Copper Effect on Cu Uptake, Earthworm AChE Activity, Chlorophyll Content, Gas Exchange Parameters of Mung bean Mitigated by Copper Tolerant Bacteria under Cu Contaminated Soil

Shazia Iqbal[1], Hafiz Khurram Shurjeel[2*], Sarvet Jehan[3], Munaza Naseem[4], Hina Sattar[5], Naila Abbas[4], Fariha Sohail[4] and Nasir Khan[6],

[1]University of Sargodha-Pakistan
[2]University of Agriculture, Swat-Pakistan
[3]PMAS Arid Agriculture University, Rawalpindi- Pakistan
[4]The women university, Multan
[5]Government College University Faisalabad- Pakistan
[6]Agriculture Research Institute, Mingora-Pakistan

Corresponding Author' email: shurjeel_38@yahoo.com

ABSTRACT:

Copper (Cu) is an essential yet dangerous element at high concentrations for plant growth as well as for human health after it enters the food chain. Cu comes from the mining of waste and creates a problem for plants in the soil. Cu tolerant bacteria may help to reduce Cu uptake by the plant and mitigate the effect of Cu on the gas exchange parameters, Cu uptake of plant. This study was planned to study the effect of Cu tolerant bacteria on the earthworm activity, Cu uptake, and physiological parameter of the mung bean crop. Three Cu tolerant bacterial strains SC1, SC2, and SC3 at two Cu levels 0 and 150 mg kg^{-1} of soil were used. Results showed that Cu application reduced the AChE assay for earthworm activity, increased the Cu uptake both in root and shoot but Cu uptake was more in root compared to shoot. Physiological gas exchange parameters were negatively affected by Cu application. Cu application at 150mg kg^{-1} soil reduced the earthworm activity (31%) compared to the control. Cu tolerant bacterial strains inoculum improved the gas exchange parameters photosynthetic rate (43%), transpiration rate (48%), stomatal conductance (39%), substomatal conductance (34%), and total chlorophyll content (28%) while reducing the uptake of Cu in the shoot (24%) and root (26%) compared to 150 mg Cu kg^{-1} of the soil application treatment. It is concluded that the use of Cu-tolerant bacterial strains as inoculum can help improve the chemical status and gas exchange parameters of crops grown in Cu-contaminated soils.

Keywords: Cu contaminated soils, Cu tolerant bacteria, Gas exchange parameters, Earthworm activity

INTRODUCTION:

By 2030, the sustainable development goals want to achieve food security. Targeting different aspects of food security, where food safety is a major factor, becomes crucial if food security is to be achieved. The safety issue is frequently disregarded as efforts are made to increase food production to meet the demands of the expanding population. The ambient environment has been affected and harmed by irrational human activities. Contaminants have accumulated in soil and water due to degradation, and this has further transferred contaminants into the food chain. Many regions of the world have reported an increase in the amount of heavy metals in our environment. Additionally, as metal concentrations in soil and water rise, these metals enter the food chain. Contrary to other metals, Cu is necessary for living things, but excessive consumption of it has been linked to toxic effects on living things (Sharma et al., 2021).

Contaminants are absorbed by crops grown on polluted lands and with wastewater. There have been reports of an increase in heavy metal content in different food crops around the world (Sarker and Oba, 2021; Tariq, 2021; Wu et al., 2021). Different plant species have varying capacities for accumulating Cu depending on the growth stage and fertilizer application. Cu toxicity primarily affects root growth and shape because Cu is quickly absorbed by plant roots and not easily translocated to aboveground parts of the plant. The significant concentration of Cu in roots effectively regulates the transfer of Cu into aboveground plant components. Previous research on plants including Phyllostachys pubescens, Amaranthus spinosus, Eclipta alba, Boehmeria nivea, Oryza sativa, Trigonella foenum-graecum, Triticum aestivum, and Willows (Salix spp.) has revealed that Cu tends to accumulate in root tissues with little upward migration towards shoots. Cucumber was found to have the highest concentration of Cu (95.31 mg/kg dry weight). The capacity of a plant to store Cu in its roots and effectively stop it from being translocated to photosynthetic areas is correlated with its tolerance to Cu (Sharma et al., 2021).

