Mitigating potential of polystyrene microplastics on bioavailability, uptake, and toxicity of micronutrients in *Zea mays* L.

Master of Philosophy

in

Environmental Sciences

by

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Registration No: 02312013005

DEPARTMENT OF ENVIRONMENTAL SCIENCES

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

ISLAMABAD, PAKISTAN

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A Dissertation Submitted in the Partial Fulfillment of the Requirements for the Degree of Master of Philosophy in ENVIRONMENTAL SCIENCES

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It is to certify that the research work presented in this thesis, titled "**Mitigating potential of polystyrene microplastics on bioavailability, uptake, and toxicity of micronutrients in *Zea mays* L**" was conducted by **Mr. Ch Muhammad Saad Bashir (Registration No. 02312013005),** 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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LIST OF ABBREVIATIONS

Abbreviation	Full form
MPs	Microplastics
mg/kg	Milligrams Per Kilogram
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
N	Nitrogen
P	Phosphorous
K	Potassium
ROS	Reactive Oxygen Species
SOD	Superoxidase Dismutase
POD	Peroxidase

SD	Standard Deviation
$CuSO_4.5H_2O$	Copper Sulphate Penta Hydrate
NARC	National Agriculture Research Council
GR	Glutathione Reductase
Fe	Iron
Mn	Manganese
Zn	Zinc
Pb	Lead
Cd	Cadmium
Hg	Mercury

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ABSTRACT

Microplastics (MPs) are widespread in agroecosystems, while the coexistence of MPs and Copper (Cu) contamination in agricultural soils is increasing dramatically with time, thus posing a serious risk to soil-crop interaction. However, little is known about how MPs interact with essential micronutrients in Cu-contaminated soils and their impact on crop growth. To fill this gap, we analyzed the immobilization potential of polystyrene microplastics (PSMPs) for micronutrient bioavailability in soil and assessed Cu uptake, and its toxicity in maize under different Cu and PSMPs concentration. To test the hypothesis, a pot experiment was carried out where maize (Zea mays L.), variety (Islamabad gold) grown in Cu spiked soil with concentrations (0, 50, 100, 200, and 400 mg/kg) and PSMPs (150-250µm) at the concentration (0%, 1%, and 3% w/w) for 60 days. Maize growth indicators consist of height, chlorophyll, fresh and dry weight, while biochemical parameters include antioxidant enzymes (SOD, POD, CAT and APX) activity and oxidative damage (MDA content) was measured in maize root and shoot. Micronutrients in soil and maize tissues were analyzed by atomic absorption spectrophotometry. Results showed that PSMPs positively while Cu application impact negatively on soil pH and other micronutrients (Mn, Zn, and Fe) concentration in post-harvesting soil. The reduction of micronutrients (Cu, Mn, Zn, and Fe) in soil decreased with increasing PSMPs in soil. Morpho-physiological traits showed that maize growth improved at Cu50, and Cu100 mg/kg then declined significantly with further increase in soil Cu concentration as compared to control. Growth traits of maize were enhanced with 3%PSMPs application. PSMPs significantly reduced the uptake of Cu and other micronutrients, while reduced the Cu-induced oxidative stress (MDA) by increasing SOD, POD, CAT, and APX activity in root and shoot particularly at higher Cu concentrations. These findings suggest that PSMPs application in soil can improve maize growth and alleviate Cu-stress, in pot experiment however, their long-term impacts can be focused in the future on soil biota and plant growth performance under field conditions.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The healthy environment is crucial for healthy plant growth and soil biota while industrial development and urbanization has led to heavy metal pollution that exceeds the environment's ability to rehabilitate itself. In addition to heavy metal contamination, another major challenge is the excessive use of single use plastics in agricultural practices, such as plastic sheets used in silage, mulching, and tunnel farming. Study showed that soils can contain up to 40,000 microplastics particles/kg of sludge-amended soil (De Souza Machado et al., 2019). Waste plastics polymers in agricultural soils degrade through processes such as photodegradation, hydrolysis, thermal oxidation, and biodegradation, and ultimately disintegrate into microplastics (MPs) and nanoplastics (NPs).

1.1 Polystyrene microplastics (PSMPs)

Plastics are the organic polymers made from petroleum-derived hydrocarbons and come in different types, such as polyethylene terephthalate (PET), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and others. Polystyrene is a thermoplastic polymer made from the monomer styrene (vinylbenzene) and is highly adsorptive in nature. The benzene ring in PS structure increases the distance between adjacent polymer chains and makes it easier for organic pollutants to diffuse into the polymer. This property enhanced the adsorptive nature of polystyrene as shown in Figure 1.1. Polystyrene microplastics (PSMPs) are known to have a porous structure, have density almost the same as that of water, and remain suspended in water, and readily bioavailable. Among different types of plastics polymer, PS, PE, and PP are the most abundant polymer types found in agricultural soil (R. Ullah et al., 2021).

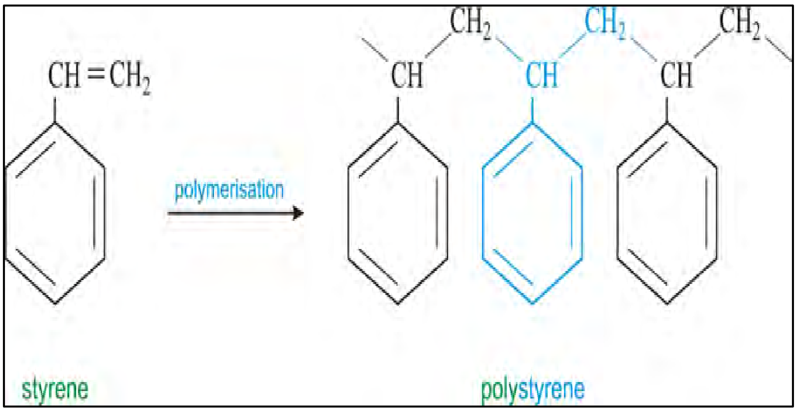

Figure 1 : 1.1. Polymerization reaction of styrene (monomer).

The size of microplastics (MPs) plays a crucial role in determining their reactivity, toxicity, bioavailability, and mobility. The toxicity potential of MPs is inversely related to their size; larger particles are less toxic, while smaller particles have showed more harmful impacts. Smaller particles have a higher surface-to-volume ratio, which increases their ability to interact with biomolecules. The Environmental Protection Agency (EPA) does not officially classify styrene, (component of polystyrene) as carcinogenic, although it has been classified as potentially carcinogenic (carcinogenicity class B2) by the International Agency for Research on Cancer (IARC) (Kik et al., 2020). PSMPs are commonly used as model polymers in research because they can be easily created in a variety of sizes, for the study of surface characteristic properties.

1.2 Sources of PSMPs in soil:

Multiple sources of plastics contribute to the contamination of terrestrial and aquatic ecosystems. These sources can broadly be divided into two classes: point sources and non-point sources. Point sources of microplastics include wastewater irrigation, open dumping of plastics waste, and microplastics-coated seeds. Non-point sources include atmospheric deposition, weathering of tires, and flood-carrying plastics. Microplastics can be generated from primary or secondary sources. PS is commonly used in daily life products such as toothbrushes, toys, and packaging, and food containers such as trays, plates, and cups (Kik et al., 2020). PSNPs are also widely used in various applications such as biosensors, photonics, and self-assembling nanostructured materials. In the agricultural sector, sludge from the treatment of wastewater is used for soil amendment, which can also contain a significant number of MPs. Moreover, sewage and sludge-amended soil contains 30.7 $\times 10^3$ MPs particles/kg of dry sludge (Lozano et al., 2021).

1.3 Impact of PSMPs on soil physiochemical properties

The alteration in physiochemical properties of soil by MPs depends on several factors such as the MPs' polymer type, size, concentration, and degradation rate. MPs can have both positive and negative effects on the soil's physiochemical properties, which can affect the plant growth. Previous literature has shown that a different polymer types, PET (0.2%), PU (0.2%), LDPE (0.3%), and PS (0.4%) concentrations had a beneficial effect on microbial activity (Lozano et al., 2021). On the other hand, breakdown of PS and PU into their hazardous monomers can have harmful effects on soil microbiota. MPs can alter soil texture, porosity, aeration, water-holding capacity, and organic matter content, which overall change soil chemistry as indicated in Figure 1.2. (De Souza Machado et al., 2019) showed that PSMPs decreased the soil bulk density, which affect evapotranspiration, water saturation, and water dynamics of soil. PES fibers due to their ability to entangle soil particles can cause soil aggregation, whereas polyamide (PA) fibers have the reverse effect (Lozano et al., 2021). The application of PS foams and PET fragments, tends to elevate soil pH, while foam's characteristic sponge-like structures can absorb water, which can increase the amount of water available for plants (Zhao et al., 2021).

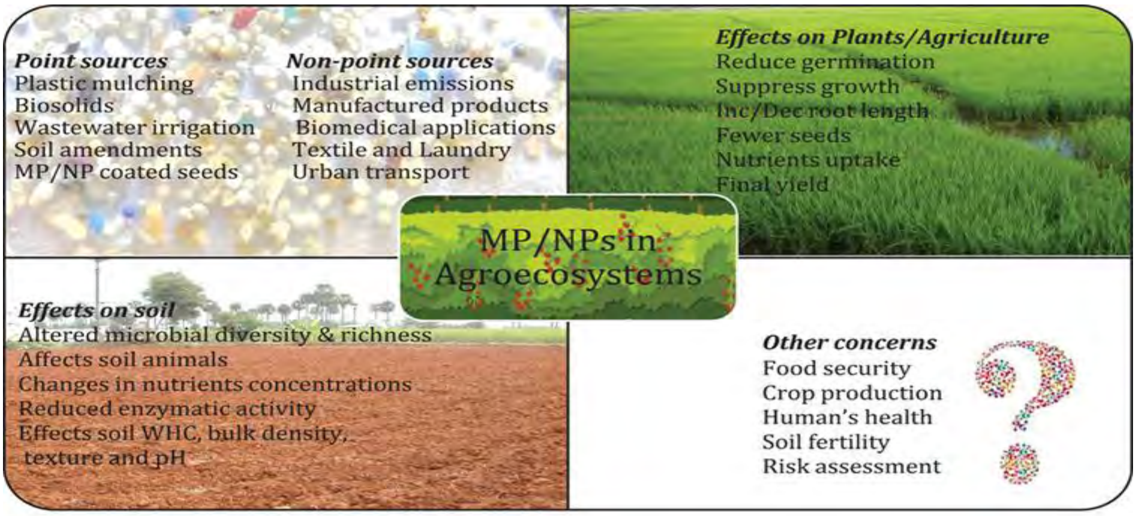

Figure 2 : 1.2. MPs & NPs in an agroecosystem. (R. Ullah et al., 2021)

1.4 Effects of PSMPs on plant growth

The effects of MPs on plant growth depend upon multiple factors such as polymer type, plant species, and soil conditions. Studies have shown that MPs can have both positive and negative effects on plant growth. Growth stimulation by PSMPs in different plant species include reduced oxidative damage, increased chlorophyll content, biomass, shoot, and root length. A study found that the shoot biomass of wild carrot increased by 27% with fibers, 60% with films, 45% with foams, and 54% with fragments as compared to control (Lozano et al., 2021). PSMPs can have a mitigating effect on wheat and increasing chlorophyll content in co-exposure with Cu and Cd (Zong et al., 2021). In hydroponic system, PSMPs (1000 mg/L) significantly reduced SOD and CAT activity while PSMPs (10 mg/L) increased the root length of rice as compared to control (Q. Zhang et al., 2021). However, other studies have found that spring onion exposed to PES and PS experienced a considerable increase in root biomass but plants exposed to HDPE, PET, and PP experienced a milder response (De Souza Machado et al., 2019). The long-term usage of plastics in agricultural fields can inhibit crop productivity. The inhibitory effects of PSMPs on growth include delayed seed germination, reduced chlorophyll, biomass, shoot, and root length, and increased oxidative stress. MPs can cause primary toxicity by clogging the cell wall, impairing water and nutrient availability, and their uptake. For instance, a concentration of microfilm increased the routes for water to travel, which accelerated soil evaporation and caused a loss in shoot and root mass due to a deficiency of water (Lozano et al., 2021). Moreover, PTFE and PSMPs caused damage to rice roots and reduced root growth (Dong et al., 2022). On the other hand, MPs uptake in plants depends on various factors such as plant species, polymer shape, size, and type. A study found that 1 µm particles could not enter the plant body, while 200nm PSMPs entered the lettuce from roots and transported toward the stem and leaves. On the other hand, HDPE of size (3 µm) can neither move to the vascular system nor translocate to the shoots (Dong et al., 2021).

