SEM-EDX)

According to Fellet et al., (2014), SEM analysis was employed to identify the surface structures of BC. The SEM images revealed significant changes in surface morphology due to different procedures and temperatures while retaining the overall particle shape. Furthermore, increasing the pyrolysis temperature led to the development of pores, which greatly enhanced the pore properties of BC. The higher temperature also resulted in increased crystallinity of mineral components and the formation of well-ordered aromatic structures within BC. SEM images provide detailed information about the distribution of micropores and mesopores, as well as the arrangement of pores in the BC. Additionally, SEM allows for the prediction of surface morphology before and after the adsorption process. To examine the elemental composition of biochar, scanning electron microscopy combined with EDX is used. SEM-EDX analysis enables the determination of various elements present on the BC surface. In many studies, SEM-EDX has been utilized to investigate the surface of BC after it has absorbed contaminants. However, it should be noted that SEM-EDX is not suitable for the analysis of organic contaminants.

In summary, SEM analysis provides valuable insights into the surface structures of BC. The SEM images demonstrate changes in surface morphology caused by different processing conditions, as well as the development of pores and increased crystallinity at higher pyrolysis temperatures. SEM also allows for the prediction of surface morphology before and after adsorption processes. Furthermore, SEM combined with EDX facilitates the examination of elemental composition on the BC surface, although it is not suitable for analyzing organic contaminants.

2.9.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR is an analytical method used to characterize the bonding structure of atoms by measuring the frequencies at which a substance absorbs IR radiation, leading to molecular vibrations. It is a versatile technique employed in various fields, including biological and composite materials analysis, as well as liquids and gases. IR spectroscopy has a historical background starting with the first IR spectrum obtained by Coblentz in 1905 and the development of the first IR spectrometer in the 1930s. FTIR revolutionized the field by combining an interferometer with the Fourier transform and computerization, allowing for fast and qualitative analysis. A FTIR spectrum represents the transmittance or absorption of IR radiation at different frequencies, expressed in wavenumbers (cm^$^{-1}$). The spectrum can be divided into three regions: near infrared (NIR), mid infrared (MIR), and far infrared (FIR), each exciting different types of vibrations. While MIR is commonly used, NIR and FIR also provide valuable information. FTIR spectrometry is non-destructive, real-time, and applicable to various sample types (solid, liquid, gas), allowing for identification and concentration determination. IR-active substances exhibit a change in the electric dipole moment during vibration, leading to absorption, while IR-inactive substances have a zero-dipole moment. Each chemical bond has a specific vibration frequency corresponding to its energy level, and the absorption occurs when the radiant energy matches the molecular vibration energy. Vibration modes can involve changes in bond length (stretching) or bond angle (bending or deformation). The number of fundamental vibrational modes in a molecule depends on its linearity, with nonlinear molecules having ($3N^{-6}$) modes and linear molecules having ($3N^{-5}$) modes (Ţucureanu et al., 2016).

2.9.3 X-ray diffraction (XRD)

XRD is a widely applicable technique used to analyze the crystallinity and structure of BC. By examining the diffractogram, valuable information about the amorphous material formed at temperatures exceeding 350 °C can be obtained. This characterization is considered reliable. Modern XRD instruments are equipped with a monochromator, radiation source, and a stepping motor for automated analysis. The XRD peaks observed in the diffractogram indicate the presence of well-defined nanocrystals, displaying sharp and strong peaks, suggesting a crystalline nature. Furthermore, as the duration of synthesis increases, the particle diameter also increases. Consequently, XRD patterns play a significant role in the production of high-quality BC, as they allow for fast, non-destructive characterization and the evaluation of sorption efficiency (Usman et al., 2015).

2.9.4 Thermogravimetric analysis (TGA)

TGA is a commonly used method for studying the physical and chemical properties of materials as they undergo temperature changes. TGA enables the characterization of thermal behavior and ignition properties of biochar and biomass/biochar blends. It also examines the potential synergistic effects between different components in the blends. These findings contribute to a better understanding of the thermal characteristics of the samples and provide insights for further experimentation. The heating process in TGA typically starts from room temperature and is gradually increased up to 1000 °C, with various heating rates reported by different researchers, such as 10 and 20°C/min, 10 K/min, and below 1000 °C (Yao et al., 2014).

CHAPTER 3

3 MATERIALS & METHODS

Numerous principles, techniques, and procedures were employed to accomplish the study's goals through the help of prior research findings relating to the study. The specifics of these are explained in this section. The production quality and significance verification of the assessment and evaluation were maintained by adhering to the standard processing methodological approach.

3.1 Site description

A pot experiment was conducted at the Institute of Environmental Science & Engineering at the National University of Science & Technology Islamabad, Islamabad Capital Territory Pakistan (Latitude 33.645572, Longitude 72.990345). According to the weather and climate, the city of Islamabad, Pakistan is situated at a height of 525.72 meters (1724.8 feet) above ocean level, Islamabad has a humid subtropical, dry winter environment (Classification: Cwa). The city's yearly temperature is 26.54ºC (79.77ºF) and it is 5.65% higher than Pakistan's midpoints. Islamabad commonly gets around 92.85 millimeters (3.66 inches) of precipitation and has 122.53 stormy days (33.57% of the time) each year.

3.2 Collection, preparation, and acid digestion of soil

The topsoil (0-20 cm) which was uncontaminated, was taken from the Islamabad Capital Territory, Pakistan. The soil was dried for seven days at room temperature. The soil was homogenized, and unwanted particles, wastes, stones, and pebbles were removed manually. After the soil was passed through a 2-millimeter sieve (Khan et al., 2020).

Taking air-dried three samples of (0.505) soil in small beakers. Added 10 ml concentration of HNO_3 in each sample and put it on the hot plate for 15 minutes at 90 °C. After this, we add 4 ml concentration of $HCLO_4$ to each sample. Again, put these samples on the hot plate until the soil sample solutions see clear. Then the final solution was made of double-deionized water after passing through filtration (Zeng et al., 2011). All the samples were analyzed through AAS (Analytic-Jena nova 800D). These analyses were performed in the lab of wastewater treatment at the Institute of Environmental Science and Engineering at NUST Islamabad. During the preparation of soil following physicochemical properties of soil were tested.

Soil pre-analysis (e.g., EC, pH, CEC, EC (1:2.5) dS/m, Exch. Na (me/100 gm), organic matter (%), Available P (ppm), Extractable K (ppm) and soil texture were done using appropriate methods. Before analysis, the soil was air-dried, impurities like debris and stone materials were removed, and finally, it was sieved through a 2 mm mesh size sieve.

3.3 Soil pH

To determine the pH in soil samples, the soil particle size fraction < 2 mm was treated by manual quartering and mechanical pulverization. The pH was measured in a 1:1 (w/v) soil/water mixture according to method 9045/D of the United States Environmental Protection Agency.

3.4 Electrical Conductivity ((1:2.5) (dS/cm)

The electrical conductivity is less than 1 (dS/cm) it is a normal soil, 1-2 (dS/cm) then critical for germination, 2-3(dS/cm) critical for the growth of salt-sensitive crops, and greater than 3 (dS/cm) it is severely injurious to crops. For conductivity of soil, dissolved 25 g of soil in 100 ml water and placed for 30 minutes. Conductivity is a measure of water capacity for conveying electric current and it is directly related to the concentration of an ionized substance in water also it estimates the total soluble salts. After processing this check electrical conductivity with the help of an Electrical conductivity meter (ECM) at Environmental biotechnology laboratory – IESE.

3.5 Organic matter (%)

To determine the organic matter content in soil, the following procedure was carried out. Initially, 1 gram of air-dried soil with a particle size of 0.15 mm was weighed into a 500-mL beaker. Next, 10 mL of 1 N potassium dichromate solution was added using a pipette, followed by the addition of 20 mL of concentrated H_2SO_4 using a dispenser. The beaker was then gently swirled to ensure proper mixing of the suspension. After allowing the mixture to stand for 30 minutes, approximately 200 mL of deionized (DI) water was added, followed by the addition of 10 mL of concentrated H_3PO_4. The mixture was allowed to cool. To facilitate stirring, 10-15 drops of diphenylamine indicator were added, along with a Teflon-coated magnetic stirring bar. The beaker was placed on a magnetic stirrer. The titration process involved using a 0.5 M ferrous ammonium sulfate solution, which was added until the color of the solution changed from violet-blue to green. Additionally, two blanks were prepared, consisting of all the reagents except for soil. These blanks were treated in the same manner as the soil suspensions (Ryan et al., 1999).

Calculations:

$M = 10/V_{blank}$

Oxidizable Organic Carbon (%) = $[V_{blank} – V_{sample}] \times 0.3 * M /Wt$

Total organic carbon (%) = 1.334 * oxidizable organic carbon (%)

Organic matter (%) = 1.724 * Total organic carbon (%)

Where:

M = Molarity of $(NH_4)\ 2SO_4.FeSO_4.6H_2O$ solution (about 0.5 M)

V_{blank} = Volume of $(NH_4)\ 2SO_4.FeSO_4.6H_2O$ solution required to titrate the blank (mL)

V_{sample} = Volume of $(NH_4)\ 2SO_4.FeSO_4.6H_2O$ solution required to titrate the sample (mL)

Wt = Weight of air-dry soil (g)

0.3 = 3 * 10-3 * 100, where 3 is the equivalent weight of C.