Additionally, it decreases earthworm activity when the soil's Cu content exceeds 50 mg kg^{-1} (Graber et al., 2006). Plant growth is significantly impacted by earthworm activity, which influences both biotic and abiotic soil properties (Van Groenigen et al., 2014). Earthworms aerate the soil and add nutrients by breaking down and digesting decaying matter (Ojha & Devokta, 2013). Cu soil contamination has been shown to decrease AChE levels in earthworm Eisenia foetida starting from a concentration of 200mg of Cu per kg of soil (Bednarska et al., 2017). Its higher concentration has a negative impact on the photosynthetic machinery and process (González-Mendoza et al., 2013; Wang et al., 2014), nitrogen assimilation (Zhang et al., 2014), cell wall metabolism, mitochondrial electron transport chain, and the development of root hair (Liu et al.,2014).

Cu-contaminated soils can be remedied using a variety of methods, including chemical, physical, and biological ones. All of these methods have some drawbacks, such as being expensive, time-consuming, or not all that environmentally friendly. The biological approach uses two strategies. Both phytoremediation and bioremediation are effective methods for cleaning up contaminated soil because they are less expensive, pleasant to the environment, and best suited for developing nations (Ghosh and Singh, 2005). Microorganisms are uniquely able to detoxify, assimilate, and mortify both organic and inorganic compounds in the body. In an incubation study, Cu-tolerant bacteria reduced the Cu level in soil samples (Samala and Kotiyal, 2013). Metal-tolerant bacteria may be able to survive in these habitats despite the toxic effects that metals have on microorganisms through a variety of mechanisms. These bacteria may then be isolated and chosen for use in the bioremediation of contaminated sites (Piotrowska-Seget et al., 2005). The ability of plants to tolerate heavy metals may also be enhanced by metal-tolerant bacteria that can produce the enzyme ACC deaminase (Glick et al., 1998). In heavy metal-contaminated soils, it has been suggested that ACC-using bacteria could encourage plant growth and shield plants from heavy metal toxicity (Madhaiyan et al., 2007). These bacteria can be used for mitigating the adverse effects of Cu in Cu-contaminated soil when plants are grown in these soils.

Mung bean is an important leguminous crop due to its high nutritional value. Previously very few studies on the effect of Cu-tolerant bacteria on mung bean plant Cu accumulation and mitigating the Cu effects are carried out. So it was hypothesized that Cu-resistant bacteria may reduce the uptake of Cu, and improves gas exchange parameters and earthworm activity. The objective of this study was to mitigate the adverse effects of Cu on gas exchange parameters and biological and chemical aspects of a mung bean plant.

MATERIALS AND METHODS:

Pot experiment

A pot experiment was conducted to evaluate the effect of Cu tolerant bacteria on gas exchange paameters, and chemical and biological parameters of mung beans under Cu-contaminated soil. The Cu tolerant bacterial strains SC1, SC2 and SC3 were usedd and a pot trial was carried out in the wirehouse. 12 Kg pots were filled with 8 kg soil and recommended doses of N, P, and K for mung were applied in all pots. Before experimentation, different levels of Cu (0, 150, mg Cu L-1) were applied to the soil by adding 2.48L of each level of Cu in the soil according to the saturation percentage of soil as mentioned in Table 7.1 with the help of a measuring cylinder and mixed thoroughly. The control treatment was not received any Cu. The leaching of soil was controlled by using a ploy and then plastic bags. Thereafter, the pots were irrigated with water and incubated for two weeks to get uniform distribution of Cu.

Inoculum preparation

Luria Bertani (LB) broth was used to make the inoculum in conical flasks. For three days, it was incubated at 28±2 ^{o}C in an orbital shaking incubator. To make the inoculation slurry, 1.25:1 w/w sterilized peat and clay were combined with 5 mL of a 15% sterilized sugar solution. Seeds treated with autoclaved slurry without inoculum were used as the control, while specific bacterial strains were mixed with it to coat the seeds. Overnight, inoculated seeds were left in the shade to dry. Each pot contained 8 kg of soil containing 5 dried seeds, which were later thinned to one plant after fifteen days of germination. All agronomic procedures, such as irrigation and plant protection techniques, were carried out consistently.

Physiological parameters

Total chlorophyll

With the aid of a portable SPAD chlorophyll meter, it was measured. The third fully expanded leaf of the mung bean plant was chosen for this purpose, and all readings were completed between 10:00 and 11:00 am.

Gas exchange parameters

On the 45th day of mung bean growth, attributes related to gas exchange, such as sub-stomatal conductance, stomatal conductance, photosynthetic rate, and transpiration rate, were measured under both normal and Cu-stressed conditions. Portable CIRUS-3, made by PP Systems in Amesbury, Massachusetts, was employed for this. All of the CIIRUS readings were collected between 10:00 and 11:00 am from the third fully expanded mung bean leaf.

