COMBINED EFFECTS OF POLYSTYRENE MICROPLASTICS AND GREEN SYNTHSIZED IRON-OXIDE NANOPARTICLES ON PHYSICOCHEMICAL PROPERTIES AND NUTRIENT PROFILE OF COPPER STRESSED SOIL

Master of Philosophy

In

Environmental Sciences

by

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QUAID-I-AZAM UNIVERSITY

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This work is submitted as a dissertation in partial fulfilment for the award of the degree of

Master of Philosophy
In
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I, Nosheen Shabir, hereby state that my M.Phil. Thesis titled as **"COMBINED EFFECTS OF POLYSTYRENE MICROPLASTICS AND GREEN SYNTHESIZED IRON-OXIDE NANOPARTICLES IN CU STRESSED SOIL"** is solely my research work with no significant contribution from any other person. Small contribution/help whatever taken has been duly acknowledged and that complete thesis has been written by me.

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Nosheen Shabir

Certificate of approval

It is to certify that the research work presented in this thesis, titled "COMBINED EFFECTS OF POLYSTRENE MICROPLASTICS AND GREEN SYNTHSIZED IRON-OXIDE NANOPARTICLES ON PHSICOCHEMICAL PROPERTIES AND NUTRIENT STATUS OF COPPER STRESSED SOIL" was conducted by Ms' Nosheen Shabir (Registration No. 023112111010), under the supervision of Prof. Dr. Riffat Naseem Malik (T.I). No part of this thesis has been submitted elsewhere for any other degree. This thesis is submitted to the Department of Environmental Sciences, in partial fulfillment of the requirements for the degree of Master of Philosophy in the field of Environmental Science, Department of Environmental Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

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Dedicated to my cherished parents, their support and belief in me are treasures I forever hold, a debt of gratitude I can never repay

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Abbreviations	Full forms
MPs	Microplastics
NPs	Nanoparticles
GION	Green Synthesized Iron oxide Nanoparticles
Mg/kg	Milligrams per kilo grams
Cu	Copper
UV	Ultraviolet
PET	Polyethylene Terephthalate
LDPE	Low-Density Polyethylene
HDPE	High-Density Polyethylene
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
PSMP	Polystyrene Microplastics
PSNPs	Polystyrene Nanoplastics
EC	Electrical Conductance
PLA	Polylactic Acid
PA	Polyamide
CEC	Cation Exchange Capacity

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Abstract

Background

Soil is a vital component of agriculture, providing essential nutrients and support for plant growth. However, soil health can be negatively impacted by various stressors, such as heavy metal pollution, microplastics contamination and nanoparticles. Soil physicochemical parameters and nutrient status are important to observe in the presence of these stressors.

Objectives

The study aimed to investigate the combined impact of polystyrene microplastics (PSMPs) and green synthesized iron oxide nanoparticles (GIONPs) on the physiochemical properties of Cu stressed soil. Furthermore, the impact of PSMPs, GIONPs were also observed on micro and macro-nutrients as well as heavy metals of Cu stressed soil.

Methodology

Different concentrations of Cu (0, 50, 100, 200, and 400mg/kg) PSMPs (0%, 1% and 3% w/w) and GIONPs (100ppm) were added to soil contains 3% w/w organic matter. Soil physicochemical properties (pH, EC) and nutrients profile was analysed via atomic adsorption spectrophotometer-(ASS). Micronutrients included (Fe, Zn, Cu, Mn), Macronutrients (No_3^- , Po_4^{3-}) and Heavy metals (Pb, Cd). Analysis carried out at two stages i.e. pre-sowing soil (14 days incubated soil) and post harvesting soil (after 60 days of maize cultivation).

Results

The results showed that the increasing concentration of Cu in soil showed a negative effect on soil physicochemical properties and the levels of micronutrients, essential for soil health. Soil pH and EC disrupted with the addition of Cu, PSMPs and GIONPs. The soil pH decreased (8.6-8.4) in both soils, while, EC increased (434 µS/cm -615 µS/cm) in pre-sowing soil with the addition of Cu. However, soil pH (8.6-8.8) and EC (426 µS/cm -580 µS/cm) increased with addition of GIONPs, PSMPs in pre-sowing soil. While, in post-harvest soil pH increased (8.9 -9.13) and EC decreased (130 µS/cm -150 µS/cm) over all with the addition of Cu,PSMPs and

GIONPs in soil. Cu, PSMPs and GIONPs adsorbed the nutrients in pre-sowing and post-harvest soil. However, PSMPs and GION showed less reduction as compared to Cu in post-harvest soil significantly ,the concentration ranged from 307 mg/kg -12 mg/kg, 8342 mg/kg- 8342 mg/kg, 389 mg/kg- 280 mg/kg, 44 mg/kg – 30 mg/kg and 19 mg/kg -10 mg/kg, 31mg/kg -19 mg/kg respectively. The adsorption capacity of GIONPs was greater as compared to PSMPs in soil. Addition of GIONPs adsorbed more as compared to PSMPs in soil. Moreover, addition of 3% PSMPs showed greater adsorption as compared to 1% PSMPs in soil.

Conclusion

Cu, PSMPs and GIONPs acted as vector of nutrients in this study, thus reduced the bioavailability of nutrients in soil. The significant difference in soil pH, EC and nutrients was observed in pre- sowing and post-harvest soil. Moreover, the adsorption capacity of GIONPs was greater as compared to PSMPs in soil as the addition of GIONPs adsorbed more as compared to PSMPs in soil. Additionally, addition of 3% PSMPs showed greater adsorption as compared to 1% PSMPs in soil.

This research highlights the need to consider the combined impact of multiple stressors on soil health and nutrient status. However, this research provides valuable insights into the complex relationships between different soil stressors and their impact on soil health and nutrient status.

Chapter 1

Introduction and Literature Review

Due to its adverse impacts on environmental safety, Soil contamination with heavy metals is becoming a worldwide threat. Soil is one of the key resources that are being polluted with excess of Copper (Cu). Cu is considered a trace element in soil-plant systems, and its average concentration in the Earth's crust is approximately 60 mg kg^{-1}. In soil, the typical range of Cu concentration varies from 2 - 50 mg kg^{-1} .Soils that naturally have elevated levels of clay minerals (such as Vertisols) or organic matter (like Spodosols and Histosols) often exhibit higher Cu content, reaching levels as high as 180 mg kg^{-1}(Cesco et al., 2021).

1.1 Sources of Cu in soil

Various sources have been identified as contributors to soil accumulation and subsequent contamination of Cu , including mining activities, former wood treatment sites, dust fall out, metal organic residues, scrap deposits and the application of Cu-based fungicides in crops (Burges et al., 2020; Coelho et al., 2020; Penteado et al., 2021). Anthropogenic activities such as coffee plantations, vineyards orchards and the cultivation of Solanaceae plants (e.g., tomato, potato) have been found to be significant sources of soil Cu contamination, primarily due to the intensive use of Cu-based fungicides like Bordeaux mixture (CuSO4 + CaO) and other Cu-based salts (Ballabio et al., 2018). Records show that Cu applications for crop disease control have been employed since 1850, leading to long-term Cu accumulation in soils (Poggere et al., 2023).

1.2 Factors Effecting Bioavailability of Cu in soil

Cu has the ability to create complexes with a wide range of ligands, including both organic and inorganic ones such as iron and manganese oxides. This interaction occurs through various mechanisms like cation exchange, precipitation, biosorption, or adsorption. These processes effectively reduce the concentration of free Cu ions and overall Cu contamination (Cui et al., 2019). Cu exhibits restricted mobility within soil and tends to accumulate primarily in the uppermost layer of soil, commonly known as the topsoil. (Araújo, Strawn, Morra, Moore, & Alleoni, 2019). The presence and accessibility of Cu in soil depend on several factors, including the soil type and its morphological as well as chemical properties. (Y.-p. Zhao et al., 2018). Moreover, the retention time of Cu in the soil is influenced by factors such as particle size, soil aggregates, and various other soil characteristics (Q.-Y. Wang, Liu, & Hu, 2016). Approximately 80% of Cu in soil exists in the form of insoluble oxides and sulfides, making it less readily available for plant uptake. (Mihaljevič et al., 2019).

The concentration of Cu (II) in soil tends to increase when the soil pH decreases, leading to a reduction in the adsorption capacity of Cu. (Cui et al., 2020). Moreover, in Cu-contaminated soils, the bioavailability of Cu is influenced to a greater extent by the rhizospheric soil pH rather than the overall soil pH. Additionally, the interplay of dissolved organic matter and rhizospheric pH has a greater impact on the dynamic speciation of Cu compared to the inorganic components present in the soil. However, the involvement of plant roots in determining Cu bioavailability is significant, yet it is influenced by factors such as the plant species and the concentration of Cu present in the soil. (Kumar et al., 2021).

1.3 Cu toxicity to soil nutrients

High levels of Cu in soil are persistent and cannot be broken down naturally, making them non-degradable. This high Cu concentration poses a threat to microorganisms present in the soil. Soil microbes, including those involved in nitrification (the process of converting ammonia to nitrate), are particularly sensitive to changes in the soil environment. As a result, it disrupts the nutrient cycling process and hinders the essential mineralization of nutrients like nitrogen (N) and phosphorus (P). Hence, they can serve as early indicators of shifts in soil ecology. The oxidization of ammonia, which is a key step in nitrification, tends to increase as the soil becomes more contaminated with Cu (Rehman et al., 2020). The results from Laboratory and Screen house experiments showed significant decrease in soil available P, Zn and Fe as rates of Cu increase over control experiment. The effect was more pronounced at application rate above 20 mg Cu kg^{-1} (Azeez, Adesanwo, & Adepetu, 2015).

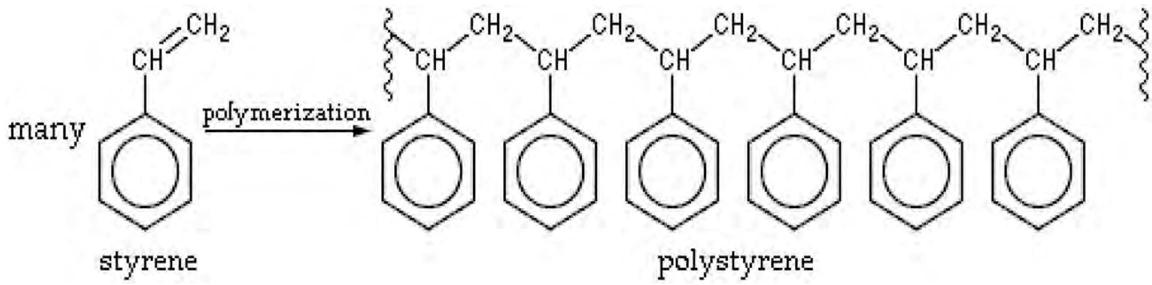
Therefore, it is important to manage and monitor Cu levels in soil to maintain healthy ecosystems and sustainable food production.