3.6 Available phosphorus (ppm)

To determine the phosphorus content in soil, the following procedure was followed:

A. Extraction: 10 mL of the clear filtrate was pipetted into a 50 mL flask, and the required amount of acid was added to all the unknown solutions. The addition of 1 mL of 5 N H_2SO_4 was sufficient to acidify each 10 mL of $NaHCO_3$ extract to pH 5. By following this procedure, the phosphorus content in the soil could be effectively extracted and measured (Ryan et al., 1999).

B. Measurement: A volume of 10 mL of the clear filtrate was pipetted into a 50 mL flask, and the necessary amount of acid was added to all the unknown solutions. The addition of 1 mL of 5 N H_2SO_4 was adequate to acidify each 10 mL of $NaHCO_3$ extract to pH 5. By adhering to this procedure, the phosphorus content in the soil could be successfully extracted and measured.

Calculation:

Extractable P (ppm) = ppm P (from calibration curve) * V/Wt * V_2/V_1

Where:

V = Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

V_1 = Volume of soil extract used for measurement (mL)

V_2 = Volume of flask used for measurement (mL)

3.7 Extractable Potassium (ppm)

Extraction

1. Weighed 10 grams of air-dried soil with a particle size of less than 2 mm into a 250-mL flask.
2. Added 50 mL of 1 N NH_4OAc solution (ratio 1:5).
3. Shook the mixture for 30 minutes on a reciprocating shaker at a speed of 200-300 rpm.
4. Filtered the suspension using a Whatman No.1 filter paper to remove any soil particles and adjusted the volume of the extract to 50 mL using 1 N NH_4OAc solution.

Alternatively:

1. Weighed 5 grams of air-dried soil with a particle size of less than 2 mm into a 50-mL centrifuge tube.
2. Added 33 mL of 1 N NH_4OAc solution and shook the tube for 5 minutes on a reciprocating shaker at a speed of 200-300 rpm.
3. Centrifuged the tube at 2000 rpm until the supernatant liquid became clear, collecting the extract in a 100-mL flask through a Whatman No. 1 filter paper to exclude any soil particles. This process was repeated two more times, and the extract was collected each time.
4. Diluted the combined 1 N NH_4OAc extracts to a volume of 100 mL using 1 N NH_4OAc solution (Ryan et al., 1999).

5. By following these procedures, the extractable potassium content in the soil could be successfully obtained and measured.

Calculations:

Extractable K (ppm) = ppm K (from calibration curve) * V/Wt

Extractable K (ppm) = meq/L K (from calibration curve) * V/Wt * 39.1

Where:

V= Total volume of the soil extract (mL)

Wt = Weight of air-dry soil (g)

3.8 Exchangeable Sodium (me/100 gm)

To determine the exchangeable sodium (Na) content in soil, the following steps were followed:

100 grams of air-dried soil were weighed.

The soil was placed in a container.

A suitable extracting solution, such as 1 N ammonium acetate (NH_4OAc), was added in a specific ratio.

The soil and solution were thoroughly mixed.

The mixture was allowed to sit for approximately 1 hour to facilitate sodium exchange.

The mixture was filtered to separate the liquid extract from the soil particles.

The filtered extract contained the exchangeable sodium ions.

The concentration of sodium ions in the extract was measured using appropriate analytical techniques.

The exchangeable sodium content was calculated as milliequivalents per 100 grams of soil (me/100 gm) based on the measured concentration and the volume of the extract (Ryan et al., 1999).

Formula:

Exchangeable Sodium (me/100 gm) = (Concentration of Sodium in the extract) × (Volume of extract used for analysis) × (Dilution factor) × (100 / Weight of soil sample).

3.9 Texture

Soil texture is done by a hydrometer. The final categorization is done by the using criteria of USDA. Soil texture determination test is done at the Institute of Environmental Sciences & Engineering, NUST Pakistan. Firstly, the hydrometer was calibrated by preparing the solution of 28 g of sugar and 176 g of distilled water and measuring the gravity of the prepared solution to 1.048. Later all the soil samples were sorted out by hydrometer test. 50 g of each soil sample was spread on sodium metaphosphate within the limit of 2 mm coarse particles of soil and then agitated. The fraction of sand, silt, and clay was calculated using the equations below. % Clay= Corrected hydrometer reading at 6hrs; 52 min x 100 wt. of sample

i. %Silt= Corrected hydrometer readings at 40-sec x 100 wt. of sample

ii. % Sand = 100% -%Silt- %Clay

The result of sand, silt, and clay was recorded in the percentage by hydrometer. The texture of the soil was determined by using the USDA triangle method. The USDA triangle method has used the determine the texture of the soil sample. There is a developed code called MATLAB in the Excel sheet. It is used for the classification of the texture of the soil.

3.10 Soil organic amendments and their characterization

A total of twenty levels of treatments were applied to have different combinations of FYM, BC, Fe-BC, and VC to the soil with appropriate controls (Table 1). VC was taken from the University of Agriculture, Faisalabad, and BC/Fe-BC was taken from GC University of Lahore. Both VC and BC were subjected to characterization by SEM, and FTIR.

3.11 Experimental design and Treatments

Control, soil organic amendments (biochar, Fe-BC, FYM, and VC), and three Cr stress levels of 25, 50, and 100 ppm kg^{-1} soil were used. Eighty (80) pots were arranged in a randomized complete block design in total. The table below lists twenty levels of treatment.

Table 1: Treatment plan

S No	Amendments	Concentration
1	Control	N/A
2	BC	0.5%
3	Fe-BC	0.5%
4	FYM	5%
5	VC	5%
6	Cr	25 ppm
7	Cr	50 ppm
8	Cr	100 ppm
9	BC + Cr	0.5%+25ppm
10	BC + Cr	0.5%+50 ppm
11	BC + Cr	0.5%+100 ppm
12	Fe-BC + Cr	0.5%+25 ppm
13	Fe-BC + Cr	0.5%+50 ppm
14	Fe-BC + Cr	0.5%+100 ppm
15	FYM + Cr	5%+25 ppm
16	FYM + Cr	5%+50 ppm
17	FYM + Cr	5%+100 ppm
18	VC + Cr	5%+25 ppm
19	VC + Cr	5%+50 ppm
20	VC + Cr	5%+100 ppm

The following growth, physiological and biochemical parameters were studied in this research. The table is followed by:

3.12 Shoot Length

For estimating the SL of the plants, the shoots were isolated, and their length was estimated by using a scale (Islam et al., 2008).

3.13 Shoot & Leaves Fresh Weight

For estimating the shoot and leaves FW of the plants,' the shoots and leaves were isolated, and their fresh weights were recorded by the digital scale at Environmental biotechnology (Islam et al., 2008).

3.14 Shoot & Leaves Dry Weight

Shoot and leaves trials were first air dried and afterward dried on a stove for 48 hours at 75°C. Information regarding the shoots and leaves dry weight was recorded by using the weighing balance (Islam et al., 2008).

3.15 Relative Water Content

The grown new leaves were used to gauge RWC. After harvesting, leaves were immediately transported in an ice cube to the laboratory and their FW was calculated. The 0.5 g leaves samples were then absorbed in 10 mL refined water for 24 h at room temperature and under low light to decide the soaking weight, after which the examples were quickly and precisely weighed with a dry paper towel, and their soaking weight (SW) was determined. After that at last, the samples were dried in a Lab Tech oven at 65 °C for 48 h to decide the DW, and the RWC was recorded to utilize the accompanying condition (Sairam et al., 2002).

$$\text{RWC} = (\text{Fresh Weight} - \text{Dry Weight}) / (\text{Soaked Weight} - \text{Dry Weight}) \times 100$$

3.16 Membrane Stability Index

The MSI was estimated by noticing the EC of leaf leachates in two-fold refined water at two unique temperatures, 40 and 100 °C (Sairam et al., 2002). Then, at that point, 0.1 g of leaf samples were cut into a little circle of even size and moved in test tubes having 10 mL of the refined water in two distinct sets. One group of the test tubes was at 40 °C for around 30 min and the other was set at 100 °C in a water shower for around 15 min then their different electric conductivities C_1 and C_2 were noted by EC meter. The upsides of MSI were determined utilizing the accompanying equation:

$$\text{MSI} = 1-(C_1/C_2) * 100$$

3.17 Total Chlorophyll Contents

TCC were recorded by the chlorophyll meter in the greenhouse. Each replicate of plants' leaves was used to measure the TCC.

3.18 Reactive Oxygen Species

For the determination of ROS, e.g., (H_2O_2) leaves samples of 0.5 g were mixed with 4 mL of 0.1% (w/v) Trichloroacetic Acid (TCA) in the ice bath. The homogenized leaves samples (0.5 g weight) were subjected to extraction using 10 mM potassium phosphate buffer (1 mL) and 2 M potassium iodide (2 mL) solution, followed by centrifugation for 20 minutes at 12000 rpm. After that, the absorbance was measured with a spectrophotometer as described by (Islam et al., 2008)**.**

Note: Same plant extract was used for lipid per oxidation. plant extract for H_2O_2 is also used for lipid contents. The replicates were placed in Eppendorf tubes.

3.19 Anti-oxidative enzyme activity

The exercises of anti-oxidative enzyme catalysts were estimated with the highest and completely fresh leaves. For this reason, 0.5 g of the frozen leaves' replicates were squashed in a 2 mL of 0.1M phosphate phosphates buffer (pH 7.0) utilizing a precooled mortar and pestle while kept in fluid nitrogen. The resultant combination was centrifuged at 10,000×g for 20 min at 4 °C. After centrifugation, the supernatant was gathered to decide for further activities of antioxidant enzymes underneath.