1.5 Effect of PSMPs on plant micronutrients uptake

Microplastics (MPs) can directly or indirectly change the properties of soil, such as pH, porosity, aeration, and microbial activity. Directly, they can immobilize contaminants by adhering heavy metals on their surface. This adsorption capacity of MPs can have dual functions; it can immobilize the contaminant, or it can bind essential nutrients that plants require and then plant is unable to uptake. PSMPs have a strong affinity with positively charged nutrients, and cationic adhesion is more dominant than anionic adhesion. The strong adsorption of Zn^{+2} and Cu^{+2} was reported on PET-MPs surface (Wang et al., 2020). According to (Zong et al., 2021), PSMPs reduced the uptake of Cu and Cd in wheat seedlings and mitigate the toxic effects of heavy metals in a hydroponic system. The application of PSMPs has also been found to reduce the uptake of Cd in Chinese cabbage (Z. Zhang et al., 2022). Due to the large surface area and adsorbing nature of PSMPs, they can also adsorb organic pollutants. Recent study found that PSMPs can diminish the uptake of phenanthrene in soybean roots and leaves (Xu et al., 2021). The presence of PSMPs has also been found to decrease the uptake of micronutrients by wheat, which may be due to the clogging of root pores or the regulation of gene expression involved in metal ion transporters by PSMPs (Lian, Wu, Xiong, et al., 2020). The results of previous studies have revealed that PSMPs adhere to the organic pollutants and nutrients in the soil and immobilize the contaminants, thereby reducing their bioavailability.

1.6 Copper (Cu): A plant micronutrient and contaminant

Heavy metal (Cu) contamination in soil is a major environmental problem that poses a threat to food safety and human health. Heavy metals are metals or metalloids, having density greater than 4 gcm^{-3} or density 5 times greater than the density of water. They are divided into two classes: essential, which is required for proper healthy functioning of the cell (e.g., Fe, Zn, Mn, and Cu), and non-essential, which is not required for plant growth (Cd, Hg, and Pb) (Kuzminov et al., 2013). Copper (Cu) is a heavy metal that is essential for plant growth at optimal levels but harmful at high levels. It can be found in two oxidation states (Cu^{+1} and Cu^{+2}), dominantly found in di-cationic form (cupric ion) which is used by plants as a micronutrient (Shabbir et al., 2020). When Cu entered the soil, it binds with different organic and inorganic ligands. However, plant Cu uptake can be reduced by increasing the contact time between soil and Cu through aging (conversion process of the available form into an unavailable form) (Lem, 2020).

1.7 Sources of Cu in soil

The background concentration of Cu in soil varies depending on the mineralogy and rock formation of a particular region (Lem, 2020). Sources of Cu include both natural and anthropogenic sources. Natural sources include weathering of mineral rocks, atmospheric deposition, forest fires and volcanic eruptions, while anthropogenic sources include smelting, industrial effluent, sewage sludge, excessive use of Cu-containing fertilizers, pesticides, paints, Cu mining, urban runoff, and coal combustion. The annual discharge of Cu in agricultural soils was 939,000 tons during the previous decades (Ahmadpour et al., 2015). However, total amount of Cu in soil does not accurately reflect how much metal is transported into plants, some amount of Cu is used in making complexes with other metal oxides. The Cu^{+2} concentration in soil depends on soil texture, organic matter, and pH. Soil with high clay content (vertisol) or with high organic matter (histosol and spodosol) carry a high amount of Cu. High concentration of heavy metals in agricultural soils was reported in South Asian countries due to the use of untreated wastewater for irrigation. Heavy metals can exist in different forms in soil, with varying degree of mobility, bioavailability, and solubility. These forms are influenced by some factors such as soil mineralogy, pH, organic matter, CEC, and microbial activity as shown in Figure 1.3. An increase in soil pH leads to a decrease in free available Cu ions in solution (Lem, 2020). However, addition of Cu to soil has been found to decrease the soil pH (Zand & Mühling, 2022).

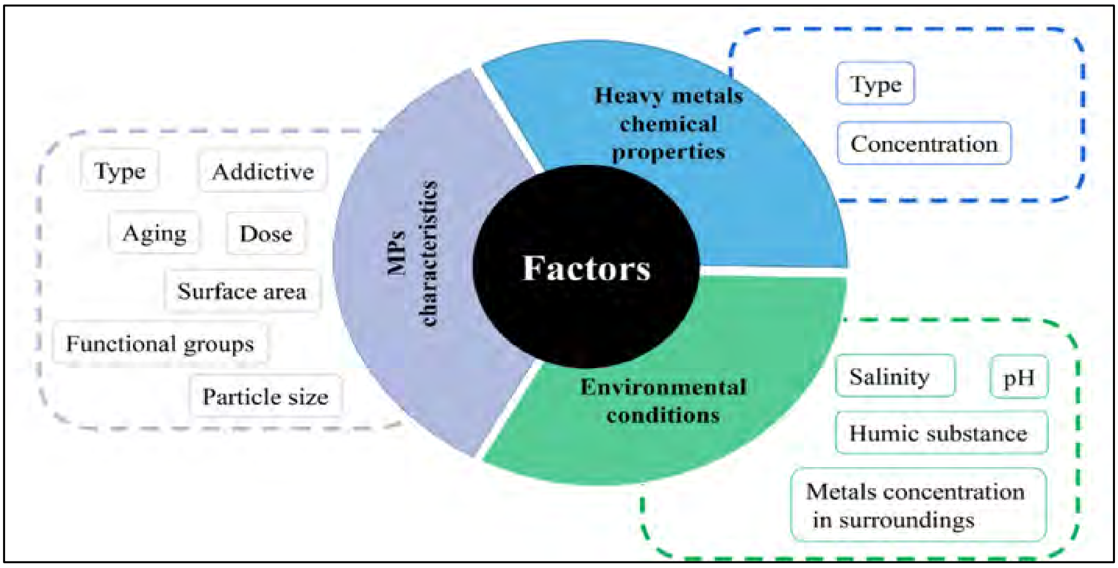

Figure 3 : 1.3. Factors influencing heavy metal adsorption on MPs.

1.8 Role of Cu in plant

Cu is an essential element for plant growth and plays a vital role in several metabolic processes, for instance, photosynthesis, oxidative stress response, mitochondrial respiration, hormone signaling, iron mobilization, and oxidative phosphorylation (Shabbir et al., 2020). In addition to this, Cu act as a cofactor in several enzymes, especially in the electron transport chain, plastocyanin, amine oxidase, and cytochrome c-oxidase (Lem, 2020). Due to its redox-active nature, Cu may form bond with biomolecules, and boost the reactive oxygen species (ROS) formation through the Fenton reaction (Shabbir et al., 2020). However, the concentration of micronutrients required for normal plant growth, while their deficiency and excess amount retard the growth (Table 1.1).

Table 1 : 1.1. Plant analysis for micronutrients on dry weight basis (Neenu & Ramesh, 2020)

Micronutrients (ppm)	Range of Concentration	Deficient Range	Normal Range	Toxic Range
Mn	10-600	15-25	20-300	300-500
Cu	2-50	2-5	5-30	20-100
Zn	10-250	10-20	27-150	100-400
Fe	20-600	<50	100-500	>500

1.9 Phytotoxicity of Cu on growth traits

1.9.1 Morphological parameters

Cu can have toxic effects on plant morphology, resulting in change in the shape of leaf blades, size of stomata, number of hairs on leaf blades, and a reduction in the distance between mesophyll cells. These symptoms can lead to a change in transpiration rate, which affects water uptake. Deficiency of Cu symptoms include necrosis, chlorosis, and twisted leaves, while excess Cu can cause more severe symptoms, such as delayed root development, dark roots, and abnormal root growth (Shabbir et al., 2020). The reduced leaf area in Cu-stressed plants is likely due to lignin accumulation in xylem tissues, which leads to an increase in the hardness and thickness of the cell wall, obstructing cell growth and expansion, and decreasing the flexibility of the cell wall. Additionally, Cu toxicity can also lead to reduced cell division, resulting in reduced root and shoot biomass.

1.9.2 Physiological parameters

Photosynthesis depends upon several cellular and molecular structures that are susceptible to metal content in the plant. Cu can alter the photosynthetic activity of plants by interfering with photosynthetic pigments and by directly impacting the dark and light reactions of photosynthesis. (Kuzminov et al., 2013) reported that the transport of photoexcited electrons from photosystem II to photosystem I is restricted by Cu and affects the lipid membranes, result in decreased photosynthetic activity. While the target of Cu toxicity in photosynthesis is majorly the reaction center of photosystem II and photosystem II is more prone to Cu toxicity than photosystem I. In the case of chlorophyll, excess Cu can replace Mg from chlorophyll molecules found in the antenna complex causing damage to the structure and functionality of the pigment.

1.9.3 Oxidative stress

Plants have developed several mechanisms to combat heavy metal stress. The first line of defense was achieved by reducing the metal uptake from the soil via the roots, then resisting their translocation into the above ground parts. In the second line of defense, plants produce of ROS, which are highly reactive free radicals and peroxides produced during chemical reactions. ROS could be superoxide radicals (O^{-} ^{2}), hydroxyl radicals (OH^{-1}), and hydrogen peroxide (H_2O_2). To combat these ROS, plants have developed antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR), which control the ROS levels under normal conditions. When excess ROS produced under stress and were not scavenged by antioxidant enzymes, then they interact with different cellular components, for instance, causing oxidation of cellular membrane, and reacting with biomolecules like DNA, and proteins. In the presence of ROS, plant energy is utilized in defending against stresses, and ultimately growth is reduced. Studies have shown that increasing Cu content in maize leaves stimulated the synthesis of SOD, POD, APX, GR, and GST (Shuang Gui Tie, 2012). Increased APX activity was reported with an increase in Cu stress in sugarcane (Tamez et al., 2019).

There are enormous studies were carried out with different polymers and combined stresses with heavy metals some of important studies tabulated in Table 1.2.

Table 2 : 1.2. Physiological, morphological, and biochemical changes in plants under different heavy metals and MPs stress.