1.4 Microplastics

MPs are small plastic particles with a size equal to or less than 5 mm. They can be classified into two main categories: primary microplastics and secondary microplastics. (Blettler, Ulla, Rabuffetti, & Garello, 2017). Primary MPs are plastic particles that are produced intentionally and manufactured at a small size. In contrast, secondary MPs are formed from the breakdown of larger plastic waste due to various environmental processes, such as ultraviolet radiation, weathering, high temperature, oxidation, freeze-thaw cycles, and biological action (Qi et al., 2020). MPs found in soil primarily originate from two main sources: human activities and natural factors. The former includes practices like plastic mulching, fertilization, and improper disposal of plastic-containing household waste. On the other hand, the latter source involves natural processes like plastic degradation and sedimentation. (Zhang et al., 2023).

1.5 PSMPs

PS is a synthetic polymer made from the monomer styrene. It is a high molecular weight material with a formula of (C8H8)n. PS can be solid or foamed and is characterized by its clear, hard, and brittle nature. It is an inexpensive resin but has a low barrier to oxygen and water vapour and a low melting point. Chemical formula of PS (Ho et al., 2017).



1.6 Sources of PSMPs

The world's total plastic production includes 6.1% of PS (Europe, 2020) is widely used in everyday items like foam lunch boxes, plastic bottles, and micro beads in cosmetics. Consequently, it becomes prevalent in wastewater and sewage sludge due to its extensive usage in these products (Ullah et al., 2023). In Taiwan, compost has been found to contain 53 items of PS microplastics per square meter, while in farmlands of Sri Lanka, the presence of PS microplastics reaches 310 to 410 items per kilogram (Premasiri, 2021). Notably, the presence of microplastics in soil is significantly higher when plastic mulching practices are employed. In fact, the number of microplastics in these soils is approximately double compared to soils where such plastic mulching practices are not utilized. (Zhou et al., 2021) and based on an analysis of 31 research papers, it has been reported that PS ranks as the fourth most abundant type of microplastic in terrestrial systems, with a particularly widespread presence in agricultural soils. (Huang et al., 2021)

1.7 Effects of PSMPs on soil properties

Studies have reported that MPs can raise the pH level of soil, as observed in an experiment where the addition of 0.4% PS foam resulted in a higher pH and reduced microbial activity. However, it's worth mentioning that the impact of PS on soil pH can vary based on factors such as size and dosage, as noted in studies by (Boots, Russell, & Green, 2019) (Li, Yu, Yu, & Xu, 2022) and (Yang, Zhang, Kang, Wang, & Wu, 2021). According to (S. Dong et al., 2021), the addition of PS and polytetrafluoroethylene (PTFE) to soil can lead to a decrease in pH, however, this effect is dose-dependent. Small-sized MPs have a more pronounced impact on soil pH compared to larger ones. The incubation time and shape of the MPs can also impact soil pH, with foam and fragmented MPs causing a more significant increase in pH than film or fibrous MPs. (T. Zhao, Lozano, & Rillig, 2021) found that over time and with exposure to PET fragments and PS foam soil pH gradually increased, highlighting the influence of both incubation time and MP shape on soil pH.

MPs impact soil nutrients directly and indirectly through multiple mechanisms. (F. Wang, Wang, Adams, Sun, & Zhang, 2022). Microplastics have the potential to directly absorb nutrients, altering their availability in the soil (Mao et al., 2020). As a result of weathering and oxidation, microplastics may undergo surface modifications, leading to the development of pores and charges. This enhanced surface area and charge capacity enable microplastics to exhibit higher adsorption capabilities, including the ability to electrostatically adsorb Cu2+ ions (Zou, Liu, Zhang, & Yuan, 2020). Studies suggest that microplastics with different surface charges have the potential to bind with both negatively and positively charged nutrients in the soil. Additionally, soil nutrient cycles are regulated by bacterial-driven biochemical processes. The presence of microplastics can influence microbial communities and their activity, thereby impacting soil nutrient dynamics. (Zou et al., 2020). Likewise, polystyrene microplastics (PSMPs) also disrupt nutrient cycles in the soil. For instance, PSMPs have been found to decrease urease and phosphatase enzymatic activities, leading to a subsequent reduction in the availability of nitrates and phosphates in the soil. (Dong, Gao, Qiu, & Song, 2021). Polystyrene microplastics (PSMPs) coexist with heavy metals in soil, with cadmium (Cd) and Cu (Cu) being the predominant types of heavy metals found. Apart from heavy metals, PSMPs are extensively distributed in the environment and are susceptible to aging processes. (Pang etal., 2023) (Zhang et al., 2023).The table below showed impact of MPs on soil properties.

Table 1 Effects of Microplastics (MPs) on soil chemical properties

MPs	**Chemical** Property	**Dose**	**Trends**	**References**
Polystyrene, Polyamides, Plycarbonate,Polystylene,Polyurethanes	pH	0.4%	Increased	Zhao et al., 2021 (A)
Polyethleneterphataltae	pH	5.0%	Increased	Ghari and Zamani Ahmedmamoudi 2022
Low-Density Polyethylene	EC		Decreased	Qie et al., 2020
Polystyrene	Soil nitrates	2.0 %	Decreased	Feng et al., 2022
Polystyrene	Nitrates	0.25%	Decreased	Dong et al., 2021
Polystyrene	Zn, Pb, As	0.25%	Decreased	Dong et al., 2021

1.8 Nanoparticles

The main distinctive characteristic of nanoparticles is their considerably greater ratio between surface area and volume, known as the S/V ratio which gives them strong reactivity and physicochemical versatility (Qi et al., 2018). In addition, the higher surface energy and the effect of size-dependent behavior of electrons and other particles of nanoparticles, in comparison to their bulk materials determines their distinct behaviour in the environment (Browne, 2015). Nanoparticles are divided into many groups according to their chemical and structural components, such as, metal oxides, quantum dots, nano-polymers, zero-valent metals lipids, semiconductors, carbonaceous materials and dendrimers (Sudha, Sangeetha, Vijayalakshmi, & Barhoum, 2018).

1.9 Green synthesized Iron oxide nanoparticles

Numerous methods exist for synthesizing nanomaterials, including the sol-gel method, co-precipitation, chemical reduction method and hydrothermal synthesis. However, the chemicals involved in these conventional methods are known to be harmful to the environment. As a result, green synthesis of nanomaterials has garnered significant attention in recent times due to its eco-friendly nature, cost-effectiveness, and simplicity. (Bhuiyan et al., 2020). Magnetite iron nanoparticles exhibit a considerably high surface-to-volume ratio at the nanoscale, leading to an increased adsorbent capacity. This unique feature promotes the efficient diffusion of metal ions onto the nanoparticle surface, facilitating effective adsorption of metal ions.(Devi, Julkapli, Sagadevan, & Johan, 2023). Green synthesis of nanoparticles utilizes plant extract as both reducing and capping agent eliminating the necessity of harmful reducing agents (Bhuiyan et al., 2020). Carica papaya belongs to the family of Caricaceae which can also use for synthesis of NPs (Jain et al., 2020).

1.10 Impact of GIONP on soil

The pH level in soil plays a significant role in the presence of heavy metals. The application of GION can lead to an alteration in pH. It can either decrease, increase or have no impacts on soil. The study by Lin et al. (2019) found that the pH level increased significantly ($p<0.05$) with increasing doses of GION. GION also affect nitrates and phosphates is soil. Another study suggested iron oxide nanoparticles had significant impact on the soil nitrification potential and ammonia oxidizing abundance, suggesting that the metabolic activity and efficiency of soil microorganisms can be influenced by iron oxide nanoparticle amendments.(Lin et al., 2019)

Microplastic pollution is a major environmental concern that has received significant attention in recent years. PSMPs, in particular, are prevalent in many ecosystems, including soil, and have been shown to have negative impacts on soil health and ecosystem functioning. Additionally, Cu contamination in soil is a common problem due to anthropogenic activities, such as agriculture and mining. This can lead to soil degradation and reduced plant growth. Iron oxide nanoparticles (IONPs) have been proposed as a potential solution for Cu remediation in soil due to their high adsorption capacity. However, there is a lack of research on the combined impact of PSMPs and IONPs on Cu-stressed soil. Therefore, the aim of this study is to investigate the potential interactive effects of PSMPs and green-synthesized IONPs on Cu-stressed soil, including their impacts on soil physicochemical properties and nutrients. The findings of this study will contribute to a better understanding of the environmental implications of microplastic pollution and provide valuable information for developing effective soil remediation strategies.

Table 2 Impact of NPs on soil properties

Soil	Nanoparticles	Heavy metal	Synthesis	Response of Soil	Reference
Nursery soil	GION	As	Euphorbia leaf extract	Decreased As in soil	Su et al., 2020
Heavy metal Contaminated soil	GION	Cd	Excoecaria leaves extract	Increased soil pH decreased nitrates and phosphates	Lin et al., 2019
Contaminates Soil	PSMPs and Ag NP	……	……..	Nitrates increased	Jiao et al., 2022

1.11 Research gaps

On a global scale, literature has extensively investigated the adsorption of organic pollutants and heavy metals by PSMPs (Godoy et al., 2019; Mao et al., 2020; Zhang et al., 2021; Zhang et al., 2022; Zong et al., 2021). Given the heightened global concern regarding the MPs pollution in aquatic environments, the first two studies conducted in Pakistan were also focused on the freshwater bodies while, research on MPs in Pakistan's soil is its nascent stage (Rafique, Irfan, Mumtaz, & Qadir, 2020).Consequently, it is imperative to investigate impact of MPS on Pakistan's soil. . Similarly, studies has also delved into the coexistence of MPs and NPs in soil system.(Jiao et al., 2022; Sun et al., 2023; Zhang, Ren, Pei, Sun, & Wang, 2022) however, the specific interaction of GION and PSMPs in the context of Cu stress within Pakistan remains unexplored. In the light of aforementioned gaps, the present study hypothesized that both MPs and GION will show greater propensity for adsorbing essential nutrients as compared to Cu in soil. As such, this study aimed to investigate impact of PSMPs and GION on soil physicochemical properties in the presence of Cu stress. Additionally, the study examines their impact on soil micro and macronutrients in pre-sowing and post-harvesting soil.