3.20 Superoxide Dismutase

SOD activity was determined by measuring the enzyme's decrease in wavelength of the superoxide-nitro blue tetrazolium complex. Every enzyme in the specimen received 3 ml of reaction mixture containing 0.1 ml of 1.5 M sodium carbonate, 0.2 ml of 200 mM methionine, 0.1 ml of 2.25 mM Nitro-blue tetrazolium, 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml distilled water, and 0.05 ml of the enzyme. A pair of tubes with no enzyme extract were used as a baseline. The process began by adding 0.1 ml riboflavin (60 M) and putting the tubes for 15 minutes beneath a light source of two 15 W fluorescent lamps. By turning off the light that covered the tubes with black fabric, the reaction was halted. Tubes lacking enzymes produced the most color. As a blank, a non-irradiated finished reaction mixture that did not develop color was used. Absorbance was measured at 560 nm, and one unit of enzyme activity was used to calculate the amount of enzyme that reduced the absorbance value of samples by 50% when compared to tubes without enzymes (Dhindsa et al., 1981).

3.21 Catalase

The standard strategy for evaluating catalase (CAT) action by (Aebi, 1984) was followed. It contained 3 ml of response compound. Ready with 50 mm phosphate cradle and 100 ml. 25 minutes brooding after catalyst extraction. After brooding, 6 mM H_2O_2 was added to the response compound and ingested. Peruse at 240nm for 2 minutes. The adjustment of CAT movement was perused, and U (unit)/mg is communicated as protein.

3.22 Peroxidase

A standard method for identifying POD action by (Hemeda & Klein, 1990) was followed. The 3 ml compound was created which is ready from 50mM phosphate buffer, 20mM of guaiacol, and protein separate. The subsequent compound hatched for 5 minutes. After hatching, 6mM of H_2O_2 was added to the response compound. The calculation was perused on a Spectrophotometer for 2 minutes and the adjustment of absorbance was assessed to work out G-POD action.

3.23 Ascorbate Peroxidase

APX was measured from the decline in absorbance at 290 nm (an absorbance coefficient of 2.8 mM"1 cm"1) as ascorbate was oxidized. The reference frequency of a Hitachi 356 double frequency spectrophotometer was fixed at 310 nm. We involved 290 nm instead of 265 nm since the absorbance of our measure blend was excessively high at the retention limit of ascorbate. The response combination for the peroxidase contained 50 mM potassium phosphate, pH 7.0, 0.5 mM ascorbate, 0.1 mM H2O2, and 0.1 mM EDTA in an all-out volume of 1 ml. The response was started by adding the compound or H2O2, and the absorbance decline was recorded 10 to 30 sec. Generally, no revision for the oxidation of ascorbate without H2O2 was fundamental, which shows the absence of or extremely low action of ascorbate oxidase in tomato leaves. The remedy was finished for the low, non-enzymatic oxidation of ascorbate by H_2O_2 (Nakano et al., 1981).

3.24 Statistical analysis

All treatments were replicated three times, and data were analyzed using one-way Analysis of Variance (ANOVA) in the Statistical Package for the Social Sciences (SPSS) 16.0 program with a significance threshold of 0.05. Excel was used to calculate the standard errors for each mean number that represents a result. Comparative graphs were made using Excel.

CHAPTER 4

4 RESULTS AND DISCUSSION

4.1 Physiochemical analysis results (Pre-test)

The preliminary analysis of several physiochemical parameters of soil is shown in Table 4.1. The results indicate that the soil conditions are favorable for the plant growth. The pH of the soil ranged from 8.12 – 8.25, demonstrating the suitability of environmental conditions for plant growth. To reveal the existing presence of Cr in the soil, the analysis of Cr was done by using the AAS model (novAA 800D, Analytik Jena, Germany) in the wastewater laboratory at IESE-NUST. The findings discovered the absence of any detectable amount of Cr in the chosen soil.

Table 2: Soil Physicochemical analyses

pH (1:2.5)	EC (1:2.5) dS/m	Exch. Na (me/100 gm)	Organic matter (%)	Av. P (ppm)	Extr. K (ppm)	Texture
8.24	0.22	0.11	0.22	4	42	SL
8.12	0.24	0.10	0.32	7	52	SL
8.25	0.22	0.12	0.25	5	42	SL

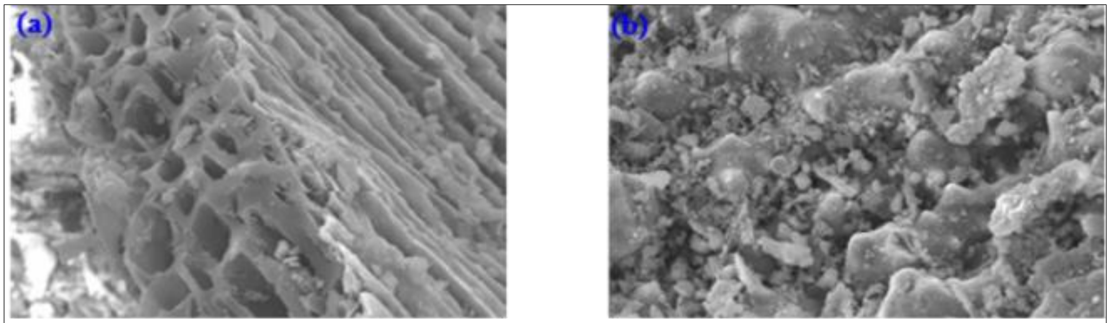
4.2 Scanning Electron Microscopy analysis

The SEM observations were completed of different soil organic amendments which are following.

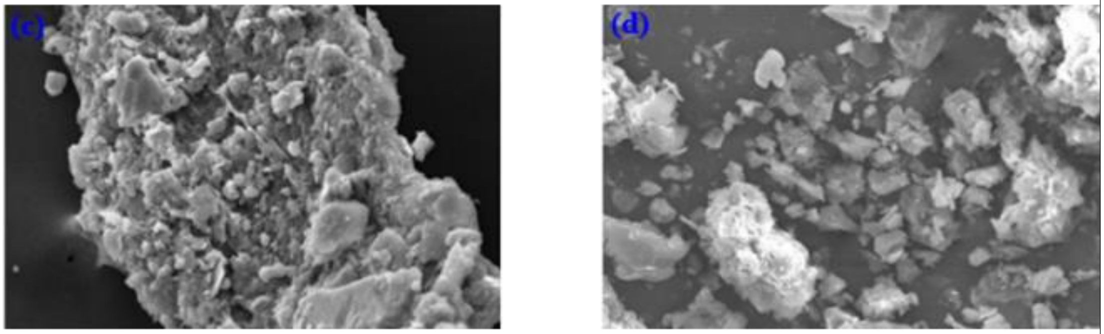
a) BC and Fe-BC: The analysis showed a well-developed porous structure in the BC samples, which demonstrates regular patterns and interconnected pores on the surface. Rice husk BC exhibited reduced variability and adequate porosity. Rice husk biochar had pores that were smaller than (6-7 m) (Binnal et al., 2022). The addition of iron in BC resulted in a deformed structure, primarily due to the deposition of iron. This led to the destruction of the pores within the Fe-BC materials.

b) FYM and VC: the SEM analysis of FYM showed a rough surface texture with irregularly shaped pores. This unique structure had potential playbacks for adsorption procedures. These materials exhibited closely associated with particles with less visible pores, indicating a relatively lower porosity compared to other materials. According to (Srivastava, et al., 2020) dispersed and permeable micromorphology of vermicompost indicates substrate deterioration. This is consistent with the observations of Wang, et al., (2022) who determined a scattered granular structure in cow dung-based vermicompost. Earthworm action and microbial breakdown are mainly responsible for fragmentation.

These findings provide a valuable understanding of the structural characteristics of the studied materials and contribute to our understanding of their potential roles and applications in the context of the research.



(a) Biochar (b) Iron-enriched biochar



(c) Farmyard Manure (d) Vermicompost

Figure 1: Scanning Electron Microscopy (SEM) analysis results of (a) Biochar (BC), (b) Iron-enriched biochar (Fe-BC) (c) Farmyard manure (FYM) and (d) Vermicompost (VC).

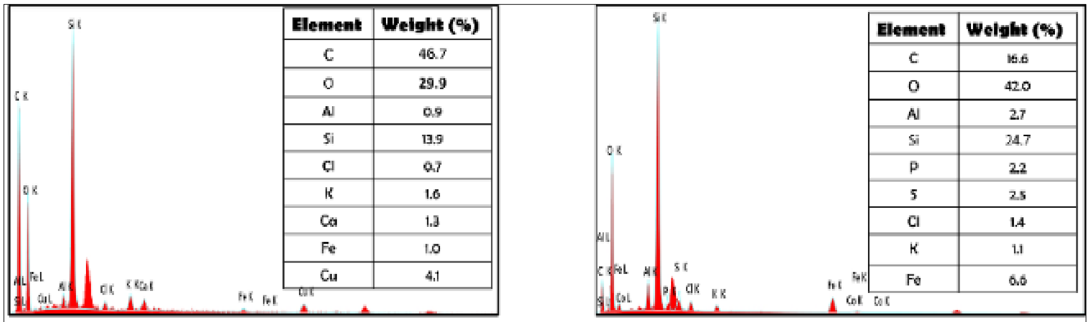
4.3 Energy Dispersive X-ray Spectroscopy (EDX)

4.3.1 Biochar (BC) and Iron-enriched biochar (Fe-BC)

The SEM-EDX of BC provides information on elemental composition in the percentages which are following: C-46.7%, O-29.9%, Al-0.9%, Si-13.9%, Cl-0.7%, K-1.6%, Ca-1.3%, Fe-1.0%, and Cu-4.1%. In contrast, the elemental composition of Fe-BC displayed the following percentages: C-16.6%, O-42.0%, Al-2.7%, Si-24.7%, P-2.2%, S-2.5%, Cl-1.4%, K-1.1%, and Fe-6.6%. The SEM-EDX findings provided a precious understanding of the elemental composition of the two BC materials. The analysis showed the presence and sharing of several elements, including carbon, oxygen, aluminum, silicon, chlorine, potassium, calcium, iron, and copper. The findings proved the elemental composition of both regular BC and Fe-BC, highlighting the substantial changes in the arrangement and the enhanced presence of iron in the end. However, Rice husk BC had 34.25% carbon, 35.16% oxygen, 28.38% silicon (97.79% of the total), and 1.94% potassium. Pretreated rice husk BC, on the other hand, had a higher content of oxygen (43.89%) and a lesser amount of carbon (25.2%). Except for Si, no minerals were detected. The findings confirmed that all minerals were removed, resulting in a minimal amount of ash (Binnal et al., 2022).