Plant	Time period	Growth media	Polymer Type	Polymer size	Polymer concentration	Heavy metal concentration	Plant Response	References
Maize	21 days	Soil (pot)	PP, PET, PVC, PS, PE	75-150 and 150-212 µm	0.02 g	No	Unstable cell membranes decreased photosynthetic pigment and generation of ROS	(Pehlivan & Gedik, 2021)
Maize	15 days	Hydroponic	PE	3 µm	0.0125, 100 mg/L	No	Reduced growth, and nitrogen content; impaired water and nutrient intake	(Urbina et al., 2020)
Rice	till grains	Soil (pot)	PS, PTFE	0.1-1 µm, 10-100 µm	0, 0.25, and 0.5% (w/w)	As (1.4, 24.7, 86.3 mg/kg)	Hemoglobin content was decreased by PSMP and PTFE. In grains, the activities of starch synthase and pyrophosphorylase are reduced by PSMP, PTFE, and As	(Dong et al., 2022)
Maize	42 days	Soil (pot)	No	No	No	Cu (0, 50, 100, 200, 300 mg/kg)	Growth characteristics of Z. mays were increased upto 50 mg/kg Cu further increasing Cu content had an inhibitory effect on plants.	(Zand & Mühling, 2022)
Maize, Wheat, Sorghum	30 days	Soil (pot)	No	No	No	Cu, Cd (17.40, 34.80mg/kg)	Declines in growth, leaf osmotic potential, and RWC by cadmium. When Cu was used alone, the effect was lesser than in combination with Cd.	(Metwali et al., 2013)
Wheat	18 days	Hydroponic	PS	1-1000 nm	0, 10 mg/L	Cd (0,20µM)	Leaf Cd levels and superoxide dismutase activity are decreased by PSNP.	(Lian, Wu, Zeb, et al., 2020)
Wheat	8 days	Hydroponic	PS	0.5 µm	100 mg/L	Cu (2 mg/L), Cd (1 mg/L)	PSMPs increased chlorophyll content, photosynthesis and reduced the accumulation of ROS, and uptake of Cu, Cd.	(Zong et al., 2021)
Chinese	45	Soil (pot)	PS	75 µm	0.5, 1.0, 1.5,	Cd (10 mg/kg)	Addition of PSMPs decreased the Cd toxicity and	(Z. Zhang et

Cabbage	days				and 2.0 % (w/w)		accumulation of Cd by plants compared with the Cd treatment alone	al., 2022)
Soybean	30 days	Soil (pot)	PS	0.1, 1, 10, and 100 µm	10 mg/kg	Phenanthrene (1mg/kg)	PSMPs reduced the uptake of Phenanthrene in soybean roots and leaves	(Xu et al., 2021)
Wild Carrot	28 days	Soil (pot)	12 types	4-5 mm	0.1, 0.2, 0.3, and 0.4% (w/w)	No	All polymer types of MPs promoted the plant biomass production and shoot length.	(Lozano et al., 2021)

1.10 Maize as a test crop

Maize (grass family; Gramineae) is a cereal crop that is widely grown in Pakistan after wheat and rice and is used as a staple food for humans and fodder for livestock. It is also a source of micronutrients for animals and is used as a fuel crop for biofuels in developed countries. Maize is a C4 plant with high photosynthesis rate due to its foliage distribution and size. It is a monocotyledon and is considered as a test plant due to its tolerance and fast-growing nature. Maize was selected in this experiment because most studies of heavy metal toxicity are done on dicotyledon plants, and this experiment aims to investigate the effects of heavy metal toxicity on maize. Exogenous Cu-containing fertilizers are commonly used which can cause primary poisoning to the plant as well as secondary toxicity to the animals through trophic transfer.

1.11 Research gaps

A closer look at the literature on heavy metal adsorption by MPs and heavy metal-contaminated soils, reveals several gaps, there was extensive work reported on the adsorption of organic pollutants and heavy metals by different MPs in soil and hydroponic system (Godoy et al., 2019; Lian, Wu, Zeb, et al., 2020; Mao et al., 2020; Q. Zhang et al., 2021; Z. Zhang et al., 2022; Zong et al., 2021). While previous literature focused on either, only heavy metal stress (Metwali et al., 2013; Zand & Mühling, 2022) or MPs stress on plants (Lozano et al., 2021; Pehlivan & Gedik, 2021; Urbina et al., 2020) but lacks the concentration-dependent effect of PSMPs on heavy metal bioavailability in soil, and their uptake in plants. As far as our current knowledge, no previous research has investigated the immobilization of Cu via the application of PSMPs in Cu-contaminated soil and the effect of PSMPs on micronutrient uptake by maize.

1.12 Problem statement

Cu contamination in agricultural soil is a major concern due to the excessive use of agrochemical products containing Cu (5000 tons/year) (Rehman, Liu, et al., 2019) and the ever increasing concentration of Microplastics (MPs) in agricultural soils (globally expanded from 4.4 million tons in 2012 to 7.4 million tons in 2019) (Li et al., 2021), alter the soil physiochemical properties and bioavailability of micronutrients which influence the crop nutrient uptake and growth while posing risks to soil and crop health, which ultimately disturbs agricultural sustainability.

So, in the present study, to fill the above-mentioned gaps in previous literature, we hypothesized that the application of PSMPs in Cu-contaminated soils reduces Cu uptake and alleviates the noxious effects of Cu toxicity in maize.

1.13 Research objectives

In our study, to investigate the above-stated hypothesis,

Objectives of the present study,

- To investigate the immobilization potential of PSMPs for Cu and other micronutrients in soil and their uptake by maize in different Cu and PSMPs concentrations.
- To study the effect of PSMPs application on physiological and biochemical parameters of maize under Cu-stressed soil.

CHAPTER 2

MATERIALS AND METHODS

The materials and methods used, in the present study "Mitigating potential of polystyrene microplastics on bioavailability, uptake, and toxicity of micronutrients in Zea mays L." was given in this chapter. The detailed materials, methods, and techniques implemented in this experiment are briefly described below. The experiment was conducted in a biological building, Quaid-i-Azam university, Islamabad under natural conditions, and further analysis was performed in Environmental Biology and Ecotoxicology lab.

2.1 Experimental design

The pot experiment was conducted (31-03-2022 to 06-06-2022) in natural conditions (33°44'55.4"N, 73°08'09.8"E) at Quaid-i-Azam university, Islamabad. The experimental setup consists of 15 no. of treatments with PSMPs (150-250 µm) (0%, 1%, and 3% w/w) and Cu concentration (0, 50, 100, 200, and 400 mg/kg) in soil with three replicates (n=3), (T1, Control/Cu0 mg/kg; T2, Cu50 mg/kg; T3, Cu100 mg/kg; T4, Cu200 mg/kg; T5, Cu400 mg/kg; T6, 1%PS+Cu0 mg/kg; T7, 1%PS+Cu50 mg/kg; T8, 1%PS+Cu100 mg/kg; T9, 1%PS+Cu200 mg/kg; T10, 1%PS+Cu400 mg/kg; T11, 3%PS+Cu0 mg/kg; T12, 3%PS+Cu50 mg/kg; T13, 3%PS+Cu100 mg/kg; T14, 3%PS+Cu200 mg/kg; T15, 3%PS+Cu400 mg/kg). Based on existing knowledge regarding the presence of MPs in soils and earlier investigations, MPs size and concentrations were selected (Z. Zhang et al., 2022). The soil used for the experiment was collected from the nursery of Quaid-i-Azam university. The physicochemical properties of soil were as follow, coarse fraction; 54%, micro-aggregate fraction; 23%, non-aggregate fraction; 23%, water holding capacity; 500 ml/L, pH; 8.6, EC; 435 μScm^{-1}, Fe; 11538 ± 288 mg/kg, Mn; 474 ± 17.5 mg/kg, Zn; 49.1 ± 1.0 mg/kg, Cu; 14.9 ± 0.0 mg/kg. Bulk soil and organic matter were air-dried and sieved (2mm). Organic matter (3% w/w) was added to the soil as fertilizer to provide sufficient nutrients for plant growth. 5 surface sterilized seeds were sowed in

(HDPE) plastic pots of size (15cm x15cm) and after one week, 3 uniform plants were selected for the experiment while the remaining plants were removed. The pots were placed under natural sunlight and their location was changed weekly and no chemical fertilizer was applied. After 8 weeks of growth, plants were harvested, and plant roots were washed thoroughly with tap water and then preserved at -80°C for further analysis.

2.2 PSMPs preparation, Cu spiking and seed pre-treatment

MPs were prepared from PS pellets (PS-330), purchased from Pak Petrochemical Industries Pvt. Ltd (Lahore). The pellets were mechanically ground and then sieved through stainless steel sieves (150 µm and 250 µm). PSMPs (150-250 µm) fraction was used in the experiment while Cu concentrations were prepared from ($CuSO_4.5H_2O$) in deionized water and then soil incubated for two weeks at 25°C. (Lozano et al., 2021). Certified seeds of maize (Islamabad Gold), from National Agriculture Research Council (NARC Islamabad), were acclimatized at -20°C for 24 hours, after that seeds were disinfected with 3% sodium hypochlorite for 5 minutes and then washed thoroughly with deionized water.

2.3 Morphological parameters

The morphological parameters include no. of leaves, shoot and root length, chlorophyll SPAD units, fresh and dry weight of maize.

2.3.1 Shoot and root length

During the experiment, weekly shoot length was measured with the help of a ruler scale. On harvesting, plants were cleaned with tap water and the length of shoot and root was measured. The height of the shoot was considered from the base of the stem to the highest leaf. The shoot length was measured for each plant and then taken an average of readings; a similar protocol was adopted for root length.

2.3.2 Fresh and dry weight

Plants after harvest were thoroughly washed with tap water and left open for air dry. The Fresh weight of the root and shoot was determined by weighing them on a digital weight balance in grams. For dry weight, plants were kept in a hot air oven at 80°C for 24 hours, and their dry weight was measured in grams.

2.3.3 Chlorophyll content

The chlorophyll content was measured by using the SPAD meter (SPAD-502) (Ling et al., 2011). SPAD values of 3 uniform-sized leaves from the top of the plant were taken for measurement. An average value of three readings was taken as chlorophyll content. The weekly chlorophyll content is measured to obtain per week variations in chlorophyll content.

2.4 Lipid peroxidation

Lipid peroxidation was estimated in terms of MDA content in maize tissues was measured (Tulkova & Kabashnikova, 2022). A fresh shoot and root sample (0.2g) was ground in a chilled mortar and pestle with 2 mL of 1% (w/v) trichloroacetic acid (TCA). After centrifugation at 15000 rpm for 10 minutes, 2 mL of the supernatant was mixed with 4 mL of 0.5% thiobarbituric acid (TBA), boiled at 95°C, then cooled. The resulting mixture was measured for absorbance at 532 and 600 nm using a spectrophotometer, and the level of TBA was calculated using an absorption coefficient of 1.55 mmol/cm.

2.5 Antioxidant enzymes assays

100 mg of root and shoot were ground with a pestle and mortar in liquid nitrogen. The sample was then added with 4 mL extraction buffer consisting of 1.4 mL of 0.07 potassium phosphate buffer, 20 µL of 200 mM ascorbic acid, 16 µL of 100 mM EDTA, and 2% polyvinyl pyridine. The slurry was added to an Eppendorf tube and centrifuged at 15000 rpm for 20 minutes (H. Ullah et al., 2022). The pellet was discarded, and the supernatant was used as an enzyme extract. A temperature of 4°C was maintained to carry out all steps of the preparation of enzyme extract. The enzyme activity was measured in units/mL of enzyme extract.

2.5.1 Ascorbate Peroxidase (APX)

Ascorbate peroxidase (APX) was assessed by the method followed by (Nakano & Asada, 1981), by estimating the decrease in optical density because of ascorbate at 290 nm at 25°C. 1mL of the reaction mixture was prepared by mixing 25 mM buffer (Potassium phosphate, pH 7.0), 100 µL H_2O_2 (10mM), 1 µL EDTA (0.1 mM), 5 µL ascorbic acid (0.25 mM), enzyme extract (50 µL). A standard curve was used to measure the activity by estimating the decrement of ascorbate.

2.5.2 Peroxidase (POD)

Peroxidase activity was found according to the (V. Velikova & , I. Yordanov, 2000) method. 5 mL assay mixture, 1.5 mL sodium phosphate buffer (100mM), 1 mL H_2O_2 (1 %), 1 mL H_2SO_4 (5N), 4% p-phenylenediamine, 450 µL deionized water, and 50 µL enzyme extract. The absorbance of the mixture was measured with the help of a spectrophotometer at 485 nm.