Soil analysis

Physical and chemical characteristics of the soil samples were determined (Table 1) using the procedures demonstrated by US Salinity Laboratory Staff (1954). Bouyoucos hydrometer method was used for particle size analysis following. Determined the AB-DTPA extractable Cu from the extract on the atomic absorption spectrophotometer. The saturation percentage of the soil sample was computed using the formula described in Method 27a. From the soil paste, pH was measured (Method 21a). The saturated extract was collected in a bottle (Method 3a). Using a digital conductivity meter, the electrical conductivity of the extract was dogged by (Method 3a and 4b). Organic matter was determined by following the method of Moodie et al. (1959). The H_2SO_4 digestion method of Ginning and Hibbard was used, and macro Kjeldahl (MRK DF-4S) was used for distillation (Jackson, 1962). Watanabe and Olsen was used for measuring the soil's available P using a spectrophotometer (UV-VIS 6000) Watanabe and Olsen, 1665). Using the Flame photometer, potassium analysis in soil was performed (Sherwood 410).

Table 1. Physicochemical properties of used soil

Characterization	Unite	Soil
Texture class		Sandy clay loam
ECe	dSm^{-1}	2.8
pHs		8.1

Organic matter	%	0.33
Saturation %	%	31
Total N	%	0.057
Available P	mg kg^{-1}	6.22
Extractable K	mg kg^{-1}	102
Total Cu	mg kg^{-1}	0.49

Chemical analysis

Oven-dried root and shoot were digested by using 3:1 nitric acid and per-chloric acid. Samples were then filtered and Cu was analyzed at atomic absorption spectrophotometer

Muscle Acetylcholinesterase Activity Level

Muscle Acetylcholinesterase Activity Level was measured at two Cu levels 0 and 150 mg kg^{-1} soil following the procedure described by Meregalli, (2017).

Statistical analysis

Two ways analysis of variance (ANOVA) was performed using STATISTIX (8.1) with two factor factorial design and Tuckey's HSD test was used for all pair wise comparison.

RESULTS:

Muscle Acetylcholinesterase Activity Level

Data for Muscle Acetylcholinesterase Activity Level is presented in Fig 1. Statistically significant results were recorded ($p \leq 0.05$). Cu application (150 mg kg^{-1} soil) reduced the Muscle Acetylcholinesterase Activity compared to the control and that reduction was 31%.

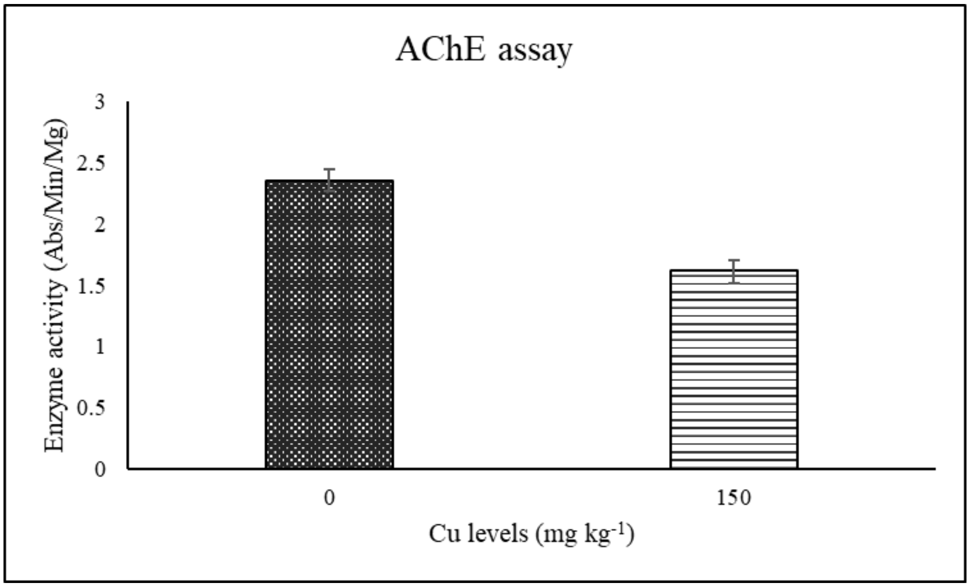

Fig 1. Effect of Cu contaminated environment on AChE assay

Effect of Cu and Cu tolerant bacteria on total chlorophyll content and gas exchange parameters

Figure 2 shows the total chlorophyll content as affected by Cu stress and Cu-tolerant bacteria. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p<0.05$). When compared to the control, a Cu application at a rate of 150 mg P kg-1 of soil reduced total chlorophyll content by 24%. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased total chlorophyll content by 8, 15, and 17%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} of soil, Cu tolerant bacteria increased total chlorophyll content by 7, 24, and 28%, respectively, compared to the application of Cu at the rate of 150 mg kg^{-1} of soil.