4.3.2 Farmyard manure (FYM) and Vermicompost (VC)

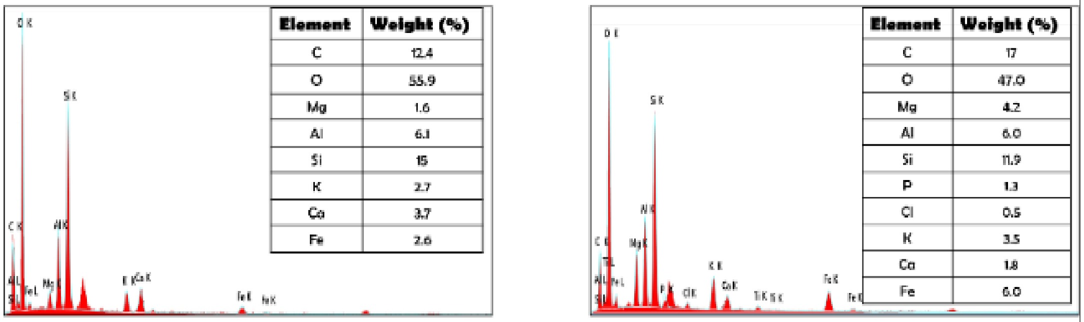
The elemental composition of FYM is presented in percentages that include C-12.4%, Mg-1.6%, Al-6.1%, Si-15%, K-2.7%, Ca-3.7, and Fe-2.6%. Similarly, the SEM-EDX of VC also showed percentages that include C-17%, O-47.0%, Mg-4.2%, Al-6.0%, Si-11.9%, P-13%, Cl-0.5%, K-3.5%, Ca-1.8%, and Fe-6.0%. Analysis of these samples provides better information about their elemental composition. FYM mainly consists of carbon, oxygen aluminum, silicon, potassium, calcium, and iron while FYM is composed of high levels of carbon, and oxygen content, along with the presence of magnesium, aluminum, silicon, phosphorus, chlorine, potassium, calcium, and iron. These elemental compositions of FYM and VC provide a better understanding of its elemental makeup and show their potential nutrient contributions. According to prior research, the mineral composition of VC varies depending on its source and manufacturing procedure. Field application delivers elements into the soil, increasing fertility. An EDX spectrum of cow manure VC exhibits greater quantities of carbonates and silicates (C, K, Ca, O, Si). The inorganic component also includes Mg, Fe, and Al (García et al., 2014).



Element	Weight (%)
C	46.7
O	29.9
Al	0.9
Si	13.9
Cl	0.7
K	1.6
Ca	1.3
Fe	1.0
Cu	4.1

Element	Weight (%)
C	16.6
O	42.0
Al	2.7
Si	24.7
P	2.2
S	2.5
Cl	1.4
K	1.1
Fe	6.6

(a) Biochar **(b) Iron-enriched biochar**



Element	Weight (%)
C	12.4
O	55.9
Mg	1.6
Al	6.1
Si	15
K	2.7
Ca	3.7
Fe	2.6

Element	Weight (%)
C	17
O	47.0
Mg	4.2
Al	6.0
Si	11.9
P	1.3
Cl	0.5
K	3.5
Ca	1.8
Fe	6.0

(c) Farmyard Manure **(d) Vermicompost**

Figure 2: Energy Dispersive X-ray Spectroscopy (EDX) analysis results of **(a)** Biochar (BC), **(b)** Iron-enriched biochar (Fe-BC) **(c)** Farmyard manure (FYM) and **(d)** Vermicompost (VC).

4.4 Thermogravimetric Analysis (TGA)

4.4.1 Biochar (BC) and Iron-enriched biochar (Fe-BC)

The TG analysis of BC and Fe-BC showed that the certain characteristics. In both BC and Fe-BC, there was an initial weight loss observed at 100 °C, indicating the loss of moisture content. Meanwhile Fe-BC showed greater strength compared to BC, staying relatively unmovable up to 400 °C. According to Binnal et al., (2022) decompostion of the rice husk BC exhibited in three stages. Initially moisture and volatile compounds were removed between 30 and 107.65°C, followed by hemicellulose and cellulose decomposition at 242.22 °C at the last lignin decomposition started a fast weight loss at 358.96 °C.

4.4.2 Farmyard manure (FYM) and Vermicompost (VC)

During the TG analysis, FYM displayed an initial weight loss at 85 °C, which can be attributed to the loss of moisture content. In contrast, VC exhibited weight loss starting at 120 °C. According to Bhat et al., (2017) A gradual decrease in mass loss during the stage of vermicomposting implies net enrichment and degradation. The final vermicompost exhibits less mass loss than the pre-vermicompost trash, indicating that it is more mature.

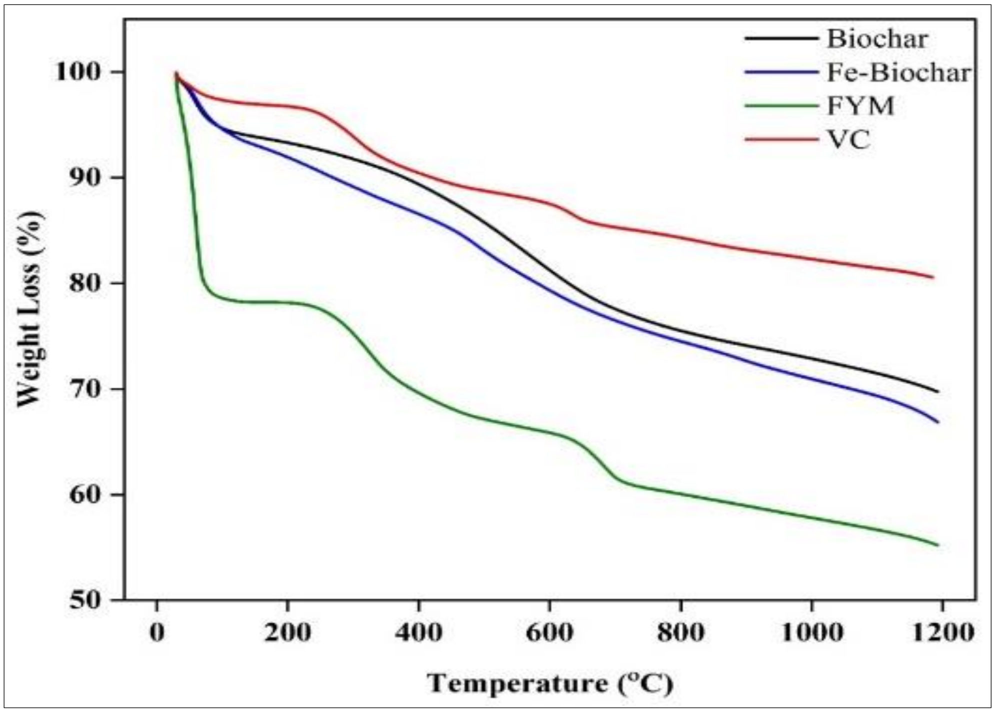


Figure 3: Thermogravimetric analysis (TGA) of Biochar (BC), Iron-enriched biochar (Fe-BC), Farmyard manure (FYM) and Vermicompost (VC).