2.5.3 Superoxide Dismutase (SOD)

Superoxide dismutase contents assessment was conducted through (Verma & Dubey, 2003) method. The assay mixture contained 1 ml of 200mM potassium phosphate buffer, 1ml NBT (250µM), 1 ml riboflavin (10µM), 1.94 ml deionized water, 10 µl TEMED, and 50 µl enzyme extract. Use supernatant as an enzyme source. Three assays were followed for this activity. Then kept the reference samples in complete darkness. Whereas placing the samples for the reaction in a light chamber for 20 min. The measurement of blank and test solution optical densities was carried out with a spectrophotometer at 560 nm wavelength.

2.5.4 Catalase (CAT)

The measurement of catalase activity was performed according to the (Aebi, 1984) method. 1 mL assay mixture was containing 714 µL of 50 mM potassium phosphate buffer, 100 µL of 10 Mm H_2O_2, 136 µL deionized water, and 50 µL enzyme extract. The optical density of CAT was measured at 240 nm using a spectrophotometer. A blank (lacking enzyme extract) was also run for comparison.

2.6 Soil analysis

2.6.1 Soil physiochemical properties

The soil pH and EC were analyzed according to the procedure given by (Sun et al., 2012). 1g dried soil mixed in 5 mL deionized water with a ratio (1:5 w/v), shaken (180 rpm, 30 minutes) and measured pH and EC, using a pH multimeter (OKATON). While the water-holding capacity of soil was determined by the method (Sun et al., 2012). The relative soil fraction was determined by the method followed by (Yu et al., 2021). The soil sample was passed through a series of sieves with decreasing mesh size (250-2000 µm, 53-250 µm and <53 µm) to obtain a particle-size distribution.

2.6.2 Heavy metal analysis of soil and plant

The heavy metals in soil and plant were analyzed by the method followed by (Ahmadpour et al., 2015) and (Sun et al., 2012) respectively. The soil samples (1g) were digested using aqua regia (HCL- HNO_3) in a ratio of (3:1 v/v) by heating on a hot plate at 100-110°C for 45 minutes, followed by the addition of 10 mL of 1.2% HNO_3 and heating for an additional 30 minutes at 80°C. The resulting solution was then brought to a volume of 50 mL with deionized water. The tri-acid (HNO_3-H_2SO_4-$HClO_4$) method was used for digestion in a ratio (10:1:4). 5 mL of concentrated H_2SO_4 was added to 0.2 g of the dried and ground shoot and root samples in a conical flask and left overnight then heated on a hotplate at 145°C for 60 minutes. After cooling, add 5 mL of the tri-acid mixture and heat at 240°C for 30-45 minutes. The resulting solution was then brought to a volume of 25 mL with deionized water. Filtered both the digested soil and plant samples through filter paper (Whatman No. 42). The concentration of Cu, Zn, Fe, and Mn in samples was measured by Atomic Absorption Spectrophotometer (Agilent 55 AA). All the glassware used was cleaned using 2% HNO_3 and then dried after being rinsed with deionized water and autoclaved. Procedural blanks were prepared and analyzed with each batch to assess the contamination in samples that might raise during the working. The samples' concentration was adjusted using the corresponding blank values. The calibration curve with R^2 value for metals was between 0.9968 and 0.9999, while the spiked metal recovery was between 85 and 110%.

2.7 Statistical analysis

Descriptive statistics were calculated using IBM SPSS Version 22 and differences between mean values were analyzed using One-way ANOVA at $P < 0.05$ using the Tukey test. Pearson bivariate correlation analysis was used to calculate the correlation coefficient (r). Data was organized in Microsoft excel and Word 365 to create tables and graphs. Heat map of correlation generated through Metabo analyst.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Impact of Cu and PSMPs on soil pH and EC

The study aimed to evaluate the influence of Cu and PSMPs on soil physiochemical properties by measuring soil pH and EC in both pre-sowing and post-harvesting soil. The results showed that soil pH decreased with increasing soil Cu concentration while pH increased with the addition of PSMPs in a dose-dependent manner in both soils (Figure 3.1). These findings consistent with prior studies that have shown an antagonistic relationship between Cu and soil pH (Romdhane et al., 2021; Zand & Mühling, 2022). The decrease in soil pH with Cu might be due to a change in soil microbial activity with increasing soil Cu (Naz et al., 2022; Yáñez et al., 2022). PSMPs increased the soil pH while decreasing EC as compared to the control. In post-harvesting soil, overall soil pH increased, and EC decreased as compared to pre-sowing soil. This increase in pH might be due to the mineralization of organic matter as nitrogen is converted into ammonium ions, which need hydrogen ions, therefore the concentration of hydrogen ions in the soil solution declined (Zhao et al., 2021). In addition to this, elevation in pH might be due to release of HCO^{-3} from maize roots to maintain the soil electrical neutrality during a cationic exchange of nutrients (Tao et al., 2003). There was a significant difference ($P < 0.05$) in pH between Control, Cu200, and Cu400 mg/kg while, the addition of 3%PSMPs significantly ($P < 0.05$) increased the soil pH as compared to Cu treatments without PSMPs in pre-sowing soil as shown in Figure 3.1(a) and (b). These results are consistent with the study in which PSMPs increased the soil pH as compared to control (Zhao et al., 2021). This increase in soil pH with PSMPs addition might be due to increased aeration, and altered microbial activity by PSMPs (De Souza Machado et al., 2019).

The study found that in pre-sowing soil, EC gradually increased while decreased in post-harvesting soil with increasing soil Cu concentration as shown in Figure 3.1(c) and (d). There was a significant difference ($P < 0.05$) in soil EC between control and Cu400 mg/kg in both soils. The increase in EC might be due to increased charged species by Cu addition (Romdhane et al., 2021). However, EC decreased non-significantly, with the addition of PSMPs as compared to soil without PSMPs in both soils. 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).

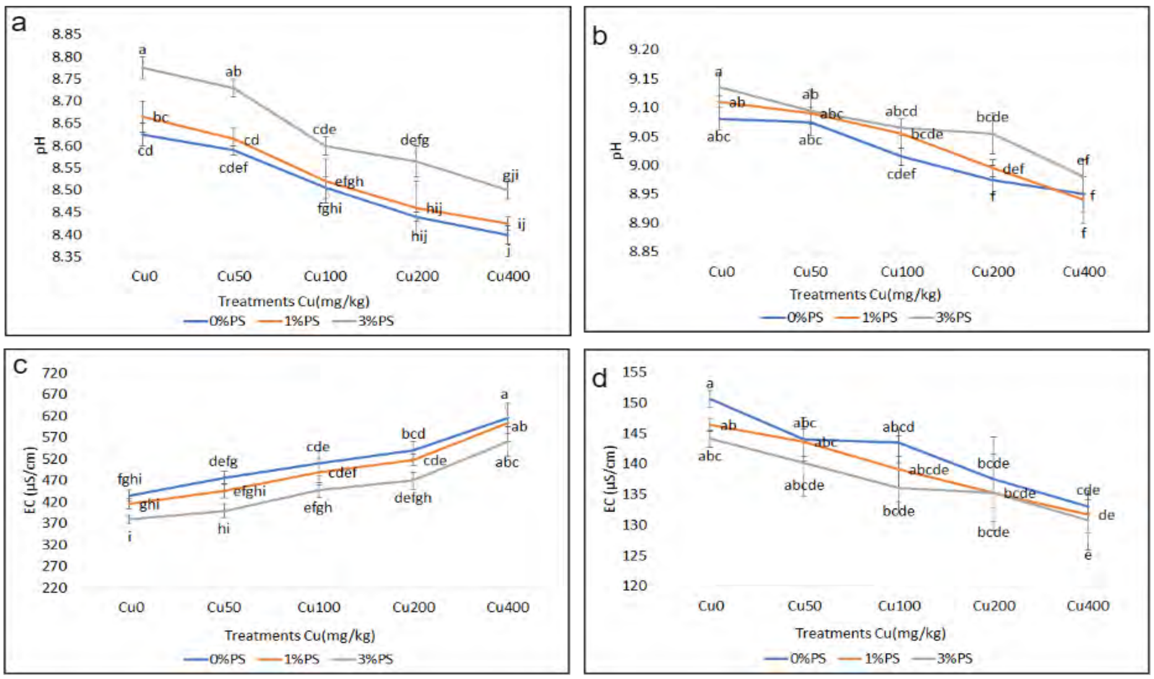

Figure 4 : 3.1 Change in soil pH and EC under different Cu and PSMPs concentrations. (a) pre-sowing soil pH, (b) post-harvesting soil pH, (c) pre-sowing soil EC, (d) post-harvesting soil EC. Data presented is average of three repeats (n=3) ± standard deviation (SD).

3.2 Change in soil fractions by PSMPs

A study found that in soil without PSMPs, 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 (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 used in the study being in the size 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 : 3.1. Relative change in soil fractions under different concentrations of PSMPs. Data tabulated is average of three repeats (n=3) ± standard deviation (SD).

Treatment	% Coarse-particulate fraction	% Micro-aggregate fraction	% Non-aggregated fraction
Control	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 Effect of Cu and PSMPs on soil micronutrients

The concentration of micronutrients in soil was governed by several factors, like soil pH, organic matter, texture, and microbial activities. In the present study, Cu concentration in pre-sowing soil ranged from (14.9 to 399.9 mg/kg), Mn (426.5 to 519 mg/kg), Fe (11538 to 13350 mg/kg), and Zn (45.1 to 52.9 mg/kg). The addition of Cu in all concentrations of PSMPs resulted in a significant ($p < 0.05$) increase in soil Cu concentration. However, there was no significant impact on the concentration of other micronutrients (Zn, Mn, and Fe). The adsorption efficiency of PSMPs is enhanced with time because aging not only increases the surface area (more exposed sites) but also increases the carboxyl and carbonyl groups by surface oxidation (Lang et al., 2020; Mao et al., 2020).

After harvest, the concentration of Cu in the soil ranged from 11.8 to 300 mg/kg. The concentration of Cu declined by 21%, 24.8%, 27.6%, 29.2% and 30.2% from Cu0 to Cu400 mg/kg respectively from pre-sowing soil without PSMPs. In presence of 1% and 3%PSMPs, Cu concentration decreased by 16%, 21.6%, 24.4%, 25.9%, 27.8% and 12.6%, 16.5%, 19.9%, 23.2%, 25% respectively. 3%PSMPs significantly ($p < 0.05$) held more Cu particularly at higher Cu concentration as compared to soil without PSMPs as shown in Figure 3.2(a) and (b). (Yu et al., 2021) found that MPs reduced the bioavailable while increased the organic bound fraction of heavy metals in soil. PSMPs-induced retention of heavy metals also has been reported by (Godoy et al., 2019). In addition to PSMPs, soil organic matter potentially affects the chemical speciation, mobility, and bioavailability of trace metals either through adsorption/desorption or dissociation/complexation mechanism (Qu et al., 2019).

Results showed that the presence of Cu and PSMPs had a significant impact on the concentration of micronutrients (Zn, Fe, and Mn) in post-harvesting soil ranged from 32 to 45 mg/kg, 7467 to 11217 mg/kg, and 286 to 392 mg/kg respectively. In soil without PSMPs, Zn reduced by 20.1%, 25.1%, 29.4%, 35.2%, and 38.1% while in presence of 1%PSMPs, reduction occurred 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. On the other hand, Mn was reduced by 27.3%, 32.6%, 37.2%, 38%, and 40.3% in soil without PSMPs, however in presence of 1%PSMPs, Mn reduction occurred by 24.2%, 28.6%, 30.3%, 31.3%, 36.1%, and 3%PSMPs reduced Mn by 18.6%, 18.3%, 27%, 20.4%, 30.2%. Moreover, Fe decreased by 20.2%, 22.1%, 28.9%, 32.5%, 37.1% in PSMPs free soil while with 1%PSMPs, Fe reduced by 23.1%, 23.3%, 24.4%, 30.5%, 33.1%, and 3%PSMPs reduced Fe by 12.6%, 11.3%, 14.7%, 21.1%, 25.2% from Cu0 to Cu400 mg/kg respectively. Moreover, the study also found that PSMPs retain more micronutrients as compared to the treatments without PSMPs in a dose-dependent manner as shown in Figure 3.2. This might be due to the increased bioavailability of micronutrients, as a result of decreased soil pH by Cu addition, therefore, the maximum reduction of micronutrients occurred in Cu400 mg/kg. Previous studies also found similar relationship between Cu and bioavailability of other micronutrients (Liu et al., 2021; Romdhane et al., 2021). In addition to this, decreased soil pH, enhances the soil CEC, consistent with literature showing that CEC and pH negatively correlate with each other (Rahal & Alhumairi, 2019; Wen et al., 2022).