1.12 Problem statement

PSMPs, in particular, are prevalent in many ecosystems, including soil, and have been shown to have negative impacts on soil health and ecosystem functioning. Additionally, Cu contamination in soil is a common problem due to anthropogenic activities, such as agriculture and mining. This can lead to soil degradation. Iron oxide nanoparticles (IONPs) have been proposed as a potential solution for Cu remediation in soil due to their high adsorption capacity. However, there is a lack of research on the impact of GION on soil properties in the presence of PSMPs and Cu. Therefore, the aim of this study is to investigate the potential interactive effects of PSMPs and green-synthesized IONPs on Cu-stressed soil, including their impacts on soil physicochemical properties and nutrients. The findings of this study will contribute to a better understanding of the environmental implications of microplastic pollution and provide valuable information for developing effective soil remediation strategies.

1.13 Objectives

This study aims to investigate the impact of PSMPs and GIONPs on physicochemical properties and, nutrients in Cu-stressed soil.

1: Impact of PSMPs, green synthesized iron-oxide nanoparticles on physicochemical properties of Cu stressed soil in pre-sowing and post-harvest soil.

2: Effects of PSMPs, green synthesized iron-oxide nanoparticles on micronutrients and macronutrients and heavy metals in Cu contaminated soil

Chapter 2

Materials and Methods

2.1 Experimental Design and soil preparation

A pot experiment was conducted at Quaid-i-Azam University, Islamabad, (33°44′55-4″ N, 73° 08′ 09, 8″ E). The experimental design included 30 treatments (as shown in table 2), involving various doses of Cu (0, 50, 100, 200, and 400 mg/kg), PSMPs (0%, 1%, and 3%), and a constant dose of GION (100 ppm) incorporated into the soil. Experiment was conducted in replication n=3, resulting in a total of 90 pots in the experimental setup.

Soil samples were collected from the nursery of Quaid-i-Azam University, Islamabad, in March 2022. After collection was completed, soil samples underwent air-drying and were then sieved through a 2 mm mesh to eliminate gravel and to obtain a finer texture. Furthermore, after the air-drying and sieving process, 3% organic matter was incorporated into the soil to enhance its fertility and nutrient content. The collected soil sample displayed following physicochemical properties: a coarse fraction of 54%, a micro-aggregate fraction of 23%, and a non-aggregate fraction of 23% (not known). The soil's water holding capacity calculated to be 500 mL/L. In addition, the initial physicochemical characteristics of the soil were recorded down, which included a pH of 8.6, electrical conductivity (EC) of 435 µScm-1, and concentrations of Cu (14.9±0.0 mg/kg), Fe (11538±288 mg/kg), Zn (49.1±1.0 mg/kg), Mn (474±17.5 mg/kg), NO^{3-} (27.3 ±0.81 mg/kg), and PO_4^{3-} (36.6±0.89). Pre-sowing soil samples were collected after 14 days of incubation. After collecting the pre-sowing soil samples, maize plants were cultivated in pots for 60 days. Soil samples were collected to obtain post-harvesting samples for further analysis

Table 2.1. Treatments used in the pot-experiment.

Treatment Id	**Treatments**
T_1	Cu 0
T_2	Cu 50
T_3	Cu 100
T_4	Cu 200
T_5	Cu 400
T_6	NP +Cu0
T_7	NP+ Cu 50
T_8	NP +Cu100
T_9	NP+ Cu 200
T_{10}	NP+ Cu 400
T_{11}	1% PS + Cu 0
T_{12}	1% PS + Cu 50
T_{13}	1% PS + Cu 100
T_{14}	1% PS + Cu 200
T_{15}	1% PS + Cu 400
T_{16}	1% PS + Cu 0 + NP
T_{17}	1% PS + Cu 50 + NP
T_{18}	1% PS + Cu 100 + NP
T_{19}	1% PS + Cu 200 + NP
T_{20}	1% PS + Cu 400 +NP
T_{21}	3% PS + Cu 0
T_{22}	3% PS + Cu 50
T_{23}	3% PS + Cu 100
T_{24}	3% PS + Cu 200
T_{25}	3% PS + Cu 400
T_{26}	3% PS + Cu 0 + NP
T_{27}	3% PS + Cu 50 + NP
T_{28}	3% PS + Cu 100 + NP
T_{29}	3%PS+Cu200 +NP
T_{30}	3%PS+Cu400+NP

2.2 MPs Preparation, Cu spiking and GION preparation

PS pellets were purchased from Pak Petrochemical Industries Pvt Ltd. MPs were prepared by grinding these pallets again and again in the grinder till their size reaches the range of 150-250um. After grinding MPs were sieved in stainless steel sieve of 150-250um. (Lozano et al., 2021). The Cu solution was prepared using Cu salt ($CuSO_4.5H_2O$). A stock solution of 1000 ppm was prepared. CuSO4. 5H2O (3.92 g) of salt was used to make 1000 ppm solution. It was further diluted to 50ppm, 100ppm, 200 ppm and 400 ppm.

Papaya plant (Carica papaya) leaves were gathered from the premises of Quaid I azam university. These freshly collected leaves underwent a rigorous washing process with both tap water and deionized water. Afterward, they were subjected to drying in an oven for an hour and subsequently ground into a fine powder. To prepare an extract, 20 grams of this finely powdered material were boiled in 1 liter of deionized water at 80°C for 30 minutes. The resulting mixture was then filtered using Whatman no 42 filter paper to remove any solid particles. The filtrate was concentrated using a rotary evaporator and finally stored at 40°C for future applications.

The synthesis of α-Fe2O3 nanoparticles utilized ferric chloride hexahydrate (FeCl3.6H2O) as the precursor. In a 1:1 ratio, 50 mL of papaya leaf extract was cautiously added drop by drop to 50 mL of a 0.1M FeCl3.6H2O solution at room temperature. Subsequently, 1 M NaOH was incrementally introduced until the pH reached 11. The resulting mixture was stirred using a magnetic stirrer for 30 minutes, and the formation of an intensely black-colored solution confirmed the synthesis of iron oxide nanoparticles. The nanoparticles were separated by centrifugation at 8000 rpm for 20 minutes and underwent a series of purification steps, including multiple washings with ethanol and water (2-3 times). Finally, the nanoparticles were dried in a hot air oven at 80°C for 3 hours and securely stored in an airtight container for future use.

2.3 Physicochemical Analysis

Soil physicochemical properties were analyzed based on the procedures described by (brown et al, 2023). pH and EC were measured using multimeter (OAKTON). Following the procedure, 5 g of air-dried soil was added to a beaker containing 25 mL of deionized water. The beaker was placed on a shaker at 180rpm for 30 minutes for complete mixing. Afterward, values of pH and EC were measured with calibrated pH and EC meter. Water holding capacity was determined following procedure by (Sun et al., 2012). While, the soil samples were passed through a series of sieves of decreasing size to get desirable soil fraction (250-2000µm, 53-250 µm and <53µm).

2.4 Micronutrients and heavy metals analysis

Micronutrients were analyzed using method of (Ahmedpour et al., 2015). Extracting solution (aquaregia) was prepared by using HCl and HNO^3 (1:3) v/v for digestion. Samples containing 5 g of sieved and air-dried soil were placed in an Erlenmeyer flask and 10ml of the extracting solution was added to each sample. Afterward, the samples were heated on a hot plate for half an hour at 90-100 c^0. The resulting solution was filtered through a Whatman No 42 filter paper into a 50 mL polypropylene vial and diluted to 50 mL with the extracting solution. A Blank was also prepared containing extracting solution. Micronutrients (Cu, Zn, Mn, Fe, Pb, and Cd) were analysed in all the soil samples using Atomic Absorption spectrophotometry.

Macronutrients analysis

Soil nitrates

Soil nitrates were analyzed using the standard chromotropic acid method (Estefan et al., 2013). Approximately, 1 g of air-dried soil was weighed and mixed with 50 mL 0.02 N $CuSO_4.5H_2O$ solution and each sample was then shaken for 15 minutes using an orbital shaker at 100rpm. The solution was filtered after mixing using Whatman No 42 filter paper and 3 ml filtrate was taken into a 50 ml conical flask using a pipette. Chromotropic acid (1ml 0.1%) was added drop by drop into the filtrate and placed on the ice bath. After mixing, 6ml concentrated sulphuric acid was added and the solution was swirled. The prepared solution was left for shaking to cool down to avoid excessive heating. After 45 minutes yellow colour was formed, and absorbance was measured at 430nm using a UV spectrometer. Afterward, a blank was prepared to contain all the above-mentioned chemicals excluding soil. Standards of NO_3, using KNO_3 dissolved in 0.02N $CuSO_4.5H_2O$ were also prepared to check the calibration.

2.5.2 Extractable Phosphates

Extractable phosphorus was measured by the method described in (Estefan et al., 2013). Briefly, 1 g of sieved air-dried soil was taken and mixed with 10 ml 0.5M $NaHCO_3$.Each sample was placed on orbital shaker for complete mixing for 30 minutes at 150rpm. Afterwards, samples were filtered through filter paper in a beaker and 0.25% w/v p-nitro phenol indictor was added drop by drop in to 5ml filtrate. Moreover, H_2SO_4 were also added into each sample until the samples became colourless. Furthermore, deionized water was used for the dilution of the samples to 40ml and after dilution 4ml of ascorbic acid was added each. A blank containing all the above-mentioned chemicals excluding soil was also prepared. Absorbance for the solution was taken on 882 nm using UV spectrometer.