4.5 Fourier Transform Infrared Spectroscopy Analysis

FTIR spectrum of BC and Fe-BC displayed characteristic peaks at specific wavenumbers, indicating the presence of several functional groups. The absorption peaks observed at 3,400 cm^{-1} to 1,886 cm^{-1} which specified the hydroxyl (-OH) and aromatic skeleton ring (-C=C-) respectively. Associated with BC the characteristic peaks intensity of each group in Fe-BC increased. Such peaks (3467cm^{-1}, 2850 cm^{-1}, 1720 cm^{-1} and 1643 cm^{-1}) that were observed by Binnal et al., (2022) which indicates OH stretching such as phenols, alcohols, ethers, esters, CH denotes such as aliphatic structures, C=O and aromatic stretching shows olefinic, aromatic compounds. The band at 964 cm^{-1} indicates C-O stretching (oxygenated chemicals) and a distinctive peak at 723 cm^{-1} confirms Si-H which is same to present findings. The rice husk BC infrared radiation spectrum demonstrates a strong peak associated with the aromatic C-H out-of-plane stretching vibration (Abrishamkesh et al., 2015). However, FYM and VC exhibited peaks in the FTIR analysis. Both samples showed peaks associated with nitrogen containing groups such as (N-H) stretching vibration in the range of 3500-3300 cm^{-1} recommended the existence of functional groups such as hydroxyl (-OH), amino (-NH). The carboxylic group appeared around 1710-1730 cm^{-1}, phenolic group around 1600-1500 cm^{-1}, and alcoholic groups around 1000-1100 cm^{-1}. The presence of more aromatic chemicals and fewer macromolecular components (carbohydrates, lipids, and polysaccharides) signifies mature vermicompost (Srivastava, et al., 2020). Our research shows that vermicompost has higher humification and stability than compost. Earthworm activity boosts humification, increasing the possibility for heavy metal stabilization in vermicompost. According to Zhang, et al., (2020) aromatic compounds with -OH, C=O, C-C, C-O, and -COO- drive Pb^{2+} adsorption by organic adsorbents

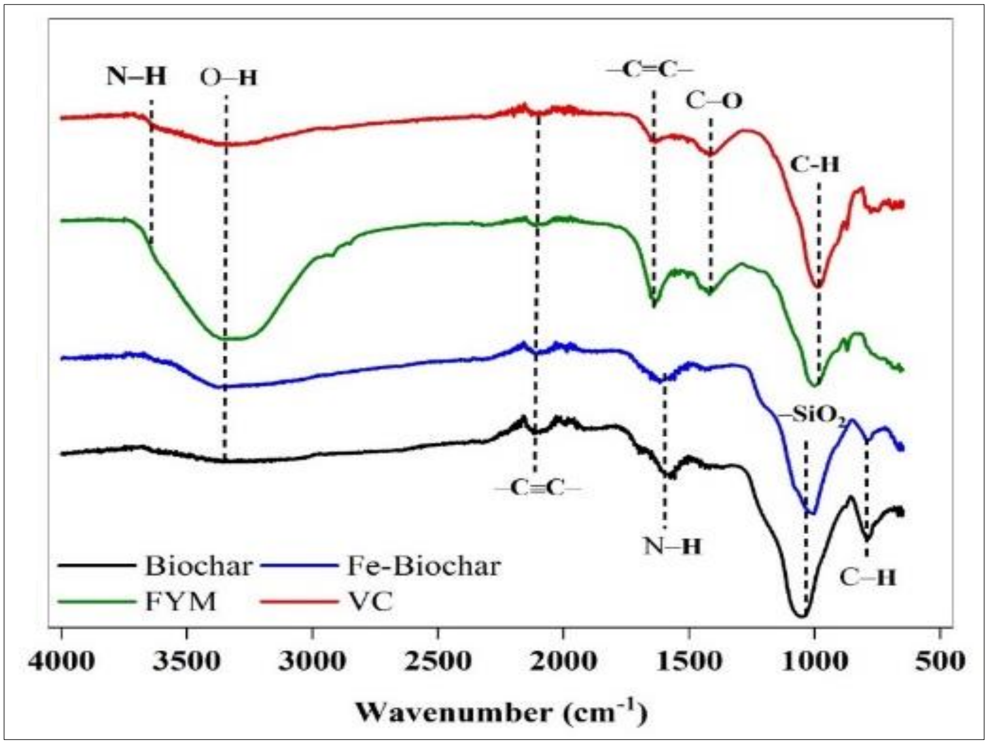


Figure 4: Fourier Transform Infrared Spectroscopy (FTIR) analysis of Biochar (BC), Iron-enriched biochar (Fe-BC), Farmyard manure (FYM) and Vermicompost (VC).

4.6 Response of tomato growth parameters

4.6.1 Shoot Length

Notable reductions in SL by 40%, 61%, and 100% (in which plant died) at 25 ppm, 50 ppm, and 100 ppm Cr levels, respectively, compared to the control (0 ppm Cr) shown in figure 22. At 25 ppm level of Cr concentration the soil organic amendments such as iron-enriched BC and VC improved SL by 1.44-fold, and VC by 1.67-fold respectively. Similarly At 50 ppm Cr concentration level the Fe-BC and VC increased the shoot length (SL) by 2.0-fold and VC by 2.27-fold respectively. However, at 100 ppm Cr level the decrease occurred by 48% and 60% in SL which were observed in Fe-BC and VC respectively, in comparison to the control (0 ppm Cr) displayed in the figure below. Cr delivery to the aerial section of the plant can have an immediate effect on shoot cellular metabolism, leading to a decrease in plant shoot length (Nematshahi et al., 2012). BC effectively mitigated Cd harmful effects on tomato plant shoot length. BC led to the longest shoot length of 19.16 cm, compared to high Cd shortest length of 11.23 cm. Low Cd levels had a minor impact and increased shoot length by 16.6 cm. Combining biochar with reduced Cd further boosted growth to 17.5 cm. These findings align with studies using BC to counteract Cr effects, emphasizing biochar's potential for enhancing plant resilience and environmental health (Dad et al., 2020). The addition of various organic amendments resulted in a significant increase in plant height ranging from 5% to 43%. The greatest substantial improvement was seen with the application of vermicompost. However, the sewage sludge-municipal compost amendment had the lowest increase. These findings are consistent with the findings of Hussain, et al., (2020) who highlighted the different impacts of several organic amendments on plant height.

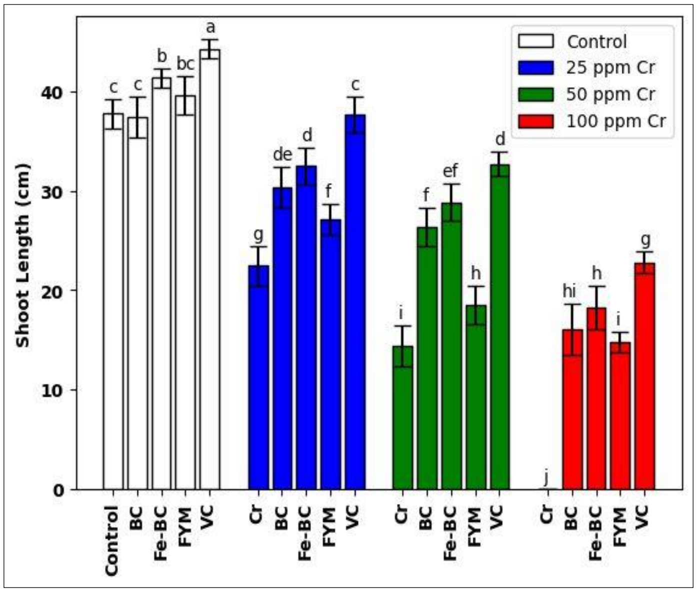


Figure 5: Shoot length (SL) responded to the different Cr concentration toxicity in soil.

4.6.2 Fresh weight

The FW (g) was noted which was decreased by 48%, 65%, and 100% (in which the plant died) at 25 ppm, 50 ppm, and 100 ppm Cr concentration levels, respectively, compared to the control (0 ppm Cr) in the below figure. However, at a 25 ppm Cr level, the application of Fe-BC and VC increased the FW by 1.77-fold and 2.34-fold respectively. At 50 ppm Cr concentration level the Fe-BC and VC increased the FW by 1.75-fold and VC by 2.0-fold respectively. But at a higher stress level of 100 ppm Cr, both Fe-BC and VC exhibited 33% and 49% reduction in FW respectively, compared to control (0 ppm Cr) which is shown in the figure below. Decreased growth was observed in terms of weight at high concentrations of Cr in soil, which might be due to this metal's adverse effect on plants' overall metabolism (Park et al., 2011). According to the study conducted by Pande et al., (2007) the application of 5 mg kg^{-1} soil of vermicompost resulted in a significant increase in herb yield. This treatment yielded the maximum herb growth, demonstrating the potential of vermicompost as a beneficial additive. Notably, the combined application of Cr and vermicompost at 5 g kg^{-1} soil significantly promoted Bacopa herb yield. Noteworthy gains were seen with vermicompost application at 5 g kg^{-1} soil, increasing yield by 44% over 50% application and by 61% over the control. The findings align with the principle that organic amendments like vermicompost can play a crucial role in enhancing plant productivity and yield.

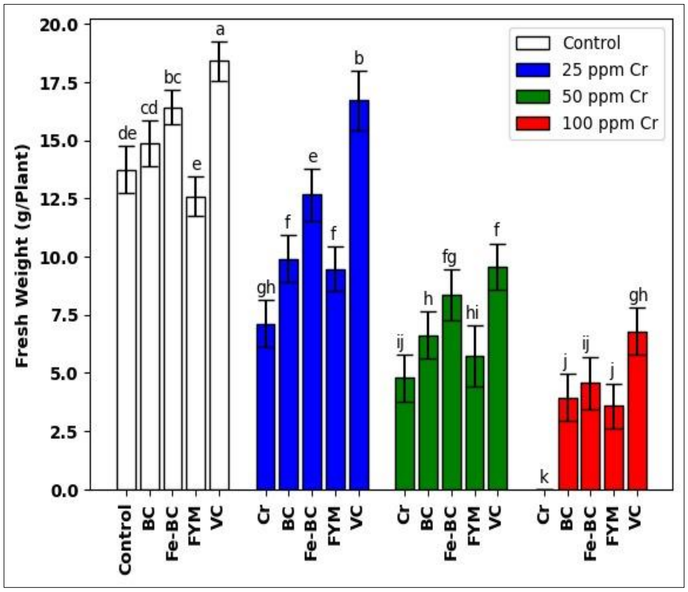


Figure 6: Fresh weight (FW) responded to the different Cr concentration toxicity in soil.