On the other hand, among different concentrations of PSMPs, the reduction of micronutrients decreased with increasing PSMPs, this might be due to adsorbing nature of PSMPs, the negatively charged functional groups on PSMPs electrostatically adhere cations on its surface. In this way, PSMPs retain micronutrients in soil and prevent them from leaching and plant uptake. Overall these findings are in accordance with a study in which PSMPs adsorb heavy metal and reduce plant uptake (Dong et al., 2022). Moreover, the mechanism behind retaining more micronutrients in soil with 3%PSMPs might be due to increased soil pH by PSMPs. In high soil pH, micronutrient sorption increased either with soil particles, organic matter, or PSMPs particles. Overall, PSMPs application, either directly or indirectly, immobilized the micronutrients in soil.

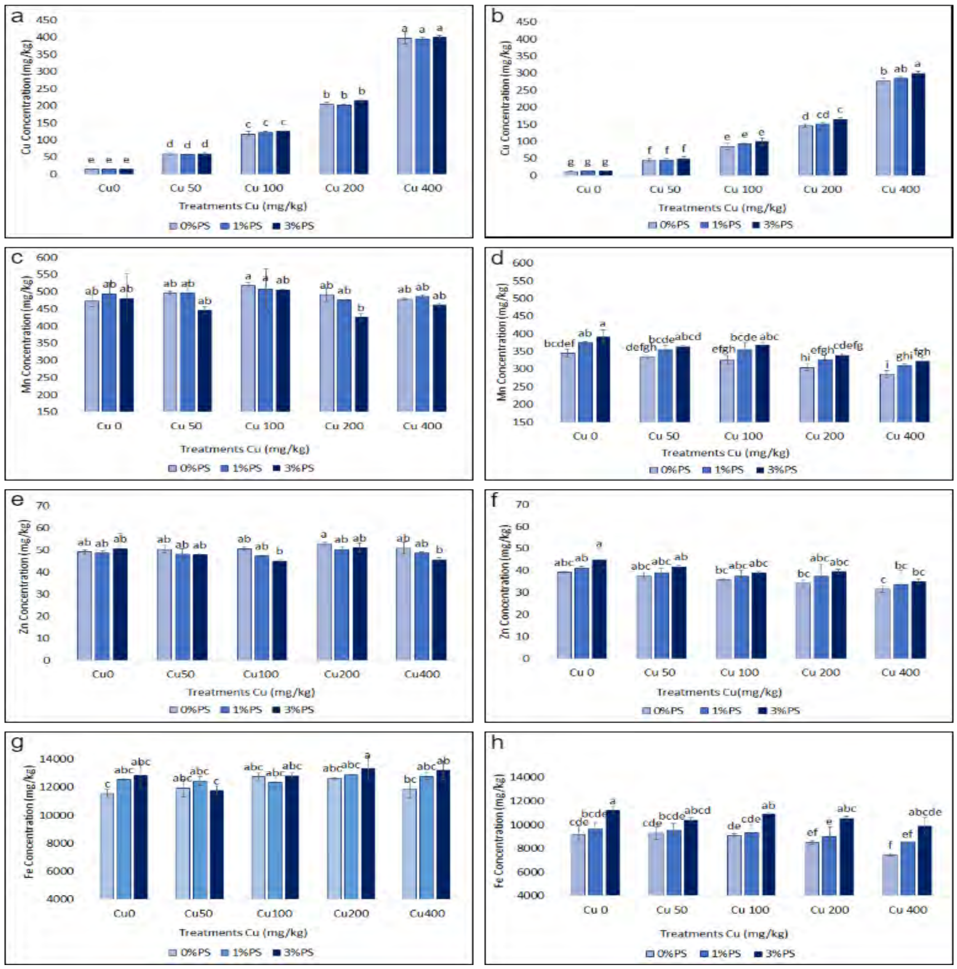

Figure 5 : 3.2. Micronutrient concentrations in soil with different concentrations of Cu and PSMPs. (a) Cu in pre-sowing soil, (b) Cu post-harvesting soil, (c) Mn in pre-sowing soil, (d) Mn in post-harvesting soil, (e) Zn in pre-sowing soil, (f) Zn in post-harvesting soil, (g) Fe in pre-sowing soil, (h) Fe in post- harvesting soil. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD). Different lowercase letters represent statistical differences among the treatments at ($p < 0.05$) according to Tukey test.

3.4 Morpho-physiological traits

The parameters evaluated as indicators of growth include chlorophyll (SPAD Units), number of leaves, plant height, fresh and dry weight (Figure 3.4). Results showed that the plant height increased at Cu50 and Cu100 mg/kg, as compared to control while further increase in soil Cu concentration cause a decline in shoot and root length as shown in Figure 3.4(a) and (b) and consistent with a previous study (Zand & Mühling, 2022). However, 1% and 3%PSMPs increased shoot length by 0.42% and 6.79% respectively, while root length increased by 8.8%, and 11.3% respectively, as compared to control. There was a significant increase ($P < 0.05$) in shoot and root length with 3%PSMPs addition in all Cu concentrations. These findings are in line with (Q. Zhang et al., 2021) where the root length of rice increased in presence of PSMPs. The increase in root length by PSMPs might be due to increased porosity and aeration of soil with PSMPs which help in root respiration and penetration in soil.

PSMPs also increased shoot fresh weight by 23% and 49.6%, while increased root fresh weight by 5.6% and 10.6% with the addition of 1% and 3%PSMPs respectively, as compared to control. On the other hand, shoot dry weight increased by 6.34%, while increased root dry weight by 13.1% and 18.9% respectively, as compared to control. However, fresh, and dry biomass increased up to Cu50 mg/kg then declined with further increase in Cu concentration. Cu induced decline in biomass was also found by (Metwali et al., 2013; Wyszkowski & Brodowska, 2020). There was a significant decline ($P < 0.05$) in both fresh and dry weight at Cu400 mg/kg as compared to control. However, PSMPs application in soil enhanced biomass production. These results are in line with a study where the biomass of wild carrot increased with the application of MPs (Lozano et al., 2021). The increase in maize biomass by PSMPs might be because MPs increased the water channels in soil resulting in more water available for plants and increased porosity of soil helps in providing space for proper root development. Moreover, the maximum number of leaves and SPAD units were found at 3%PS+Cu50 mg/kg as shown in Figure 3.4(g) and (h). Further increase in Cu concentration decreased the SPAD units, this might be because excess Cu damage the structure and function of chlorophyll. Cu interacts with protein structures and interferes with Rubisco in the Calvin cycle (Kuzminov et al., 2013).

Results of the Pearson correlation showed a strong negative correlation between Cu uptake and growth parameters, shoot length (r = -0.82), root length (r = -0.85), dry shoot weight (r = -0.91), dry root weight (r = -0.83), chlorophyll (r = -0.91), and no of leaves (r = -0.79) as shown in Figure 3.9. Previous study found a negative correlation between Cu content and growth parameters in, different plant species (Ahmadpour et al., 2015). Increased Cu content in maize, inhibits growth, as Cu cause toxicity, by interfering with photosynthetic pigments, which ultimately reduces biomass and plant height (Wyszkowski & Brodowska, 2020).

Results indicate that overall growth increased at Cu50, and Cu100 mg/kg as compared to the control, because Cu act as a micronutrient in these concentrations which is required for plant growth, however, further increase in soil Cu act as a contaminant and retard the maize growth (Figure 3.3). On the other hand, PSMPs application improves the physiological parameters of maize. These results are directly in line with previous literature in which agronomic parameters increased in the presence of PSMPs (Lozano et al., 2021; Zong et al., 2021).

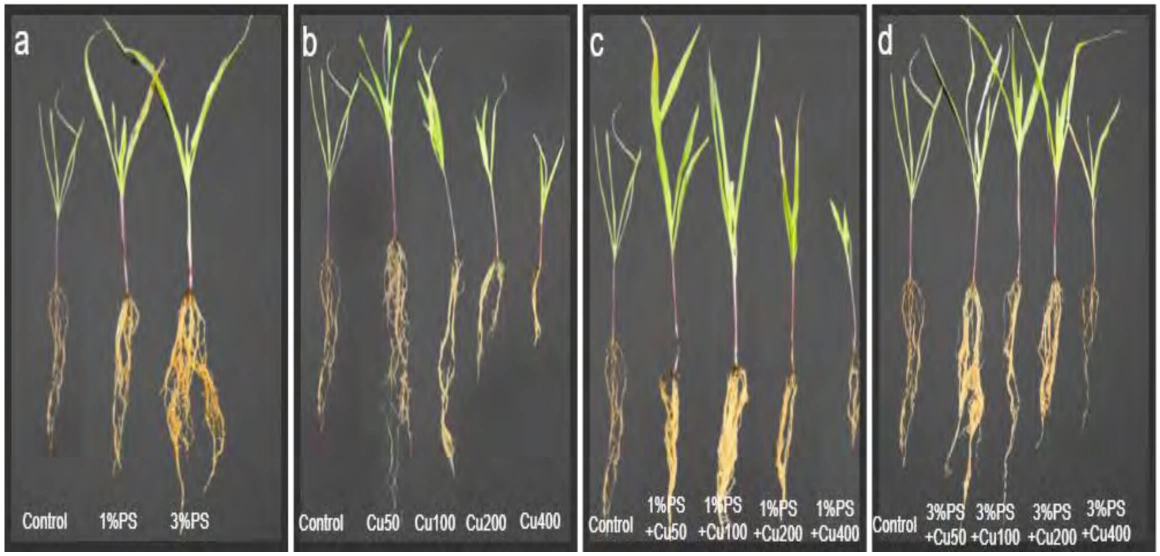

Figure 6 : 3.3. Effect of PSMPs on the growth of maize under Cu-stressed soil. (a) PSMPs combination without Cu, (b) Cu combination with 0%PSMPs, (c) Cu combination with 1%PSMPs, (d) Cu combination with 3%PSMPs