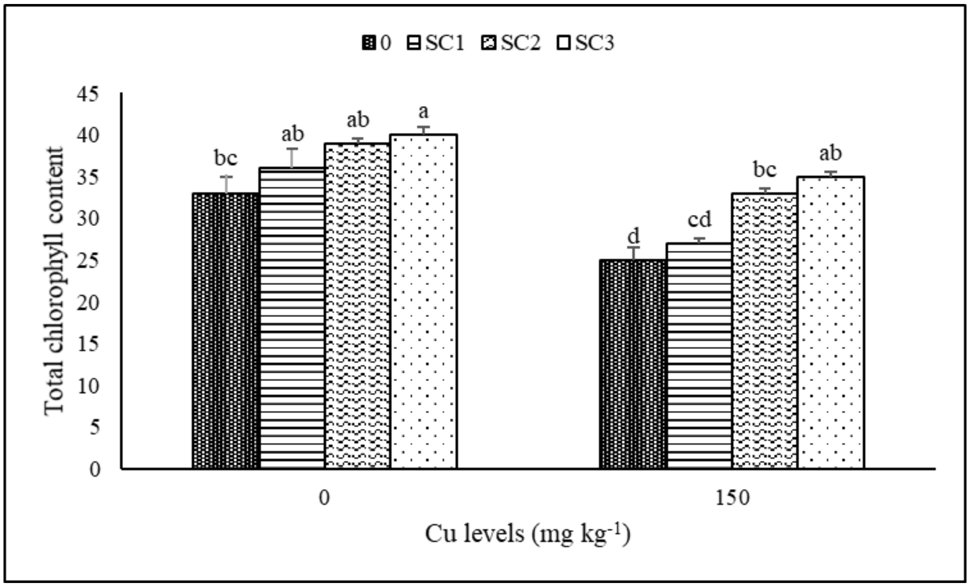

Fig 2. Total chlorophyll content affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at $p \leq 0.05$ (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

The photosynthetic rate affected by Cu stress and Cu-tolerant bacteria is shown in Fig 3. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p \leq 0.05$). When Cu application was done at a rate of 150 mg P kg^{-1} of soil, a reduction of 24% was observed in the photosynthetic rate compared to the control treatment. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased photosynthetic rates by 8, 21, and 26%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} soil, Cu tolerant bacteria increased their photosynthetic rate by 28, 37, and 43%, respectively, compared to the application of Cu alone at the rate of 150 mg kg^{-1} of soil. The maximum photosynthetic rate was observed when bacterial strain SC3 has used alone (25.3 µmol CO_2 m^{-2} s^{-1}) or at 150 mg Cu kg^{-1} of soil (24.6 µmol CO_2 m^{-2} s^{-1}).

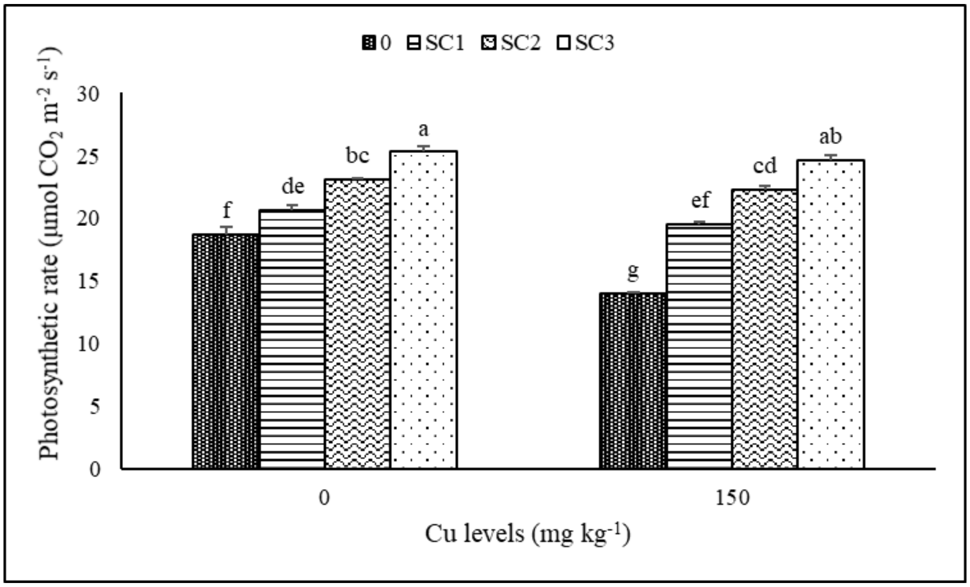

Fig 3. Photosynthetic rate affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at $p \leq 0.05$ (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