4.6.3 Dry weight

Significant reductions were observed in the DW (g) of tomatoes plant at different Cr stress levels. There was a notable decrease in DW by 17%, 78%, and 100%, at 25 ppm, 50 ppm, and 100 ppm Cr stress levels respectively, compared to control. While using soil amendments treatments, the application of Fe-BC and VC at 25 ppm Cr stress level led to a 1.28-fold and

1.38-fold increase in DW respectively. At 50 ppm Cr stress level Fe-BC and VC resulted in a significant increase in DW by 2.61-fold and 2.99-fold respectively. However, at the highest Cr stress level (100 ppm) both Fe-BC and VC showed a decrease in DW compared to the control. Fe-BC exhibited a 28% decrease, while VC showed a higher decrease of 37%. VC and Fe-BC addition stabilized Cr content in the soil, which may be because of its key attributes like surface heterogeneity, different functional groups, and a large surface area that adsorbed the heavy metals on the soil surface (Nematshahi et al., 2012). According to Velli et al., (2021) the application of biochar with or without compost led to an increase in the dry weight of both aboveground and belowground plant tissues. Notably, significant enhancements were observed specifically in the stem (79.9%) and roots (59.8%), compared to the control group. The addition of various organic amendments resulted in a significant increase in dry weight-plant^{-1} ranging from 2% to 37%. The greatest substantial improvement was seen with the application of vermicompost. However, the sewage sludge-municipal compost amendment had the lowest increase.

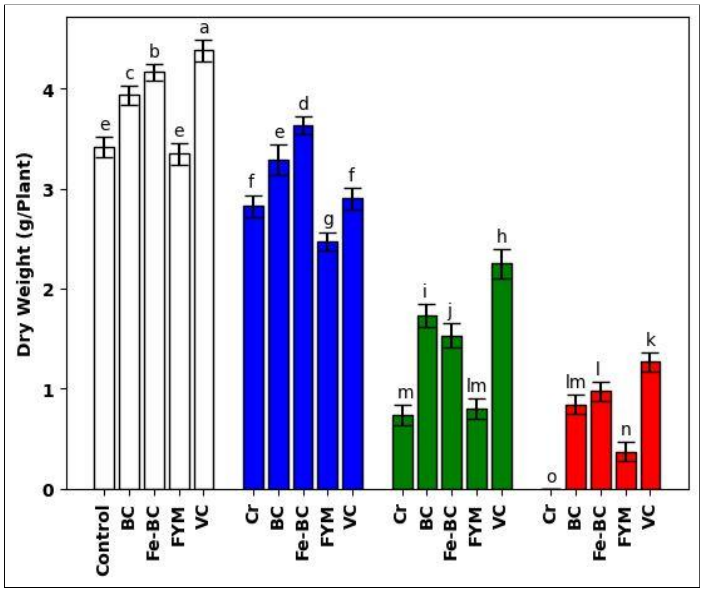


Figure 7: Dry weight *(*DW*)* responded to the different Cr concentration toxicity in soil.

4.6.4 Membrane Stability Index

A notable decrease in MSI (%) was observed at different Cr concentrations. At 25 ppm, 50 ppm, and 100 ppm Cr there was decrease by 22%, 52%, and 100% respectively, as compared to control (0 ppm Cr) shown in below figure. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr concentrations, resulted in to increase in MSI, with 1.16-fold and 1.21-fold respectively and at 50 ppm Cr concertation resulted in to increase in MSI, with 1.36-fold and 1.73-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial reductions in MSI compared to control (0 ppm Cr). Both Fe-

BC exhibited a 58% decrease while VC showed a higher decrease of 62%. Fe-BC application enhanced the plants' MSI, as compared to the control (Abbas et al., 2022). Heavy metals such as Cd, Pb, and Zn damage cell membrane stability, decreasing membrane permeability and generating an ion imbalance in the cell. This affects chlorophyll levels and photosystem II activity, limiting photosynthesis and generating metabolic abnormalities. This phenomenon has been noticed in forest tress as well as grasses and crops (Farooq, et al., 2022).

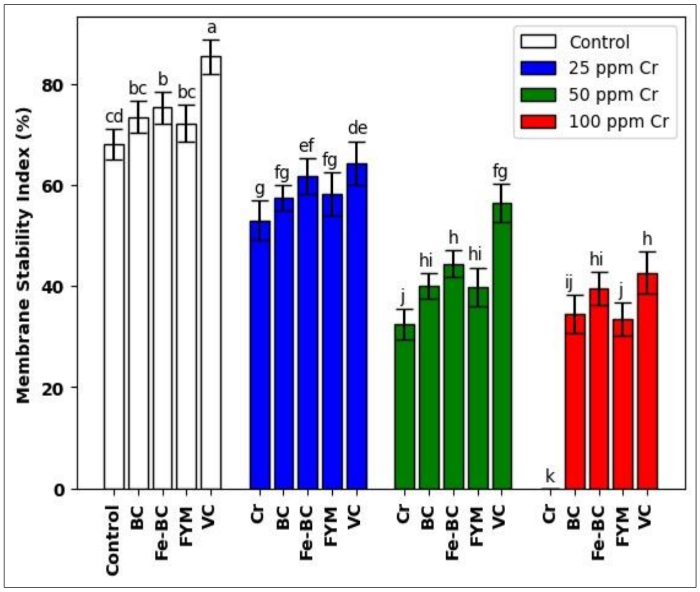


Figure 8: MSI (%) responded to the different Cr concentration toxicity in soil.

4.6.5 Relative Water Contents

A notable decrease in RWC (%) was observed at different Cr concentrations. At 25 ppm, 50 ppm, and 100 ppm Cr there was decrease by 13%, 31%, and 100% respectively, as compared to control (0 ppm Cr) shown in below figure. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr stress level resulted in to increase in RWC, with 1.10-fold and 1.12-fold respectively. And at 50 ppm Cr concertation resulted in to increase in RWC, with 1.09-fold and 1.19-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial reductions in RWC compared to control (0 ppm Cr). Both Fe-BC exhibited a 57% decrease while VC showed a higher decrease of 67%. The decrease in RWC was observed due to decreased root surface area (Stanton & Mickelbart, 2014). The impact of Cd stress was visible in the lowering of RWC, but the use of organic fertilizers improved RWC. The RWC ranged from 74.4% in non-stressed plants to 59.7% in soil with 30 mg Cd kg^{-1}. However, the use of organic fertilizers improved the RWC. The use of BC and VC, either individually or in combination, played a significant influence in improving RWC. The maximum RWC value of 71.40% was obtained by combining BC with VC (Khosropour et al., 2021). Lower leaf RWC results from decreased turgor pressure, limited water uptake, and restriction of root water absorption during stress (Sarker & Oba, 2018).This reduction conserves energy, allowing plants to survive. Organic substrates increase water content, which improves biochemical processes and promotes growth and productivity (Merwad et al., 2018). The results we obtained are consistent with greater RWC in canola cultivars after VC application (Feizabadi et al., 2021).

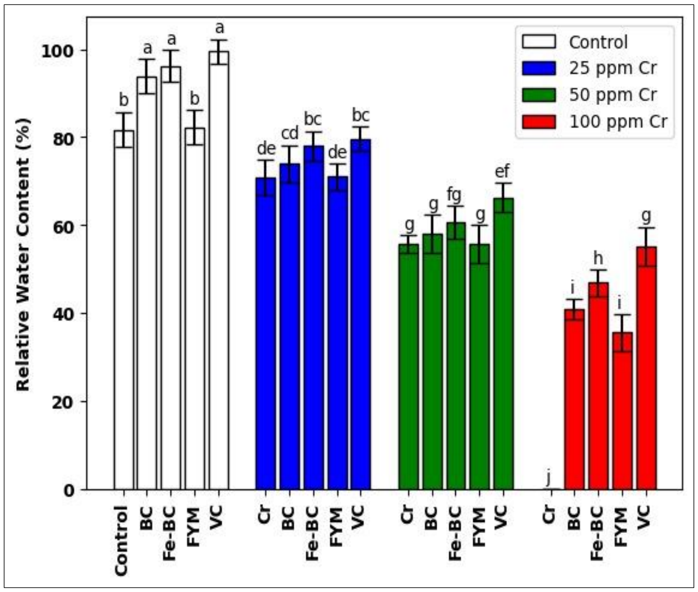


Figure 9: Relative water content (RWC) responded to the different Cr concentration toxicity in soil.

4.7 Response of biochemical parameters

4.7.1 Total Chlorophyll contents

A notable decrease in TCC was observed at different Cr concentrations. At 25 ppm, 50 ppm, and 100 ppm Cr there was decrease by 25%, 49%, and 100% respectively, as compared to control (0 ppm Cr) shown in below figure. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr stress level resulted in to increase in TCC, with 1.18-fold and 1.52-fold respectively. And at 50 ppm Cr concertation resulted in to increase in TCC, with 1.40-fold and 1.87-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial reductions in TCC compared to control (0 ppm Cr). Both

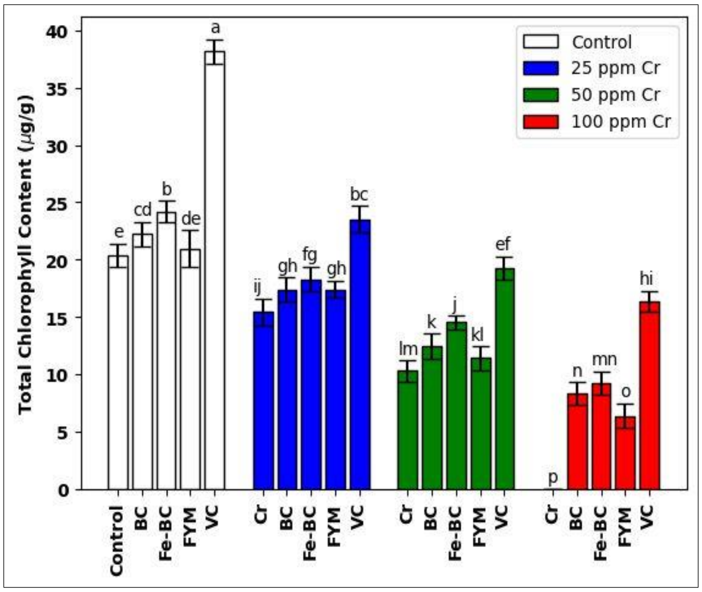


Figure 10: Total chlorophyll content (TCC) responded to the different Cr concentration toxicity in soil.