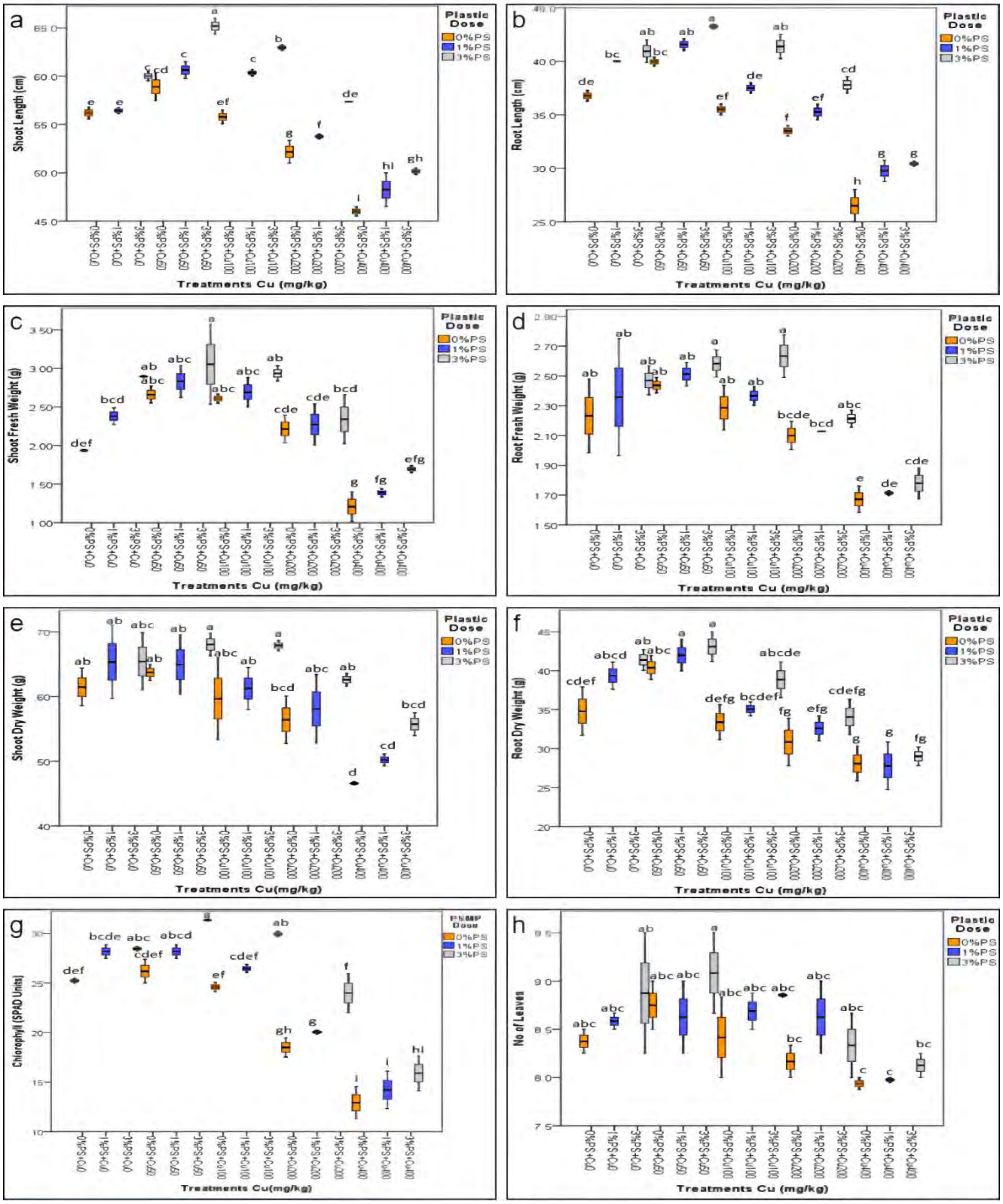

Figure 7 : 3.4 Growth traits of maize under different Cu and PSMPs concentration. (a) shoot length, (b) root length, (c) shoot fresh weight, (d) root fresh weight, (e) shoot dry weight, (f) root dry weight, (g) chlorophyll (SPAD) units, and (h) No. of leaves. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD). Different lowercase letters represent statistical differences among the treatments at ($p < 0.05$) according to Tukey test.

3.4.1 Temporal variation on growth performance of maize

The results revealed that shoot length increased in all treatments with time, but the rate of growth was higher in the first four weeks than following weeks (Figure 3.5). Chlorophyll (SPAD units) initially increased up to the fifth week after that decreased till the eighth week in all treatments and was correlated with shoot length. A similar pattern of results was obtained in a previous study (Ahmadpour et al., 2015). This pattern of growth might be because in the first month, maize roots have sufficient nutrients in the soil but over time, nutrients diminish in the soil, so plants in a single pot face competition and compete for nutrients. Additionally, with time, maize Cu content increased enough in the second month to diminish the growth rate. On the other hand, PSMPs application increased the shoot length and chlorophyll as compared to treatments without PSMPs. Overall, these findings are consistent with previous research that found that PSMPs can improve plant growth (Lozano et al., 2021; Pehlivan & Gedik, 2021).

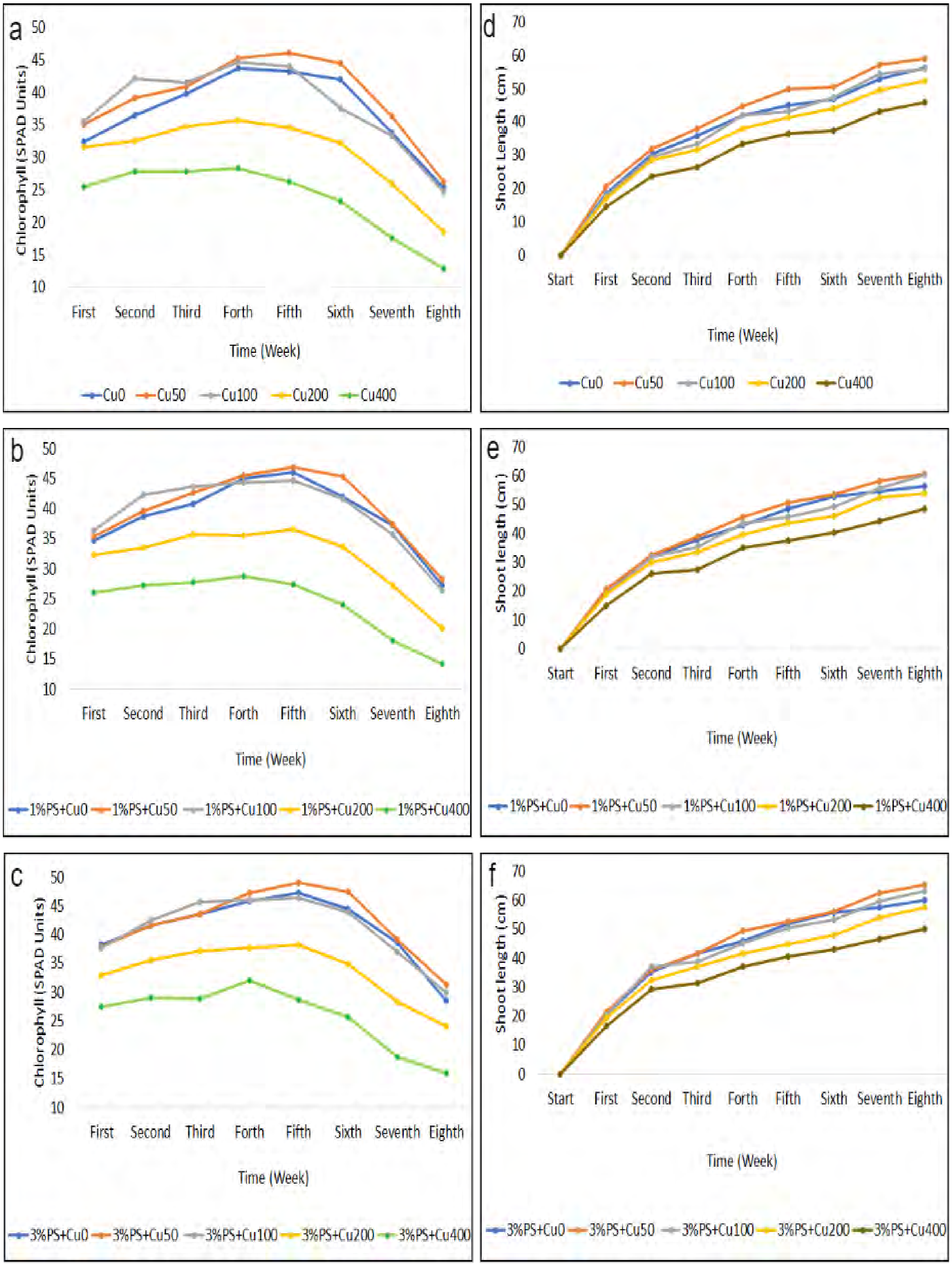

Figure 8 : 3.5. Weekly change in chlorophyll SPAD units (a), (b), (c) and shoot length (d), (e), (f) under different Cu and PSMPs concentration. Data presented in graph is average of three repeats (n=3).

3.5 Micronutrients uptake in maize

In the present study, the application of PSMPs in soil had a considerable impact on Cu uptake by maize in Cu-contaminated soil. Cu accumulation ranged from (3.44 to 84.1 mg/kg DW) and (14.7 to 265 mg/kg DW) in shoot and root respectively as shown in Figure 3.6(a) and (b). Cu uptake in root and shoot increased significantly ($P < 0.05$) with an increase in soil Cu concentration, these results are consistent with studies (Wyszkowski & Brodowska, 2020; Zand & Mühling, 2022) where maize Cu uptake increased with an increase in soil Cu concentration. The accumulation of Cu was substantially higher in root than in shoot. However, the application of 1%PSMPs slightly reduced the maize Cu uptake, while soil amended with 3%PSMPs significantly ($P < 0.05$) reduced the Cu uptake, particularly at higher Cu concentrations by 47.6%, 22.5%, 38.8%, 29.6%, and 26% in shoot, while in root Cu accumulation decreased by 32%, 23%, 15%, 11%, and 18% from Cu0 to Cu400 mg/kg respectively as compared to treatments without PSMPs. These findings suggest that the presence of PSMPs hinders the uptake of Cu in maize and is consistent with previous studies that found that PSMPs reduced the uptake of Cu and Cd in wheat (Zong et al., 2021).

However, it has been reported that increasing Cu in soil disturbed the uptake of other mineral nutrients. Correlation analysis revealed a negative correlation between soil pH and micronutrients in shoot and root except for Zn root uptake. While there was a positive correlation between Cu and other micronutrient content in the shoot and root, except Zn root uptake (Figure 3.9). Zn, Fe, and Mn content in the shoot increased with an increase in soil and shoot Cu content. On the other hand, Fe and Mn increased while Zn decreased with an increase in root a. These results are in line with previous studies, which found that Cu stress increased the uptake of Fe and Mn in root and shoot of sugarcane and maize with an increase in soil Cu (Wyszkowski & Brodowska, 2020; Zeng et al., 2019). Moreover, (Tamez et al., 2019) reported that sugarcane root absorbed more Mn and Fe with an increasing Cu. An increase in Mn and Fe content in maize root might be due to the increased bioavailability of other micronutrients by Cu addition (Liu et al., 2021). The increased bioavailability of other micronutrients by Cu addition is thought to be the reason for the increase in Mn and Fe content in maize root. However, Zn decreased in maize root with an increase in soil Cu, possibly due to Cu^{+2} and Zn^{+2} ions competing for uptake by the same membrane transporters and both have the same ionic charge and size, competing for uptake in the plant (Romdhane et al., 2021).

PSMPs application significantly reduces the uptake of micronutrients in maize in a concentration-dependent manner. The reduction in uptake increased soil pH caused by PSMPs reduced the bioavailability of other micronutrients, resulting in reduced uptake in maize. In the case of Fe, there was a non-significant decrease in uptake among different concentrations of PSMPs. This might be because the chelation strategy is less sensitive to pH. Maize release phytosiderophores through their efflux transporters and uptake Fe present in the rhizosphere in the form of Fe^{+3}-phytosiderophore complex.

In addition to this, PSMPs act as super adsorbing agents, adhering cationic nutrients and pollutants in the soil on their surface by the electrostatic force of attraction, making it harder for plants to absorb them. A previous study (Z. Zhang et al., 2022), found that PSMPs reduce the uptake of Cd uptake in Chinese cabbage. It was concluded that the application of PSMPs had a positive effect on Cu uptake while negatively affecting the uptake of other mineral nutrients in Cu-contaminated soil.