Chapter 3

Results and Discussions

3.1 Soil physicochemical properties

The soil pH decreased (8.6 to 8.4) as the rate of Cu increased over control in both pre-sowing and post-harvesting soil. The effect was more pronounced at Cu 400mg/kg as shown in Figure 3.1. These results are in line with previous studies (Marques et al., 2023; Vázquez-Blanco, Arias-Estévez, Bååth, & Fernández-Calviño, 2021). The decrease in soil pH with Cu might be due to a change in soil microbial activity at higher Cu concentrations (Naz et al., 2022; Yáñez et al., 2022). Contrary, the soil pH increased with addition of 0%, 1% and 3% PSMPs in soil as shown in Figure 3.1. Adding PET fragments and PS foam leads to an increase in soil pH. (T. Zhao et al., 2021). Reason is expected to change soil microbial community structure because of the MPs, which indirectly affect soil pH (Rong et al., 2021). Additionally, the surface characteristics of MPs, including their surface charges, can lead to the selective adsorption of positively or negatively charged substances, which can disrupt ion exchange in soil solution and contribute to changes in soil pH (Rong et al., 2021). Addition of GION showed similar trends with soil pH as of PSMPs in both pre-sowing and post-harvest soil. These results are in line with previous studies, GION extracted from excoecaria cochinchinensis leaves increased soil pH (Lin et al., 2019; Su, Lin, Owens, & Chen, 2020). The increase in pH by the addition of GION can be attributed to Fe (II) hydrolysis. The Hydrolysis reaction involves utilizing H^+ ions and releasing OH- ions. As a consequence of these hydrolysis reactions, H^+ ions are consumed, reducing their concentration, while OH^- ions are released, thereby increasing the pH of the solution (Lin et al., 2019). In post-harvesting soil after cultivation of maize plant and incubation of 60 days the overall pH of soil increased, as compared to pre-sowing soil, with the addition of Cu, PSMPs and GION, as shown in Figure 3.1. These results are in line with previous research (M. H. Saleem et al., 2020). One possible reason can be the increase in uptake of anions and discharge of hydroxyl ions from plant roots (Rehman et al., 2021).The addition of 3%PSMPs showed greater increase among all the treatments. The research has showed shape and dose-dependent response of different MPs reflecting more prominent impact at higher doses (Feng, Wang, Sun, Zhang, & Wang, 2022). The present study revealed a gradual increase in EC in pre-sowing soil while it was decreased in post-harvesting soil with increasing Cu treatments. The increase in EC might be due to greater charged species by Cu addition (Romdhane et al., 2021). However, the addition of PSMPs reduced EC in both pre-sowing and post-harvesting soils as shown in Figure 3.1. This decrease in EC might be due to the adsorptive nature and large surface area of PSMPs which adsorbs nutrients and reduces the concentration of free ions in soil solution (Godoy et al., 2019).The significance difference between pre-sowing soil and post-harvesting soil is shown in T-test as mentioned in S table 1.

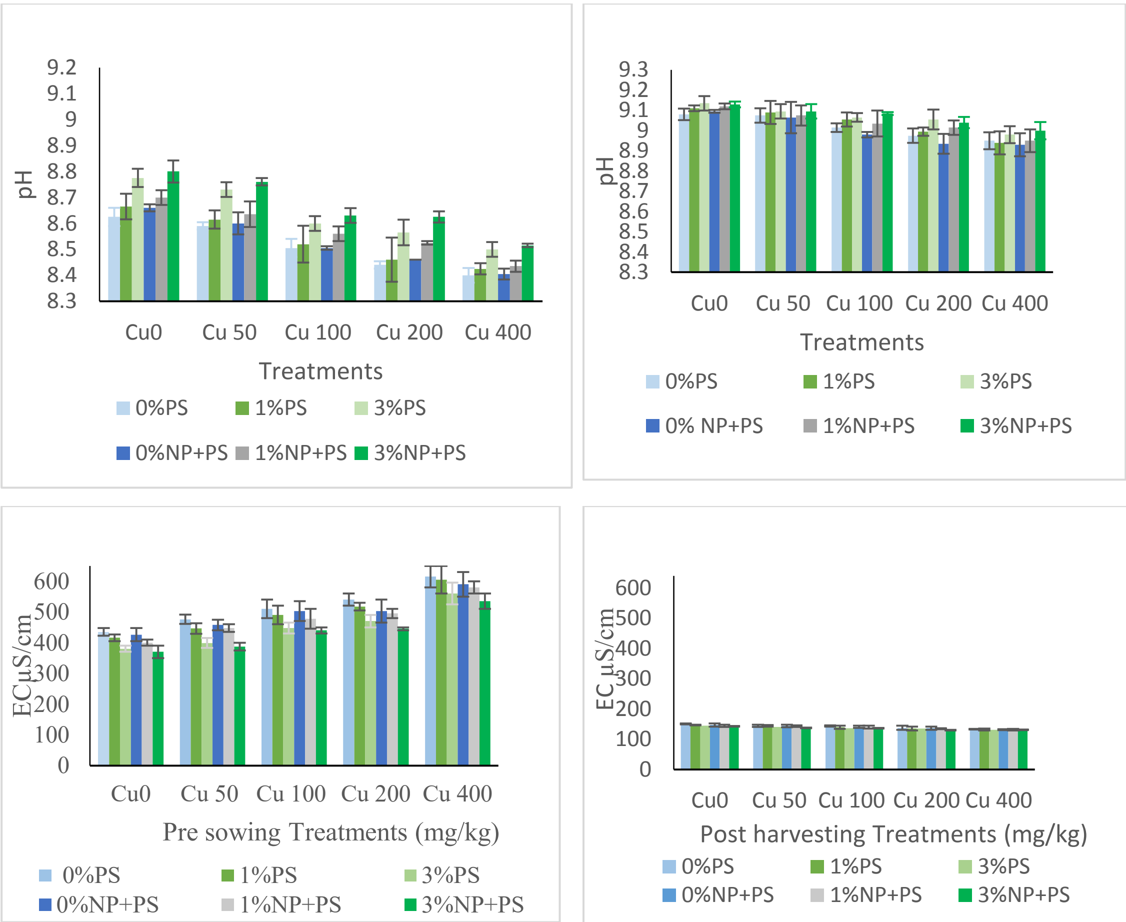


Figure 3.1. Impact of treatments on soil pH and EC. a) pH in pre-sowing soil b) pH in post-harvesting soil c)EC in pre-sowing soil d) EC in post-harvesting soil. Data in graph is an average of three repeats (n=3) ± standard deviation (SD).

3.2 Change in soil fractions by PSMPs

The present study found that 250-2000 µm (coarse-particulate fraction), 53-250 µm (micro-aggregate fraction), and <53 µm (non-aggregated silt and clay fraction) were 54%, 23%, and 23% respectively in control (Table 3.1). The addition of 1% and 3% PSMPs increased the relative proportion of the micro-aggregate fraction possibly due to the size of PSMPs within the range of the micro-aggregate fraction and reducing the relative fraction of silt and clay in the soil thus changing the chemical speciation of heavy metals (Yu et al., 2021).

Table 3.1. Relative change in soil fractions under different concentrations of PSMPs. Data tabulated is average of three repeats (n=3) ± standard deviation (SD).

Control	% Coarse-particulate fraction	% Micro-aggregate Fraction	% Non-aggregated Fraction
0%PSMPS	54 ± 5.7	23 ± 1.4	23 ± 4.2
1%PSMPS	55 ± 7.1	25 ± 4.8	20 ± 5.1
3%PSMPS	52 ± 5.7	34 ± 2.8	14 ± 2.8

3.3 Soil Micronutrients Content

Micronutrients in soil decreased significantly in post-harvesting soil as compared to pre-sowing soil as shown in Figure 3.2. The concentration of Cu reduced in post-harvesting soil by addition of Cu doses. The reduction was greater at Cu 400 mg/kg showed dose-dependent response of Cu in post-harvest soil. It reduced by 21%, 24.8%, 27.6%, 29.2%, and 30.2% from Cu0 to Cu400 mg/Kg respectively. The addition of 1%PSMPs and 3%PSMPs declined Cu by 16%, 21.6%, 24.4%, 25.9%, 27.8%, and 12.6%, 16.5%, 19.9%, 23.2%, 25% from Cu0 to Cu400 mg/Kg respectively. The addition of 3%PSMPs from Cu0 to Cu400mg/kg showed greater retention and less reduction of Cu as compared to 0% or 1% PSMPs in post-harvesting soil. Similarly, GION reduced Cu by 16 %, 23 %, 25%, 28% and 28% from Cu0 to Cu400mg/kg in post-harvesting soil. Combine addition of GION, 1%PSMPs and GION, 3%PSMPs showed decline by 14%, 20%, 20 %, 24%, 26% and

11 %, 20 %, 19%, 21%, 24 % from Cu0 to Cu400 mg/kg respectively. It has shown that GION along with 3%PSMPs from Cu0 to Cu400 mg/kg showed lesser reduction and greater adsorption.

Zn, Mn and Fe showed similar trends as of Cu in post-harvesting soil. There was a significant decrease in Zn, Mn and Fe concentrations. Zn was reduced by 20.1%, 25.1%, 29.4%,

35.2%, and 38.1% from Cu0 to Cu400 mg/Kg respectively. The addition of 1%PSMPs reduced Zn by 15.2%, 19%, 20.8%, 25.6%, 30.6%, and 3%PSMPs reduced Zn by 11.6%, 12.8%, 13.6%, 22.7%, 23.3% from Cu0 to Cu400 mg/kg respectively. Similarly, GION reduced Zn in post-harvesting soil by 19 %, 23%, 27%, 32%, and 35 % from Cu0 to Cu400 mg/kg. Addition of GION along with 1% PSMPs reduced Zn by 13% , 13% , 17%, 25%, and 25% from Cu0 to Cu400 mg/kg, while, GION and 3% reduced Zn by 10%,11%,20 %20 %, and 22 % from Cu0 to Cu400mg/kg. The addition of 3%PSMPs from Cu0 to Cu400mg/kg showed greater adsorption and less reduction of Cu as compared to 0% or 1% PSMPs in post-harvesting soil. Similarly, GION along with 3%PSMPs from Cu0 to Cu400 mg/kg showed lesser reduction and greater adsorption than all the treatments in post-harvesting soil. The significance difference in micronutrients in pre-sowing and post-harvesting soil is shown in S table 1 using T-test.

The solubility of most micronutrients will decrease, leading to low concentrations in soil solution with increasing soil pH. This might be due to the increased bioavailability of micronutrients as a result of low soil pH in alkaline soils by Cu addition (Liu et al., 2021; Romdhane et al., 2021). Furthermore, a decrease in soil pH can enhance the soil cation exchange capacity (CEC) since it has a negative correlation with pH (Rahal & Alhumairi, 2019; Wen et al., 2022).