Fe-BC exhibited a 45% decrease while VC showed a higher decrease of 80%. TCC are responsible for absorbing the light energy of definite wavelengths necessary for photosynthesis and its decreased activity was associated with higher Cr (Mishra & Tripathi, 2009). The addition of various organic amendments resulted in a significant increase in chlorophyll content ranging from 3% to 31%. The greatest substantial improvement was seen with the application of vermicompost. However, the sewage sludge-municipal compost amendment had the lowest increase. These findings are consistent with the findings of Hussain et al., (2020), who highlighted the different impacts of several organic amendments on chlorophyll content. Chlorophyll content decreases under Cr stress due to either reduced production or a rise in enzyme activity that destroys chlorophyll (Ahemad, 2015).

4.7.2 Reactive oxygen species

A notable increase in H_2O_2 was observed at different Cr concentrations. At 25 ppm, and 50 ppm there was increase by 1.9-fold, 2.7-fold respectively, while at 100 ppm Cr (plant died), and had 0-fold increase as compared to control (0 ppm Cr) shown in below figure. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr stress level resulted in to increase in H_2O_2, with 0.25-fold and 0.50-fold respectively. And at 50 ppm Cr concertation resulted in to increase in H_2O_2, with 0.19-fold and 0.53-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial increase in H_2O_2, compared to control (0 ppm Cr). Both Fe-BC exhibited a 3.72-fold increase while VC showed an increase of 2.73-fold. Accumulation of stress related metabolites is an indication of oxidative stress caused by Cr toxicity (Anjum et al., 2017). Gill et al., (2015) revealed that elevated chromium levels within plant cells prompt morphological and physiological shifts due to excessive ROS production. It interacts with lipids, proteins, and DNA, resulting in

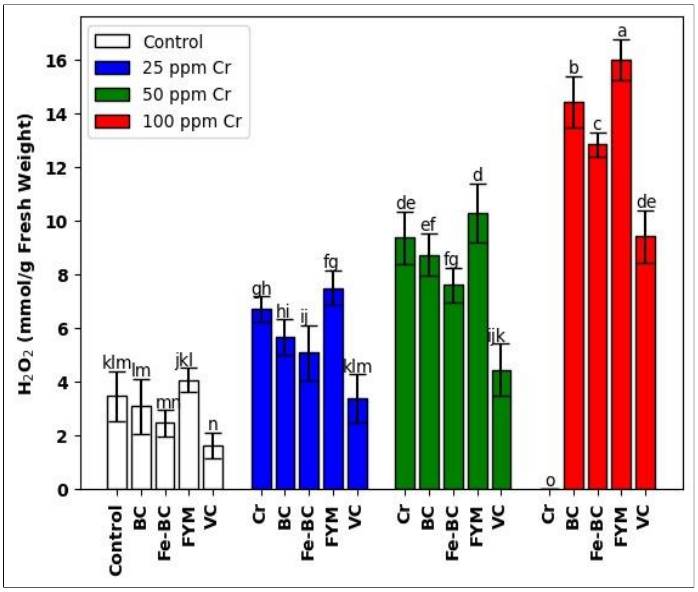


Figure 11: Reactive oxygen species (H_2O_2) responded to the different Cr concentration toxicity in soil.

membrane permeability and enzyme inactivation (Pourrut et al., 2013). Additionally, Panda & Choudhury, (2005) observed that ROS from Cr (VI) triggered oxidative harm and structural changes in Oryza sativa root cells. While ROS-induced biomolecule degradation is typically irreversible, certain molecules like cysteine, DNA, and methionine can undergo repair (Pourrut et al., 2011).

4.7.3 Superoxide dismutase

The biochemical investigation revealed notable changes in SOD levels in response to different Cr concentrations associated to the control. At 25 ppm Cr, there was a substantial 1.58-fold increase in SOD, demonstrating a raised antioxidant response. Similarly, at 50 ppm Cr, SOD levels showed a considerable 2.61-fold increase, further highlighting the antioxidant activity induced by Cr stress. Concerning soil amendment treatments, the application of Fe-BC and VC) at 25 ppm Cr stress level resulted in to increase in SOD, with 0.25-fold and 0.47-fold respectively. And at 50 ppm Cr concertation resulted in to increase in SOD, with 0.22-fold and 0.43-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial increase in SOD, with respect to control (0 ppm Cr). both Fe-BC exhibited a 3.11-fold increase while VC showed an increase of 2.64-fold. Fe-BC and VC application significantly decreased the antioxidant enzyme activity of tomato plants in Cr polluted soils (Park et al., 2011). SOD is an indicator of stress in the environment and a vital part of the plant's antioxidant system. It transforms superoxide anion O^{2-} to H_2O_2 in the cytosol, mitochondria, and chloroplast and aids in cellular defense processes against the possibility of OH production. Under heavy metal stress, high ROS generation raises SOD for activating antioxidative defense enzymes which slow down oxygen radical buildup and de-novo synthesis of enzymatic proteins (Dheeba et al., 2014).

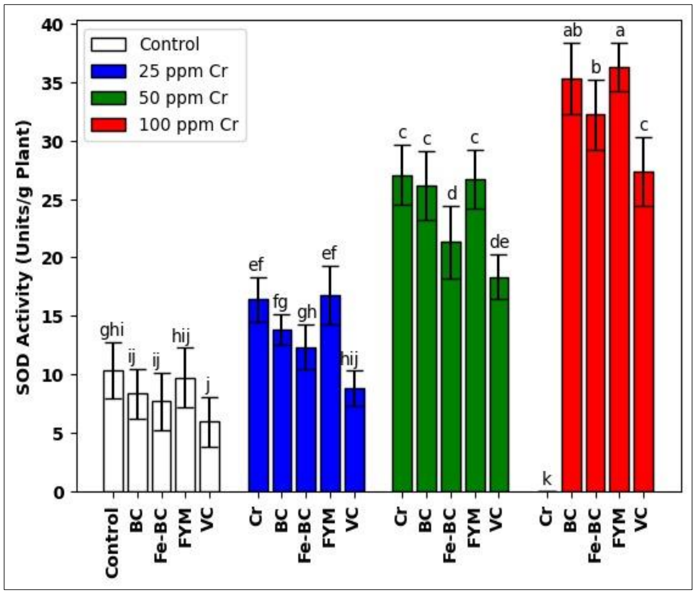


Figure 12: Superoxide dismutase (SOD) responded to the different Cr concentration toxicity in soil.

4.7.4 Peroxidase

The biochemical investigation revealed notable changes in POD levels in response to different Cr concentrations associated with the control. At 25 ppm Cr, there was a substantial 1.62-fold increase in POD, demonstrating a raised antioxidant response. Similarly, at 50 ppm Cr, POD levels showed a considerable 2.21-fold increase, further highlighting the antioxidant activity induced by Cr stress. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr stress level resulted in to increase in POD, with 0.29-fold and 0.37-fold respectively. And at 50 ppm Cr concertation resulted in to increase in POD, with 0.16-fold and 0.25-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial increase in POD, with respect to control (0 ppm Cr). both Fe-BC exhibited a 2.95-fold increase while VC showed an increase of 2.23-fold. Enhanced antioxidant defense system could be attributed as one of the major mechanisms to tolerate Cr toxicity in tomatoes (Saeed et al., 2019). Notably, peroxidase activity increased modestly with increasing chromium levels, correlating with prior observations in chromium-treated wheat plants. The activation of this enzyme may cause changes in the cell wall. Such enzymatic changes could be caused by the chemical properties of heavy metals, which stimulate oxidase. The increased peroxidase activity may equate to a decrease in growth rate, which is consistent with data under other heavy metal treatments (Ganesh, et al., 2008).

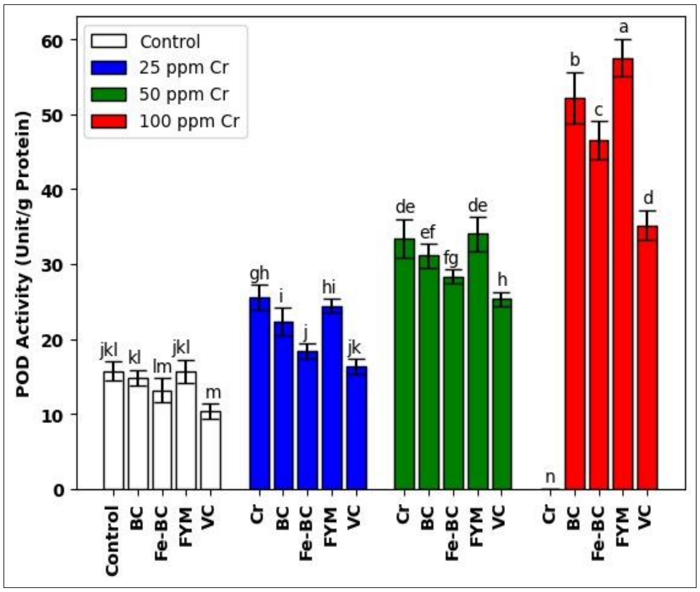


Figure 13: Peroxidase (POD) responded to the different Cr concentration toxicity in soil.