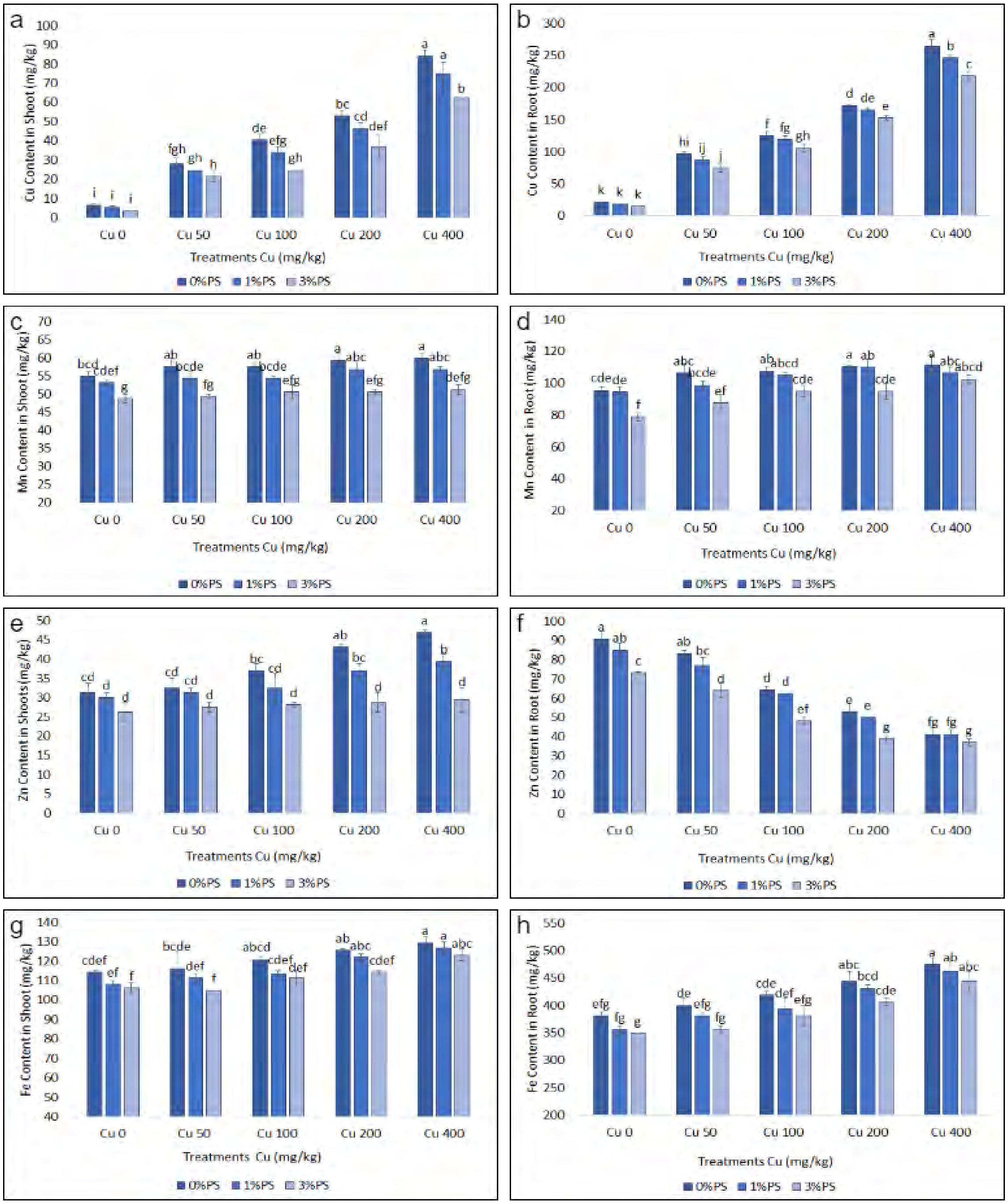

Figure 9 : 3.6. Micronutrients uptake under different Cu and PSMPs concentration, (a) Cu in shoot, (b) Cu in root, (c) Mn in shoot, (d) Mn in root, (e) Zn in shoot, (f) Zn in root, (g) Fe in a shoot, and (h) Fe in root . Data presented in graph is average of three repeats (n=3) ± standard deviation (SD). Different lowercase letters represent statistical differences among the treatments at ($P < 0.05$) according to the Tukey test.

3.6 PSMPs and Cu impact on enzymatic assays

Results depicted that, overall antioxidant enzyme activities in root and shoot increased up to Cu200 mg/kg, however subsequent, increase in Cu concentration (Cu400 mg/kg) decreased enzymatic activity in all PSMPs concentrations (Figure 3.7). In treatments without PSMPs, SOD activity in shoot increased by 40.3%, 87.6%, 61.8%, and 5.4%, while in roots, SOD activity increased by 27%, 57%, 62%, and 21% from Cu50, Cu100, Cu200, and Cu400 mg/kg respectively as compared to control as shown in Figure 3.7(a) and (b). Similarly, CAT activity in shoot increased by 2.9%, 14.4%, and 2.6%, and decreased by 41.6%, while in roots increased by 13.6%, 23.3%, 12.8% and decreased by 25.8% Cu50, Cu100, Cu200 and Cu400 mg/kg respectively as compared to control as shown in Figure 3.7(e) and (f). Overall these findings are consistent with previous literature that found Cu increased the production of antioxidant enzymes in plant tissues (Shuang Gui Tie, 2012).

POD activity in shoot enhanced by 4.9%, and 10.1%, and decreased by 11% and 39.2%, while in root increased by 18.4%, 39.1%, 46.2%, and 3% in Cu50, Cu100, Cu200, and Cu400 mg/kg respectively as compared as shown in Figure 3.7(g) and (h). These results are similar to the study (Rehman, Maqbool, et al., 2019) in which SOD and POD activity increased up to Cu200 mg/kg and then decreased at Cu300 and Cu400 mg/kg as compared to the control in ramie. APX activity in shoot increased by 10.8%, 23.7%, and 33.6%, and decreased by 24.4%, while in roots increased by 22.7%, 48.3%, 62.9%, and 22.9% in Cu50, Cu100, Cu200 and Cu400 mg/kg respectively as compared to control as shown in Figure 3.7 (c) and (d). APX activity increased non-significantly with an increase in Cu till Cu200 mg/kg but significantly ($P < 0.05$) declined at Cu400 mg/kg as compared to control. A similar finding was reached by the reported study on sugarcane, in which Cu addition, increased APX activity (Tamez et al., 2019).

Results showed that PSMPs nullify the Cu-induced oxidative stress by increasing the production of antioxidant enzymes at particularly higher Cu concentration, which may be due to less Cu uptake in the presence of PSMPs. Antioxidant enzyme production is correlated with stress tolerance and was an adaptive response against Cu toxicity. In the presence of PSMPs, maize tolerates more stress at higher Cu concentrations, resulting in more antioxidant enzyme production to scavenge ROS. These findings are tied well with the previous study in which PSMPs increased antioxidant enzyme production in maize (Gong et al., 2021).

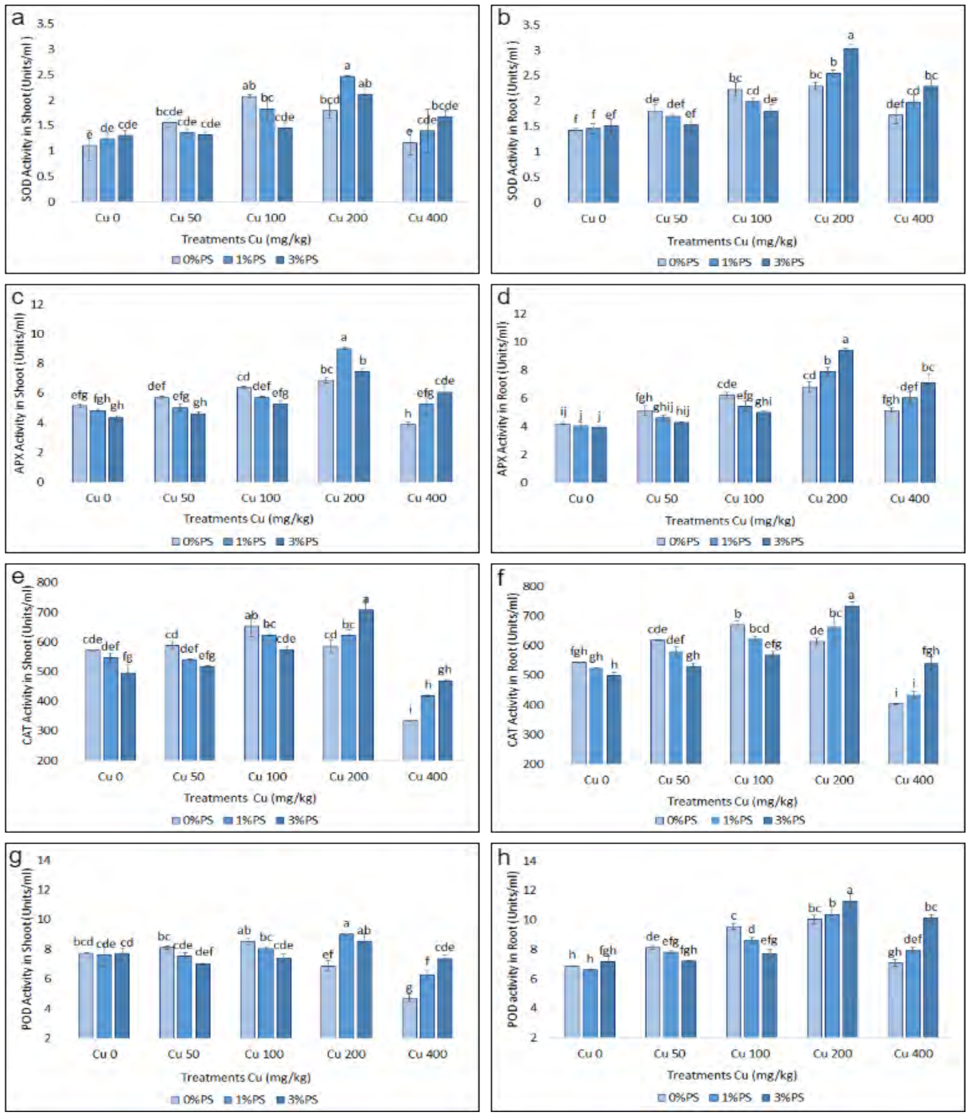

Figure 10 : 3.7. Antioxidant enzyme production under different Cu and PSMPs concentration. (a) SOD activity in shoot, (b) SOD activity in root, (c) APX activity in shoot, (d) APX activity in root, (e) CAT activity in shoot, (f) CAT activity in root, (g) POD activity in shoot, (h) POD activity in root. Data presented in graph is average of three repeats (n=3) ± standard deviation (SD). Different lowercase letters represent statistical differences among the treatments at ($p < 0.05$) according to the Tukey test.

3.7 Lipid peroxidation

In the present study, lipid peroxidation (MDA content) in maize shoot and root significantly increased with Cu concentration while decreased with PSMPs application by concentration-dependent manner (Figure 3.8). Results showed that MDA content in shoot was relatively more than in roots, despite of more Cu accumulation in maize roots. MDA content increased significantly at higher Cu concentrations as compared to control. These results are consistent with previous studies where Cu-induced high level of MDA was observed in sugarcane and ramie (Rehman, Maqbool, et al., 2019; Zeng et al., 2019). MDA content was positively correlated with Cu uptake, however, PSMPs reduce Cu uptake, consequently, alleviate the Cu-induced oxidative damage of cellular membranes. These findings are in line with previous study where PSMPs reduce the MDA content in root and shoot of Chinese cabbage (Z. Zhang et al., 2022). The Cu-triggered lipid peroxidation might be due to the reaction between Cu ions and hydrogen peroxide through Fenton reaction and produce ROS which can further react with unsaturated fatty acids in cellular membranes cause lipid peroxidation and produce MDA as a byproduct (Rehman, Maqbool, et al., 2019; Ullah et al., 2022)

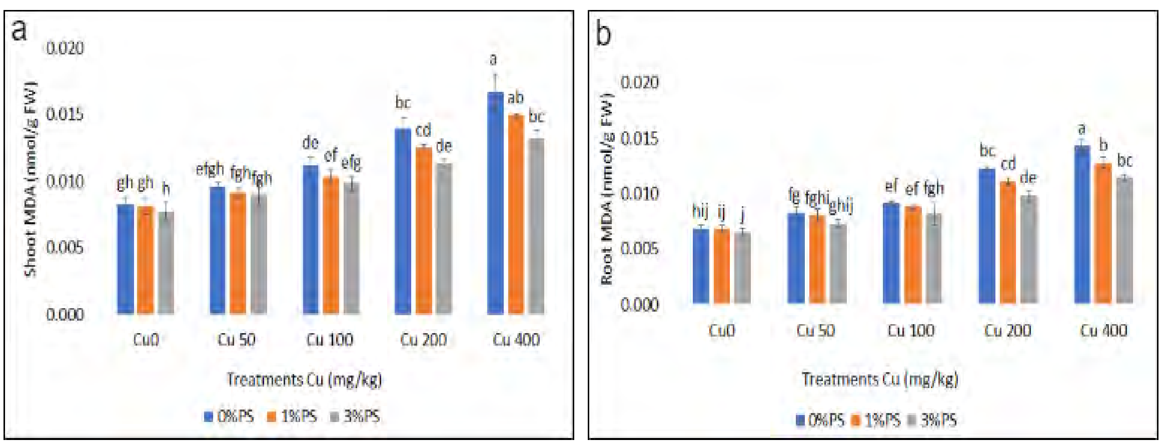

Figure 11 : 3.8. MDA level in maize under different concentrations of Cu and PSMPs (a) shoot and (b) root. Data presented in a graph is the average of three repeats (n=3) ± standard deviation (SD). Different lowercase letters represent statistical differences among the treatments at ($p < 0.05$) according to the Tukey test.