PSMPs showed lesser reduction as compared to Cu, it can be due to the fact that an increase in pH enhanced adsorption of cations (Khalid, Aqeel, Noman, Khan, & Akhter, 2021). The adsorption behavior is promoted between negatively charged MPs and positively charged metal ions through electrostatic attraction. (Torres, Dioses-Salinas, Pizarro-Ortega, & De-la-Torre, 2021). PS (aromatic polymer) exhibits mainly π–π interactions, However, occurrence depends on the polymer type; e.g., PE (aliphatic polymer) exhibits van der Waals interactions (Liu et al., 2021). Moreover, functional groups also play their role in adsorption, for instance, Carboxyl and carbonyl groups were observed on the surface of the aged PS. As both groups have high electron density, the adsorption capacity of aged PS towards Cu^{2+} increased (Chen et al., 2022).

GION showed greater adsorption and lesser reduction of micronutrients in post-harvesting soil can be due to the fact that green synthesized nanoparticles provide more surface charges hence, greater adsorption (Lin et al., 2019). GNPs has capability to decrease nutrient losses during fertilization or leaching (S. Saleem & Khan, 2023). Additionally, on a nanoscale, magnetite iron nanoparticles have a higher surface to volume ratio, which leads to an increased adsorbent capacity. This attribute leads to better metal ion diffusion on the adsorbent surface, leading to better metal ion adsorption (Devi, Julkapli, Sagadevan, & Johan, 2023). This effect was more prominent with the addition of 3%PSMPs and GION from Cu0 to Cu400mg/kg in post harvesting soil as both of components has active sites and adsorbing capacity.

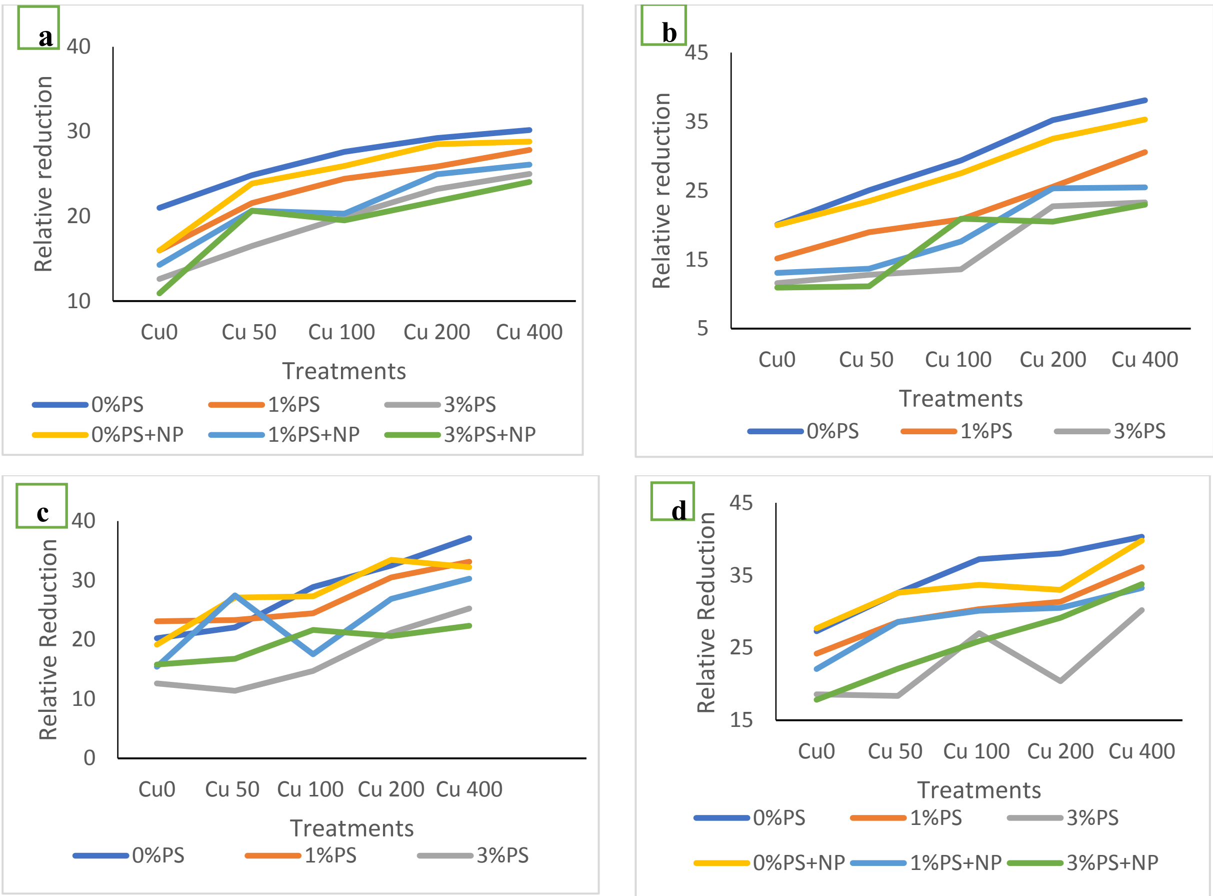


Figure 3.2. Micronutrient concentrations in soil with different treatments.(a) Relative reduction of Cu in post-harvesting soil, (b) Relative reduction of Zn in post-harvesting soil, (c) Relative reduction of fe in post-harvesting soil (d) Mn in post-harvesting soil. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD).

3.4 Soil macronutrients

In our study nitrates and phosphates decreased significantly in pre and post-harvesting soil with the addition of Cu0 to Cu400mg/kg as shown in Fig 3.3 and 3.4.The significance difference in shown in S table 1 using T-test as well. As Cu increased over control in Screen house experiments and laboratory significant decrease in soil available P was observed. The effect was more pronounced at application rate above 20 mg Cu kg^{-1} (Azeez, Adesanwo, & Adepetu, 2015). The reduction in available phosphates can due to the antagonistic relation between Cu and phosphates.

In post harvesting soil addition of PSMPs also reduced concentration of nitrates and phosphates from Cu0 to Cu400mg/kg as shown in Fig 3.3 and 3.4. MPs can play a major role in impacting soil nutrients by influencing microbial activity and communities, for example PSMPS can slow down phosphate and urease enzymatic activities which consequently reduce bioavailability of phosphates and nitrates in soil (Dong, Gao, Qiu, & Song, 2021). The studies of Wang et al., 2023 is also in line with this study. Thy study showed that an increase of PS decreased nitrates and phosphates content in soil.

Similarly, GION decreased the concentration of Nitrates and phosphates in pre and post-harvesting soil. GION to the soil could potentially influence microbial populations. Some studies have suggested that iron oxide nanoparticles might impact the microbial community, altering their metabolic activities resulting in decline in NO_3- and PO_4^3 $_4$ i n soil (Lin et al., 2019).

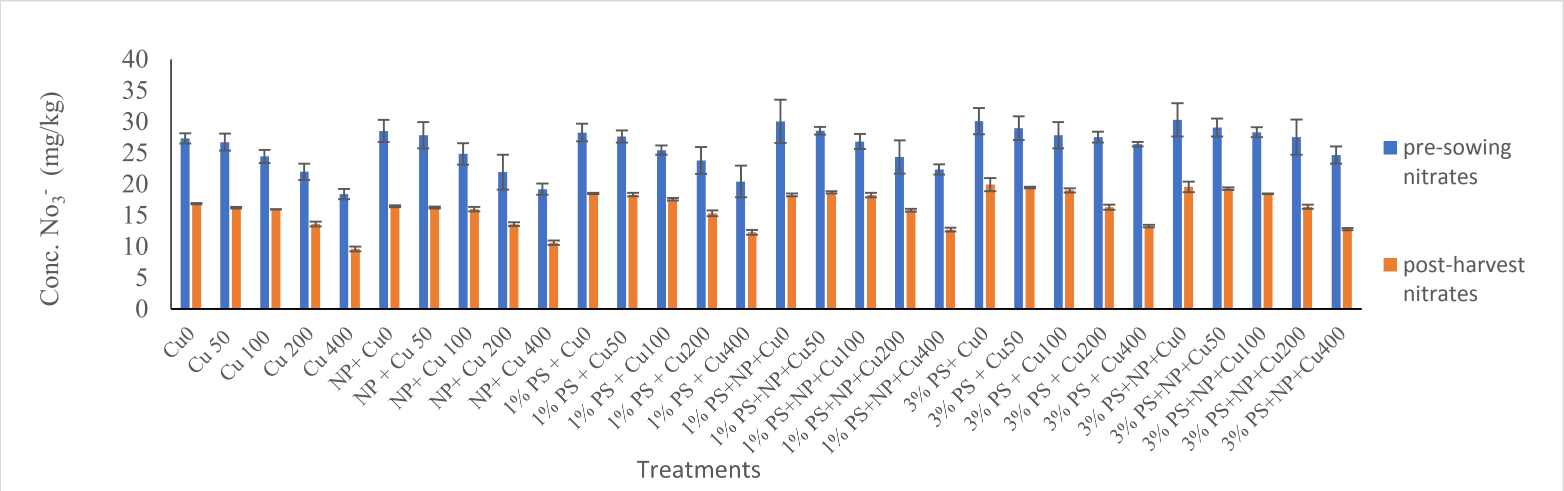


Figure 3.3 Representing No_3^- Conc. In pre-sowing and post-harvesting soil. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD).

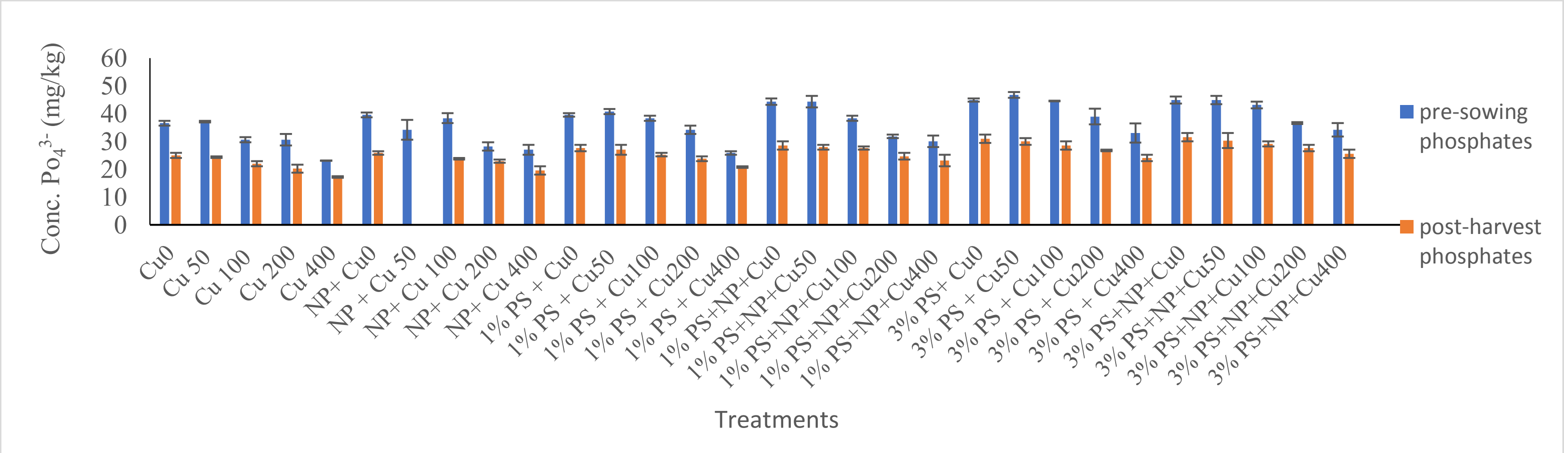


Figure 3.4 Representing Po_4^{3-} Conc. In pre-sowing and post-harvesting soil. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD).