Transpiration rate as affected by Cu application and Cu tolerant bacteria is presented in Fig 4. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p \leq 0.05$). When Cu application was done at a rate of 150 mg P kg^{-1} of soil, a reduction of 25% was observed in transpiration rate compared to the control treatment. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased transpiration rate by 8, 21, and 26%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} of soil, Cu-tolerant bacteria increased transpiration rate by 28, 34, and 43%, respectively, compared to the application of Cu alone at the rate of 150 mg kg^{-1} of soil. Maximum transpiration rate was observed when bacterial strain SC3 was used alone (5.5 mmol H2O m^{-2} s^{-1}) or at 150 mg Cu kg^{-1} of soil (4.5 mmol H2O m^{-2} s^{-1}).

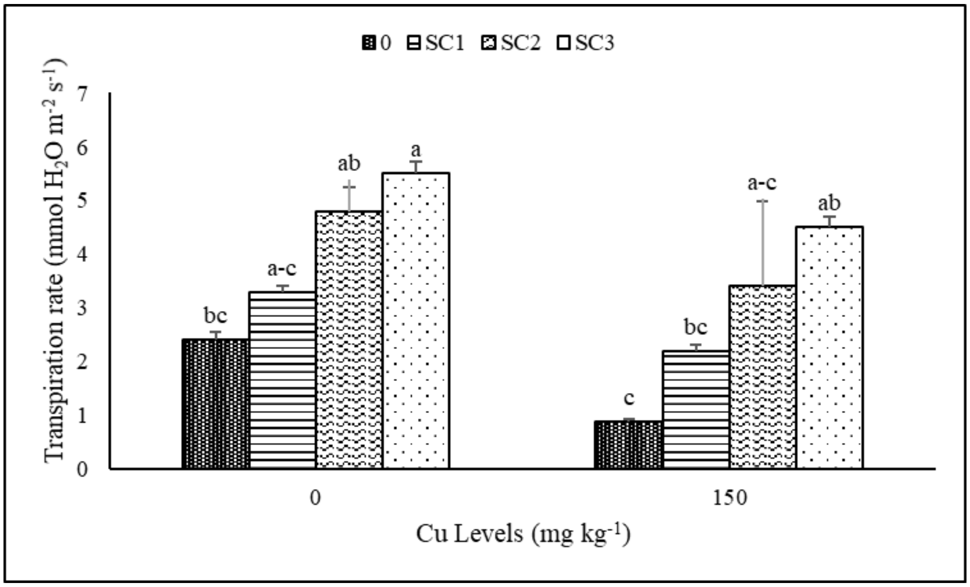

Fig 4. Transpiration rate affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at $p \leq 0.05$ (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

Figure 5 shows the stomatal conductance affected by Cu stress and Cu-tolerant bacteria. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p \leq 0.05$). When compared to the control, a Cu application at a rate of 150 mg P kg^{-1} of soil reduced stomatal conductance by 15%. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased stomatal conductance by 21, 30, and 37%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} soil, Cu tolerant bacteria increased stomatal conductance by 10, 34, and 39%, respectively, compared to the application of Cu alone at the rate of 150 mg kg^{-1} of soil.