4.7.5 Catalase

The biochemical investigation revealed notable changes in CAT levels in response to different Cr concentrations associated with the control. At 25 ppm Cr, there was a substantial 1.25-fold increase in CAT, demonstrating a raised antioxidant response. Similarly, at 50 ppm Cr, CAT levels showed a considerable 1.53-fold increase, further highlighting the antioxidant activity induced by Cr stress. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr stress level resulted in to increase in CAT, with 0.26-fold and 0.49-fold respectively. And at 50 ppm Cr concertation resulted in to increase in CAT, with 0.24-fold and 0.43-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial increase in CAT, with respect to control (0 ppm Cr). both Fe-BC exhibited a 1.14-fold increase while and VC showed an increase of 1.05-fold. Vermi-BC is an effective alternative for HMs immobilization in polluted soils because of its binding with HMs through adsorption, ion exchange, and complexation. The reduced metal uptake and the subsequently lowered oxidative damage improves growth performance of Cr stressed plants (Park et al., 2011). The increase in CAT activity can be attributed to increased SOD activity, which results in the formation of H_2O_2. This rise in H_2O_2 activates CAT, helping the detoxifying phase that sustains cellular redox balance. CAT plays a critical function in plant defense mechanisms, particularly in mitochondria and peroxisomes. It is important in scavenging for free radicals, particularly H_2O_2, produced during photorespiration and under stressful circumstances. CAT catalyzes the conversion of H_2O_2 into H_2O and O_2 via a two-electron transfer process, protecting vital cellular components such as nucleic acids, proteins, and lipids from oxidative damage caused by ROS (Dheeba et al., 2014).

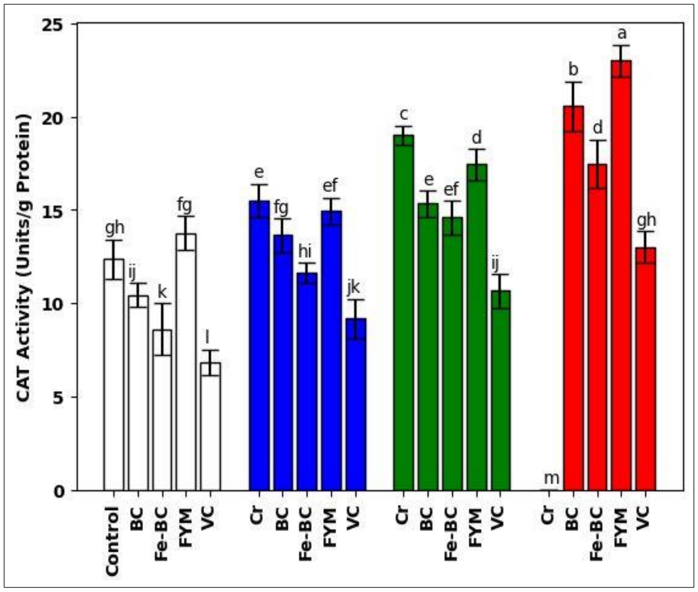


Figure 14: Catalase (CAT) responded to the different Cr concentration toxicity in soil.

4.7.6 Ascorbate peroxidase

The biochemical investigation revealed notable variations in APX levels in response to different Cr concentrations associated with the control. At 25 ppm Cr, there was a substantial 1.17-fold increase in APX, demonstrating a raised antioxidant response. Similarly, at 50 ppm Cr, APX levels showed a considerable 2.07-fold increase, further highlighting the antioxidant activity induced by Cr stress. Concerning soil amendment treatments, the application of Fe-BC and VC at 25 ppm Cr stress level resulted in to increase in APX, with 0.37-fold and 0.61-fold respectively. And at 50 ppm Cr concertation resulted in to increase in APX, with 0.19-fold and 0.45-fold respectively. However, at 100 ppm Cr concentration, both Fe-BC and VC showed substantial increase in APX, with respect to control (0 ppm Cr). both Fe-BC exhibited a 1.76-fold increase while VC showed an increase of 1.46-fold and provided enough nutrients for plant growth. Therefore, it showed an overall improvement in plant growth coupled with a strengthened antioxidative defense system (Laxman et al., 2014). APX in combination with two molecules of ascorbate, aids in the conversion of H_2O_2 to H_2O. Because of this reaction two monohyroascorbate molecules are produced. Notably, Cr exposure increases APX activity in a variety of plants, including *Pisum sativum, Gossypium hirsutum, Ocimum tenuiflorum* and *Corchorus olitorius*. Contrary findings such as a reduction in APX activity in *Triticum aestivum* (Shahid et al., 2017).

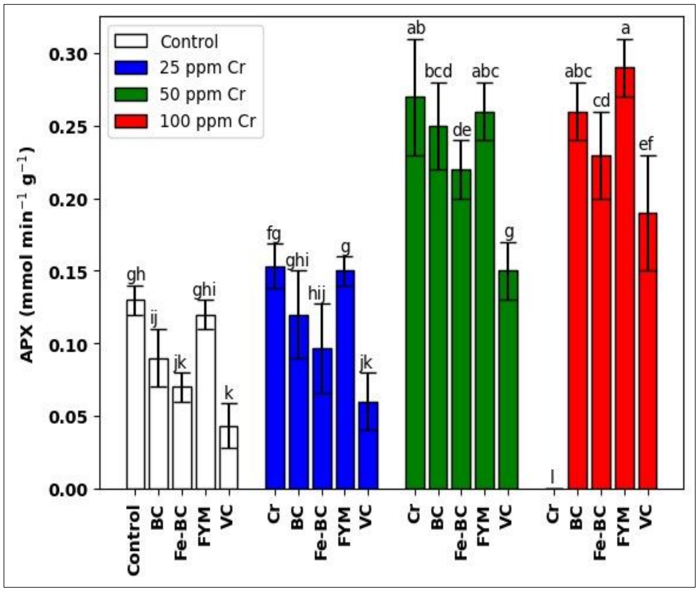


Figure 15: Ascorbate peroxidase (APX) responded to the different Cr concentration toxicity in soil.

These outcomes highlight the impact of Cr stress on biochemical parameters such as H_2O_2, SOD, POD, CAT and APX levels and explain the complex effects of Fe-BC and VC amendments on antioxidant activities. While Fe-BC and VC showed potential in reducing biochemical parameters levels at lower Cr concentrations, their effects were altered at the highest Cr concentration.

CHAPTER 5

5. CONCLUSIONS AND RECOMMANDATIONS

5.1 Conclusions

It is concluded that 50-ppm Cr stress level resulted in a substantial reduction in plant growth, MSI, RWC, and TCC. Moreover, at the highest 100 ppm Cr stress level plant was incapable to survive and died. The observed reduction in plant growth can be recognized as Cr-induced oxidative damage, as demonstrated by a notable 2.7-fold increase in H_2O_2. However, the application the application of organic soil amendments showed to be effective in improving several tomato growth parameters. Fe-BC and VC amendments led to a notable increase in 2.60- and 2.90-fold in DW, 1.36 and 1.73-fold increase in MSI, and a 1.40 and 1.87-fold increase in TCC, respectively. These results suggest that soil organic amendments positively affect tomato plant growth parameters. The improvement in plant growth can be attributed to the reduced oxidative stress facilitated by enhanced antioxidative activities. SOD activity reduced by 0.80-fold, while APX and CAT activities reduced by 0.55-fold. These enhancements played a substantial role in mitigating the adverse effects of Cr-induced oxidative stress and contributed to improved plant growth in the presence of soil organic amendments. Notably, the application of soil organic amendments allowed plants to tolerate the highest Cr concentration (100 ppm). Among the amendments assessed, VC demonstrated the most pronounced effectiveness in enhancing plant resilience to the highest levels of Cr stress.

5.2 Recommendations

Considering the findings, several recommendations can be made regarding the use of organic soil amendments and further research in this field:

1. Soil organic amendments offer a cost-effective approach to alleviate Cr uptake by plants and reduce its transport into the human body through the food chain. Therefore, it is suggested to consider the application of soil organic amendments as a sustainable approach for treatment Cr-contaminated soils.
2. Given the global production of agricultural waste, there is a potential to use this plentiful feedstock for the synthesis of organic soil supplements. Attempts should be made to

investigate and develop novel technologies for transforming waste from agriculture into efficient amendments, which will increase waste value and resource productivity.

3. Although the present research provides useful information, enormous-scale field trials are required to confirm the efficacy of organic soil amendments. This will assist in determining their realistic applicability, and their implementation.
4. Additional studies ought to concentrate on evaluating physiological and genetic changes occurring in plants in response to organic amendments to fully understand the mechanisms behind these amendments' effects. This understanding will aid routes and molecule targets that influence how plants respond to Cr tolerance.
5. Investigating the long-term destiny of Cr that is lessened by organic soil additions is essential. The stability, bioavailability, and possible leaching or buildup of Cr in soil treated with organic substances should be studied over an extended period. This knowledge is crucial for evaluating the long-term efficacy and ecological impacts of remediation solutions based on organic amendments.

By following these suggestions, we may improve our knowledge of the effectiveness of organic soil amendments, make the best use of them in real-world situations, and make sure that soils polluted with Cr are managed sustainably.

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