3.4 Heavy Metals

In our study Cd and Pb decreased significantly in post-harvesting soil with the addition of Cu0 to Cu400mg/kg as shown in Fig 3.5 and 3.6.

In post harvesting soil addition of PSMPs also reduced concentration of Pb and Cd from Cu0 to Cu400mg/kg as shown in Fig 3.5 and 3.6. In their study, Gao et al. (2019a) demonstrated that polystyrene microplastics (PSMPs) exhibited the capacity to adsorb various heavy metals, including lead (Pb), copper (Cu), and cadmium (Cd). Interestingly, it was observed that as the pH of the solution rose, there was a substantial and rapid increase in the adsorption capacity of PSMPs. This phenomenon is believed to be attributable to electrostatic interactions, as suggested by the findings of (Yu et al., 2021).

At higher pH levels, there is a tendency for greater adsorption of positively charged cadmium (Cd) onto the surfaces of GION. This can be attributed to the increased negative charge on the GION surfaces. Consequently, this effect is expected to diminish the bioavailability of Cd, as noted in the research conducted by (Lin et al., 2019). The increase in negative charge on the GION surfaces at higher pH levels can be explained by the behavior of surface functional groups. These functional groups on the GION surfaces can undergo ionization, resulting in the creation of negatively charged sites. As the pH rises, the prevalence of hydroxide ions (OH-) in the solution also increases, facilitating the deprotonation of these functional groups. Consequently, the surfaces of GION become more negatively charged (Tao et al., 2023).

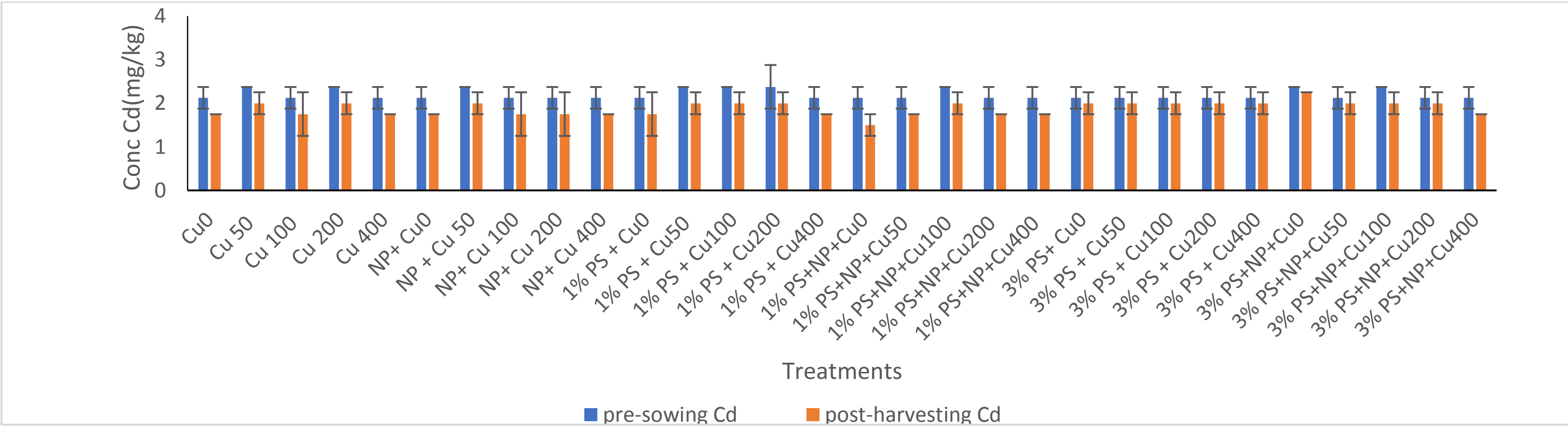


Figure 3.5. Representing Cd Conc. In pre-sowing and post-harvesting soil. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD).

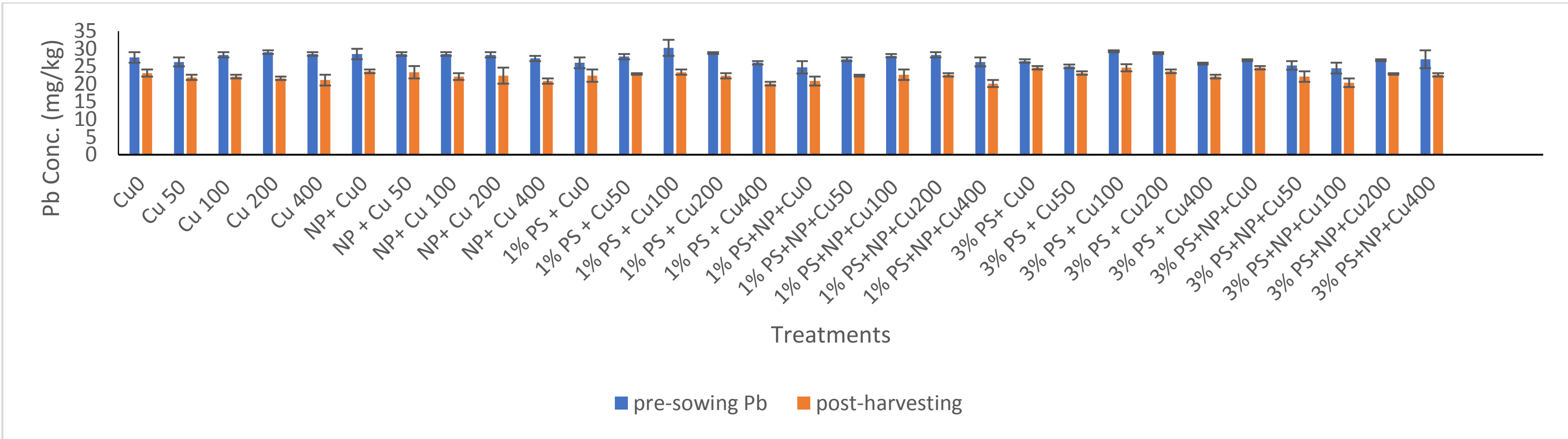
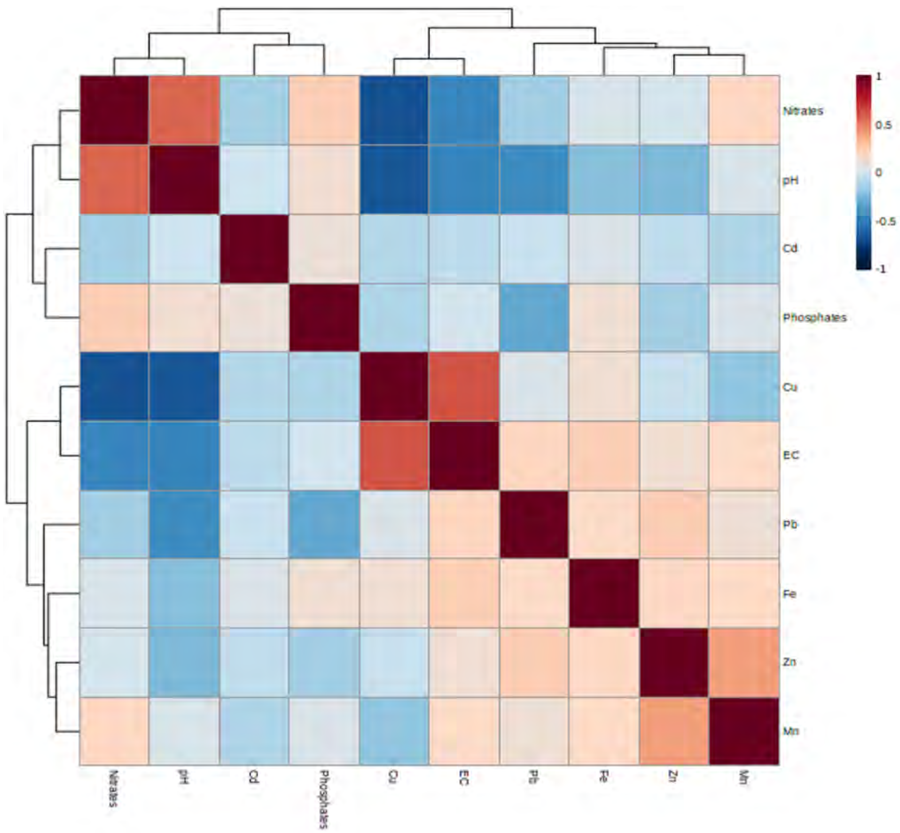


Figure 1.6. Representing Pb Conc. In pre-sowing and post-harvesting soil. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD).