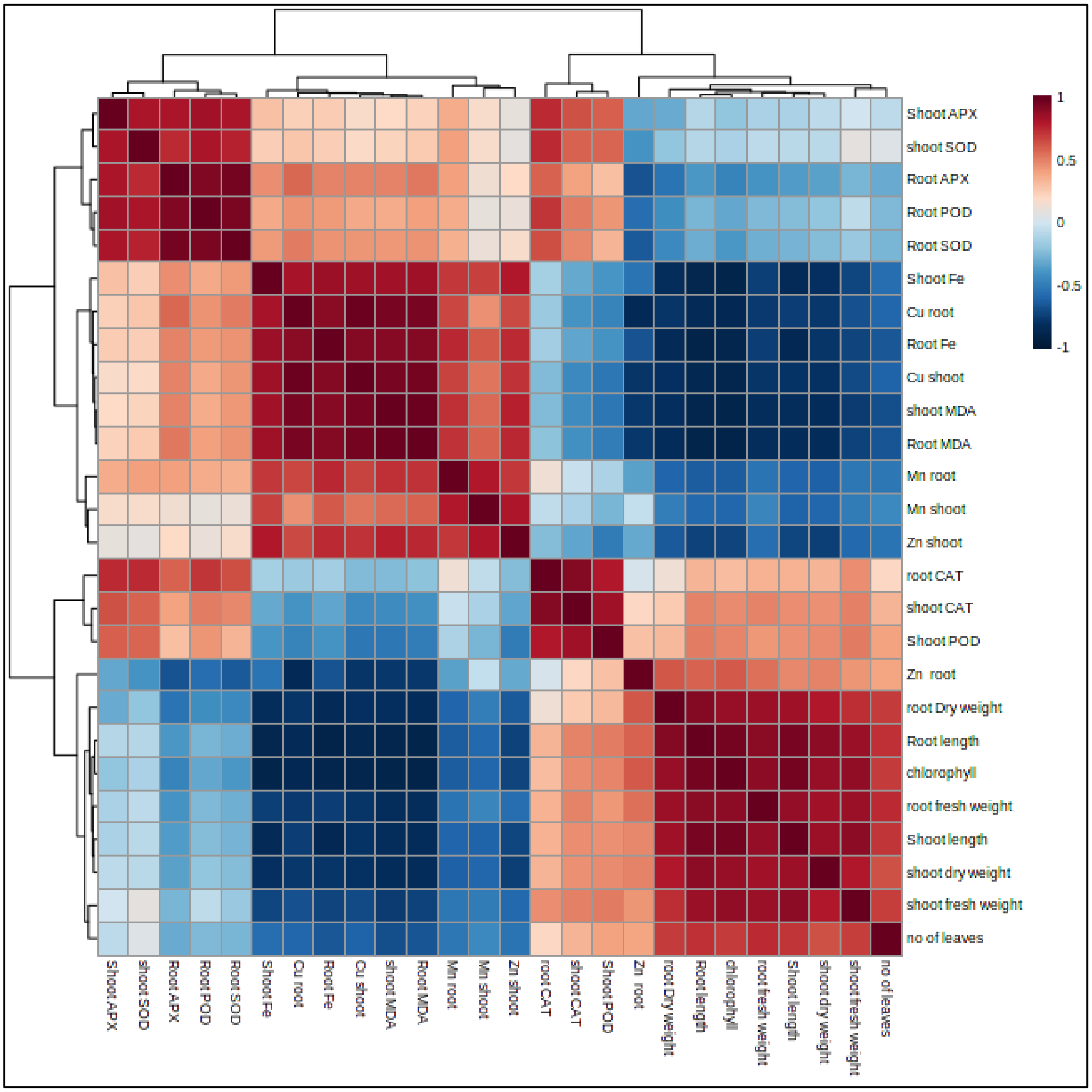

Figure 12 : 3.9. Heat map of Pearson correlation between micronutrient uptake, physiological, and biochemical parameters in maize shoot and root.

3.8 Conclusions

- It was concluded that Cu had an antagonistic relationship with soil pH and a positive correlation with soil 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.
- PSMPs application improved targeted growth parameters of maize in a concentration-dependent manner, and mitigated Cu-induced stress by reducing Cu uptake and increasing the production of antioxidant enzymes in particularly at higher concentrations of Cu.
- Change in maize growth is a function of Cu and PSMPs concentration in soil.

3.9 Recommendations

- PSMPs adsorb the micronutrients in Cu-contaminated soil, but this opens the gate for future research on PSMPs induced micronutrient deficiency in plants.
- Fresh PSMPs immobilize the micronutrients in soil efficiently, but further studies were required to understand the change in the adsorption efficiency of virgin MPs with time.
- In a short period, PSMPs improve plant growth, but in the future, their long-term impacts can be focused on soil biota and plant growth performance under natural field conditions.
- PSMPs toxicity in plants and microbes is size-dependent, so in upcoming studies, this area can be covered.

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Thesis v1

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Mitigating potential of polystyrene microplastics on bioavailability, uptake, and toxicity of micronutrients in *Zea mays* L.

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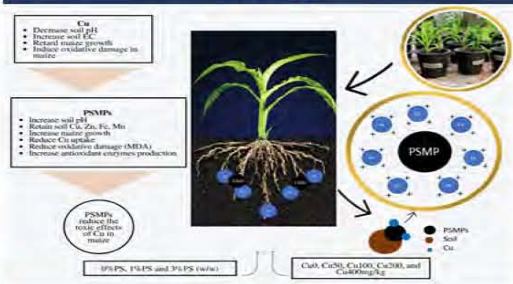
Abstract



Objectives

- To investigate the immobilization potential of PSMPs for Cu and other micronutrients in soil and their uptake by maize in different Cu and PSMPs concentrations.
- To study the effect of PSMPs application on physiological and biochemical parameters of maize under Cu-stressed soil.

Methodology

Results

Soil physiochemical properties

Results showed that soil pH decreased with increasing soil Cu concentration while pH increased with PSMPs application at both stages in a dose-dependent manner. These results are consistent with the studies where Cu and soil pH have an antagonistic relationship (Romdhane et al., 2021; Zand & Mühling, 2022). Decreased soil pH with Cu might be due to a change in soil microbial activity with increasing soil Cu (Naz et al., 2022; Yáñez et al., 2022).

The addition of 1% and 3% PSMPs increased the relative proportion of the micro-aggregate fraction, possibly due to the size of PSMPs used in the study being in the size range of the micro-aggregate fraction and reducing the relative fraction of silt and clay in the soil.

Cu, PSMPs and soil micronutrients

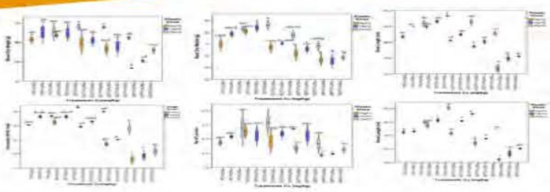
PSMPs positively while Cu impact negatively on other micronutrient content (Zn, Fe, and Mn concentration) decreased with increasing soil Cu and increased with increasing PSMPs concentration in a dose-dependent manner. Results showed that PSMPs retain micronutrients.

Maize growth

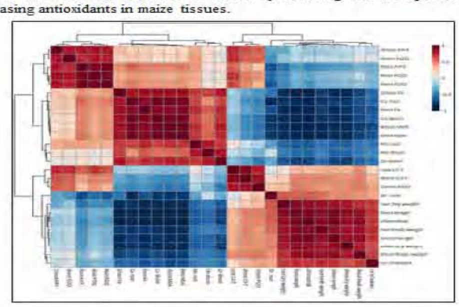
Results showed that the rate of growth is higher in the first four weeks than following weeks in all treatments. The phenotypic traits of maize slightly increased at Cu50 and Cu100 mg/kg, as compared to control while further increase in soil Cu concentration cause a decline in growth, while PSMPs increased chlorophyll content, biomass production, and maize height as compared to treatments without PSMPs. The increase in maize growth with PSMPs might be due increased porosity and aeration of soil with PSMPs which help in root respiration and penetration.

Micronutrient uptake

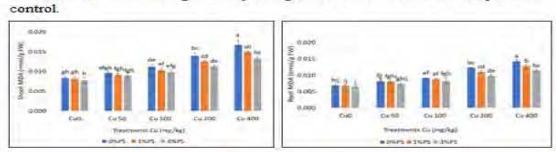
PSMPs reduced the uptake of (Cu, Zn, Mn and Fe) in maize root and shoot. However, Cu increased the uptake other micronutrients (Zn, Mn and Fe) in shoot and root except uptake of Zn in roots. PSMPs act as super adsorbing agent, adhere cationic nutrients in the soil on their surface by the electrostatic force of attraction, making it harder for plants to absorb them (Zong et al., 2021). PSMPs decreased the Cu induced oxidative damage (MDA content) as compared to treatments without PSMPs. Results of Pearson correlation revealed a negative correlation between copper uptake and phenotypic parameters while positive correlation with MDA content.



Antioxidant enzymes production (APX, CAT, POD, and SOD) increased in maize root and shoot up to Cu200 mg/kg while PSMPs increased the production of antioxidant enzymes particularly at higher Cu concentration. PSMPs increased the tolerance of maize by reducing the Cu uptake and increasing antioxidants in maize tissues.



Lipid peroxidation (MDA content) in maize shoot and root significantly increased with Cu concentration while decreased with PSMPs application by concentration-dependent manner. Results showed that MDA content in shoot was relatively more than in roots, despite of more Cu accumulation in maize roots. MDA content increased significantly at higher Cu concentrations as compared to control.



Conclusions

- In the present study, Cu showed a negative relationship with soil pH and a positive correlation with soil EC in pre-sowing soil while in post-harvesting soil, antagonistic relation with soil pH, EC, and other micronutrients.
- PSMPs showed a positive correlation with soil pH (and micronutrients in post-harvesting soil) and negatively correlate with EC in both soils. Maize withstands up to Cu100 mg/kg of soil without any significant decline in its morpho-physiological parameters, while further increase in Cu concentrations (Cu200 and Cu400 mg/kg) exhibit growth retardation.
- The PSMPs application improved the targeted growth parameters in a concentration-dependent manner. PSMPs mitigate the Cu-induced oxidative stress by reducing Cu uptake and increasing the production of antioxidant enzymes in maize, at higher soil Cu concentrations.
- Change in maize growth is a function of Cu and PSMPs concentration in soil.

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