3.5 StatisticalAnalysis



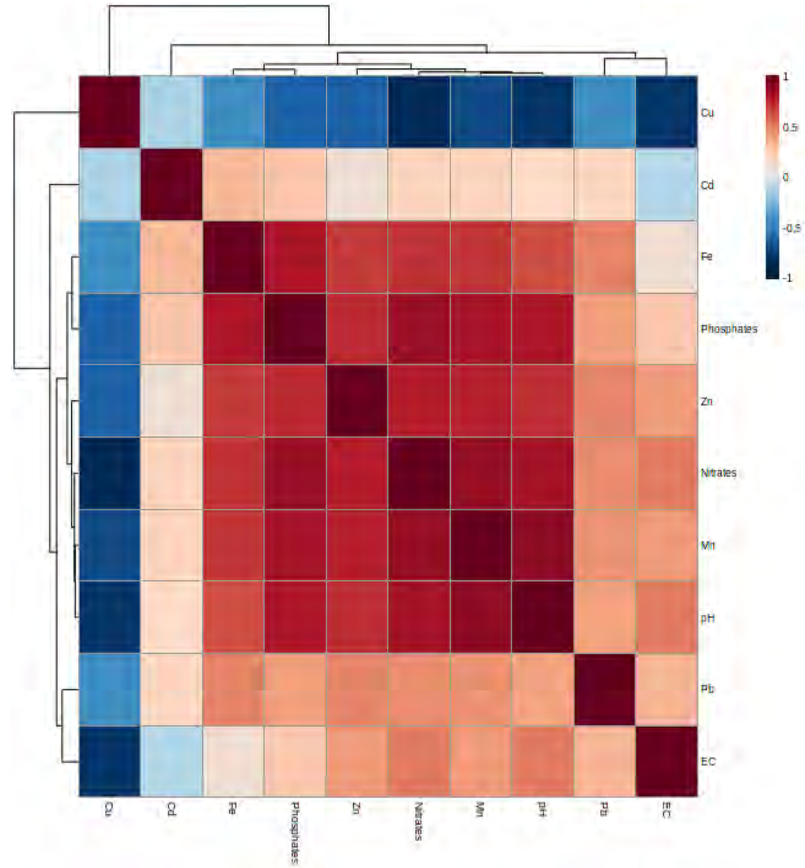


Figure 3.7. Heat map of Pearson correlation between different soil variables in pre-sowing and post-harvesting soil

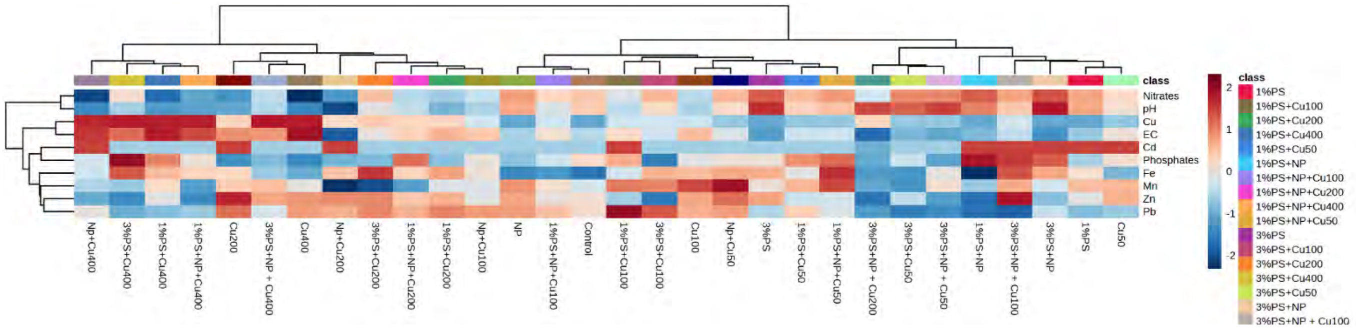


Figure 3.8. Hierarchical cluster analysis between different soil parameters and treatments in pre-sowing soil

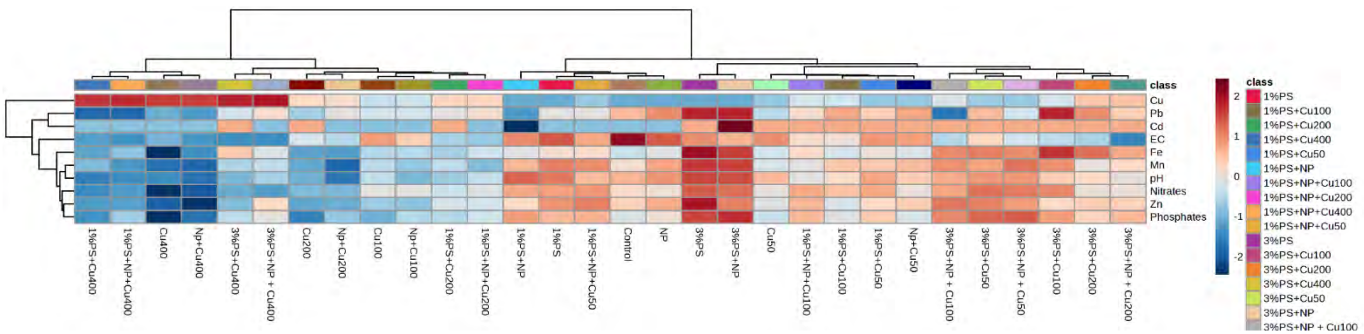


Figure 3.9. Hierarchical cluster analysis between different soil parameters and treatments in post-harvest soil

3.6 Conclusions

- It was concluded that in the study copper (Cu) had an antagonistic relationship with soil pH and a positive correlation with soil electrical conductivity (EC) in pre-sowing soil, and an antagonistic relationship with soil pH, EC, and other micronutrients in post-harvesting soil.
- PSMPs showed a positive correlation with soil pH (and micronutrients in post-harvesting soil) and negatively correlate with EC in both soils.
- Green-synthesized iron oxide nanoparticles (GION) and polystyrene microplastics (PSMPs) demonstrated significant positive effects on soil pH
- GION and PSMPs displayed superior nutrient adsorption compared to copper stress, highlighting their nutrient retention capacity.

3.7 Recommendations

- Consider integrating GION and PSMPs as soil amendments in copper-stressed agricultural systems.
- Conduct comprehensive field trials to validate the efficacy of GION and PSMPs in real-world agricultural scenarios, considering varying soil types, crops, and climatic conditions.
- Undertake long-term studies to assess the prolonged effects of GION and PSMPs on soil health, nutrient availability, and overall crop performance over multiple growing seasons.

Supplementary Data

Correlation analysis

Pre-sowing soil

mg/Kg	**Cu**	**Fe**	**Mn**	**Zn**	**Pb**	**Cd**	**NO_3-**	**PO_4^{3-}**	**pH**	**EC**
Cu	1									
Fe	0.116	1								
Mn	-0.33	0.06	1							
Zn	-0.03	0.17	0.15	1						
Pb	0.03	0.09	0.17	0.27	1					
Cd	-0.32	0.06	0.36	0.29	0.21	1				
NO3-	-0.59**	-0.01	0.21	-0.16	-0.34	0.11	1			
PO_4^{3-}	-0.66**	-0.07	0.22	-0.18	-0.29	0.18	0.93**	1		
pH	-0.60**	-0.11	0.14	-0.18	-0.44*	0.03	0.93**	0.91**	1	
EC µS/cm	0.69**	0.01	-0.13	0.09	0.28	-0.11	-0.93**	-0.94**	-0.96**	1

Post-Harvesting soil

	Cu	**Fe**	**Mn**	**Zn**	**Pb**	**Cd**	**NO_3-**	**PO_4^{3-}**	**pH**	**EC**
Cu	1									
Fe	-0.48**	1								
Mn	-0.74**	0.83**	1							
Zn	-0.710**	0.83**	0.92**	1						
Pb	-0.56**	0.64**	0.57**	0.59**	1	1				
Cd	-0.19	0.57**	0.41*	0.38*	0.50**	0.39*				
NO3-	-0.85**	0.81**	0.90**	0.89**	0.57**	0.41*	1			
PO_4^{3-}	-0.64**	0.91**	0.89**	0.92**	0.55**	0.32	0.90**	1		
pH	-0.84**	0.75**	0.94**	0.81**	0.56**	-0.04	0.89**	0.85**	1	
EC µS/cm	-0.81**	0.22	0.56**	0.52**	0.31*	1	0.61**	0.36*	0.69**	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 2: T-Test performed to analyse the impact of Cu, PSMPs and GIONPs on soil physicochemical properties and nutrients in pre-sowing and post-harvest soil

Pre-sowing soil										
Treatments	Cu(mg/kg)	Fe(mg/kg)	Zn(mg/kg)	Mn(mg/kg)	Pb(mg/kg)	Cd(mg/kg)	$N0_3$- (mg/kg)	$PO_4{}^{3-}$ (mg/kg)	pH	EC(μS/cm)
Cu0	14.9±0	11538.1±287.5	49.1±1	474±17.5	27.5±1.5	2.1±0.25	27.3±0.81	37.6±0.89	8.6±0.025	434.5±12.5
Cu50	59.9±5	11938.1±612.5	50.4±1.75	496.5±5	26.25±1.25	2.4±0	26.7±1.38	36.2±0.30	8.6±0.01	476±15
Cu100	117.4±7.5	12763.1±262.5	50.6±1	519±7.5	28.25±0.75	2.1±0.25	24.4±1.05	31.7±0.89	8.5±0.025	510±30
Cu200	204.9±5	12613.1±112.5	52.9±0.75	491.5±20	29±0.5	2.4±0	22.0±1.30	28.7±2.08	8.4±0.01	540±20
Cu400	397.4±17.5	11875.6±650	50.9±2.75	479±2.5	28.5±0.5	2.1±0.25	18.4±0.81	23.1±0.09	8.4±0.02	615±35
NP+ Cu0	14.9±0	11663.1±137.5	50.6±4.5	504±22.5	28.5±1.5	2.1±0.25	28.5±1.78	39.6±0.89	8.7±0.01	426±21
NP + Cu 50	62.4±2.5	12863.1±112.5	51.6±1.5	531.5±45	28.5±0.5	2.4±0	27.8±2.11	34.2±3.75	8.6±0.03	457.5±17.5
NP+ Cu 100	124.9±5	12500.6±75	48.6±0	476.5±0	28.5±0.5	2.1±0.25	24.8±1.70	38.4±.79	8.5±0.005	502.5±32.5
NP+ Cu 200	192.4±7.5	12725.6±200	51.1±1.5	419±2.5	28.25±0.75	2.1±0.25	21.9±2.80	28.3±1.49	8.5±0	502.5±37.5
NP+ Cu 400	389.9±10	12300.6±700	46.4±2.25	466.5±0	27.25±0.75	2.1±0.25	19.2±0.89	27.1±1.79	8.4±0.015	590±40
1% PS + Cu0	14.9±0	12538.1±37.5	48.6±1	494±32.5	26±1.5	2.1±0.25	28.3±1.42	39.6±0.60	8.7±0.035	416±11
1% PS + Cu50	57.4±2.5	12438.1±312.5	48.1±2.5	496.5±30	27.7±0.75	2.4±0	27.6±0.97	40.8±0.89	8.6±0.025	446±17
1% PS + Cu100	122.4±2.5	12325.6±475	47.4±0.25	509±57.5	30.25±2.25	2.4±0	25.4±0.73	38.4±0.89	8.5±0.05	490±30

1% PS + Cu200	202.4±2.5	12900.6±25	50.4±1.25	476.5±0	28.75±0.25	2.4±0.5	23.8±2.15	34.2±1.49	8.5±0.06	517.5±12.5
1% PS + Cu400	394.9±5	12775.6±225	48.6±0.5	486.5±5	26±0.5	2.1±0.25	20.4±2.55	25.9±0.60	8.4±0.015	605±45
1% PS+NP+Cu0	14.9±0	11150.6±200	44.9±1.75	461.5±25	24.75±1.75	2.1±0.25	30.0±3.45	44.3±1.19	8.7±0.02	400±10
1% PS+NP+Cu50	59.9±5	13375.6±375	48.4±0.25	519±57.5	27±0.5	2.1±0.25	28.6±0.61	44.3±2.08	8.6±0.035	447.5±12.5
1% PS+NP+Cu100	122.4±2.5	11750.6±525	48.9±1.75	484±12.5	28±0.5	2.4±0	26.8±1.22	38.4±0.89	8.6±0.02	477.5±32.5
1% PS+NP+Cu200	199.9±10	12650.6±50	49.9±4.25	449±27.5	28.25±0.75	2.1±0.25	24.3±2.68	31.8±0.60	8.5±0.005	495±15
1% PS+NP+Cu400	392.4±7.5	12638.1±637.5	45.6±0.5	449±12.5	26.25±1.25	2.1±0.25	22.4±0.81	30.1±2.08	8.4±0.015	580±20
3% PS+ Cu0	14.9±0	12838.1±912.5	50.6±7	481.5±70	26.5±0.5	2.1±0.25	30.1±2.11	44.9±0.60	8.8±0.025	380±10
3% PS + Cu50	59.9±5	11750.6±325	47.9±0.25	446.5±10	25±0.5	2.1±0.25	29.0±1.90	46.7±1.04	8.7±0.02	399±16
3% PS + Cu100	124.9±0	12813.1±187.5	45.1±0.5	506.5±0	29.25±0.25	2.1±0.25	27.8±2.11	44.6±0.12	8.6±0.02	447.5±17.5
3% PS + Cu200	214.9±5	13350.6±850	51.1±2	426.5±10	28.75±0.25	2.1±0.25	27.5±0.89	39.0±2.83	8.6±0.035	470±20
3% PS + Cu400	399.9±5	13213.1±662.5	45.6±1	461.5±5	25.75±0.25	2.1±0.25	26.4±0.36	33.0±3.45	8.5±0.02	560±35

3% PS+NP+Cu0	14.9±0	12875.6±150	46.9±1.75	474±2.5	26.75±0.25	2.4±0	30.3±2.68	44.9±1.28	8.8±0.03	370±20
3% PS+NP+Cu50	59.9±0	12438.1±562.5	46.1±0	484±2.5	25.25±1.25	2.1±0.25	29.1±1.42	44.9±1.49	8.8±0.01	387.5±12.5
3% PS+NP+Cu100	127.4±7.5	13225.6±475	53.1±4	499±67.5	24.5±1.5	2.4±0	28.3±0.81	43.2±1.19	8.6±0.02	440±10
3% PS+NP+Cu200	217.4±10	12775.6±225	49.4±2.25	491.5±10	26.75±0.25	2.1±0.25	27.5±2.84	36.6±0.33	8.6±0.015	445±5
3% PS+NP+Cu400	404.9±0	12063.1±462.5	50.6±2	494±27.5	27±2.5	2.1±0.25	24.6±1.38	34.2±2.44	8.5±0.005	535±25

Post-harvest soil

Treatments	Cu(mg/kg)	Fe(mg/kg)	Zn(mg/kg)	Mn(mg/kg)	Pb(mg/kg)	Cd(mg/kg)	NO_3- (mg/kg)	PO_4 $^{3-}$ (mg/kg	pH	EC(µS/cm)
Cu0	11.8±0.75	9204.5±662.5	39.25±1	344.6±11.2	23.1±	1.75±0.00	16.8±0.09	25.0±0.8	9.1±0.02	150.55±1.3
Cu 50	45±5	9304.5±537.5	37.75±1.75	334.6±3.75	21.85±	2±0.25	16.2±0.12	24.4±0.29	9.05±0.02	144±3.6
Cu 100	85±10	9079.5±137.5	35.8±1	325.9±12.5	22.1±	1.75±0.50	16.0±0.02	22.0±1.48	9.03±0.01	143.4±2.2
Cu 200	145±5	8517±150	34.3±0.75	304.6±8.75	21.6±	2±0.25	13.6±0.39	20.2±0.29	9±0.02	137.45±6.9
Cu 400	277.5±7.5	7467±100	31.5±2.75	285.9±10	21.1±	1.75±0.00	9.6±0.37	17.3±0.8	8.92±0.03	133±1.1
NP+ Cu0	12.5±0.5	9429.5±837.5	40.5±4.5	364.6±8.75	23.6±	1.75±0.00	16.4±0.14	25.9±0.2	9.1±0.00	146.3±5.4
NP + Cu 50	47.5±7.5	9379.5±662.5	39.5±1.5	358.4±10	23.35±	2±0.25	16.2±0.37	25.0±0.89	9.01±0.05	143.25±4.1

NP+ Cu 100	92.5±2.5	9092±750	35.3±0	315.9±5	22.1±	1.75±0.50	16.0±0.29	23.8±1.48	8.97±0.01	140.7±3.5
NP+ Cu 200	137.5±7.5	8467±400	34.5±1.5	280.9±7.5	22.35±	1.75±0.50	13.6±0.37	22.9±0.29	8.97±0.03	135.75±5.0
NP+ Cu 400	277.5±2.5	8342±250	30±1.5	280.9±7.5	20.85±	1.75±0.00	10.6±0.09	19.6±0.59	8.97±0.04	131.2±1.6
1% PS + Cu0	12.5±0.5	9642±550	41.3±2.25	374.6±3.75	22.35±	1.75±0.50	18.5±0.27	27.7±0.89	9.12±0.1	146.4±1.1
1% PS + Cu50	45±5	9542±575	39±1	354.6±11.2	22.85±	2±0.00	18.3±0.19	27.1±0.29	9.13±0.04	143.65±.45
1% PS + Cu100	92.5±2.5	9317±700	37.5±2.5	354.6±21.2	23.35±	2±0.50	17.6±0.47	25.3±0.59	9.03±0.02	139.1±5.4
1% PS + Cu200	150±5	8967±875	37.5±0.25	327.1±11.2	22.35±	2±0.25	15.3±0.39	23.8±0.89	9.01±0.01	135.3±6.3
1% PS + Cu400	285±5	8542±375	33.8±1.25	310.9±5	20.1±	1.75±0.25	12.3±0.22	20.8±0.29	8.98±0.03	131.7±3.1
1% PS+NP+Cu0	12.75±0.25	9429.5±412.5	39±0.5	359.6±6.25	20.85±	1.5±0.25	18.3±0.19	28.6±1.19	9.13±0.04	144.2±3.9
1% PS+NP+Cu50	47.5±7.5	9704.5±162.5	41.8±1.75	370.9±15	22.35±	1.75±0.00	18.7±0.47	28.0±1.48	9.04±0.02	142.9±2.9
1% PS+NP+Cu100	97.5±2.5	9692±300	40.3±2.5	338.4±15	22.6±	2±0.25	18.2±0.39	27.7±0.29	9.08±0.02	139.7±4.8
1% PS+NP+Cu200	150±5	9254.5±662.5	37.3±4.25	312.1±13.7	22.6±	1.75±0.00	15.8±0.22	24.7±1.19	9.04±0.01	134.35±1.9
1% PS+NP+Cu400	290±10	8817±525	34±0.5	299.6±1.25	20.1±	1.75±0.25	12.7±0.19	23.2±1.48	8.99±0.02	131±2.8

3% PS+ Cu0	13±0.5	11217±300	44.8±7	392.1±18.7	24.6±	2±0.25	19.9±0.47	31.0±0.29	9.16±0.03	144.1±1.4
3% PS + Cu50	50±5	10417±175	41.8±0.25	364.6±1.25	23.1±	2±0.25	19.4±0.39	30.1±1.19	9.07±0.04	140.2±5.5
3% PS + Cu100	100±10	10929.5±87.5	39±0.5	369.6±18.7	24.6±	2±0.25	19.0±0.22	28.6±1.48	9.08±0.04	136.1±4.1
3% PS + Cu200	165±5	10529.5±187.5	39.5±2	339.6±1.25	23.6±	2±0.25	16.3±0.39	26.8±0.29	9.09±0.03	135.2±2.3
3% PS + Cu400	300±5	9879.5±712.5	35±1	322.1±6.25	22.1±	2±0.25	13.3±0.19	24.1±1.19	9.01±0.03	130.8±4.09
3% PS+NP+Cu0	13.25±2.5	10842±375	41.8±1.75	389.6±3.75	24.6±	2.25±0.00	19.6±0.84	31.5±1.48	9.12±0.01	142.25±3.5
3% PS+NP+Cu50	47.5±2.5	10354.5±587.5	41±0	377.1±8.75	22.1±	2±0.25	19.3±0.19	30.4±2.67	9.07±0.00	137.15±3.2
3% PS+NP+Cu100	102.5±7.5	10367±375	42±4	369.6±6.25	20.35±	2±0.25	18.4±0.04	29.2±0.89	9.09±0.01	135.7±3.4
3% PS+NP+Cu200	170±5	10142±575	39.3±2.25	348.4±11.2	22.85±	2±0.25	16.4±0.32	27.7±1.19	9.06±0.02	130.1±3.9
3% PS+NP+Cu400	307.5±7.5	9367±775	39±2	327.1±18.7	22.6±	1.75±0.00	12.8±0.17	25.6±1.48	9.03±0.03	130.5±1.8
T-test*(p<0.05)	**0.002±**	**0.002**	**0.024**	**0.000**	**0.085**	**0.173**	**0.011**	**0.000**	**0.000**	**0.000**

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