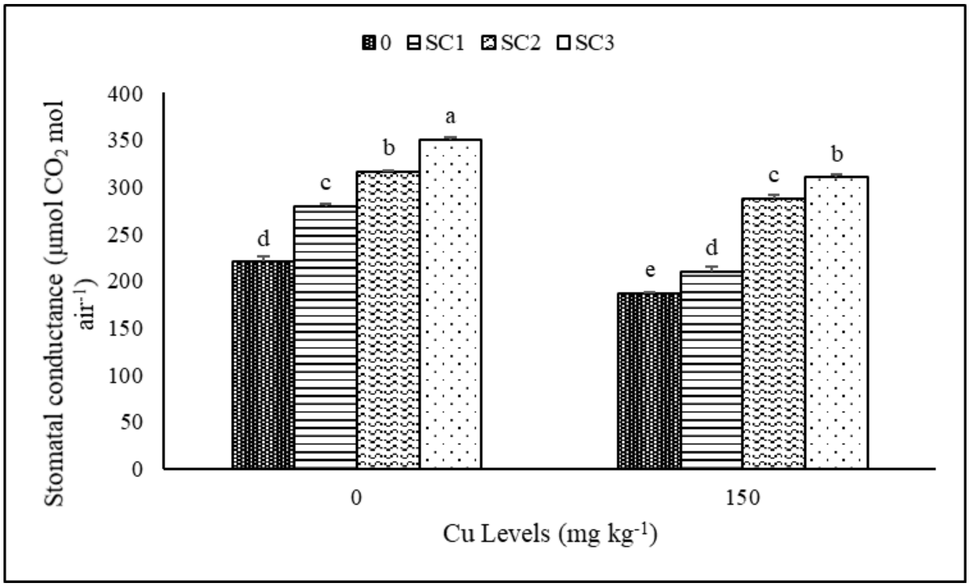

Fig 5. Stomatal Conductance affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at $p \leq 0.05$ (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

Figure 6 shows the sub stomatal conductance affected by Cu stress and Cu-tolerant bacteria. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p \leq 0.05$). When compared to the control, a Cu application at a rate of 150 mg P kg^{-1} of soil reduced sub stomatal conductance rate by 13%. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased sub stomatal conductance by 21, 35, and 43%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} of soil, Cu tolerant bacteria increased sub stomatal conductance by 4, 25, and 34%, respectively, compared to the application of Cu alone at the rate of 150 mg kg^{-1} of soil.

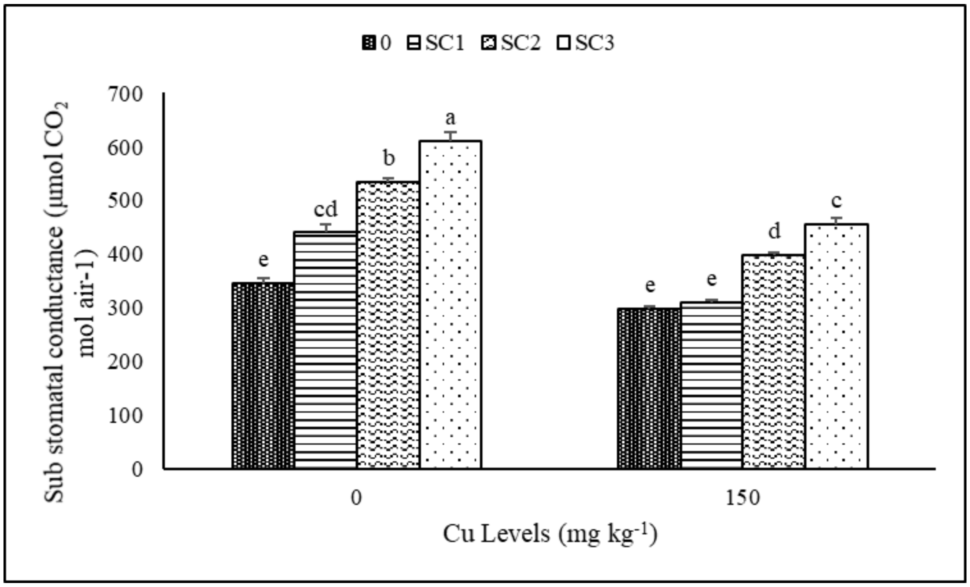

Fig 6. Substomatal Conductance affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at $p \leq 0.05$ (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

Effect of Cu and Cu tolerant bacteria on Cu uptake by root and shoot

Figure 7 shows the root Cu content affected by Cu stress and Cu-tolerant bacteria. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p \leq 0.05$). Root Cu content increased with the addition of Cu but Cu tolerant bacterial strains decreased the uptake of Cu. When compared to the control, a Cu application at a rate of 150 mg P kg^{-1} of soil reduced root Cu content by 38%. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased root Cu content by 6, 16, and 23%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} of soil, Cu tolerant bacteria increased root Cu content by 6, 17, and 26%, respectively, compared to the application of Cu alone at the rate of 150 mg kg^{-1} of soil. Minimum shoot Cu content was observed when bacterial strain SC3 has used alone (210 mg kg^{-1} dwt) or at 150 mg Cu kg^{-1} of soil (325 mg kg^{-1} dwt).

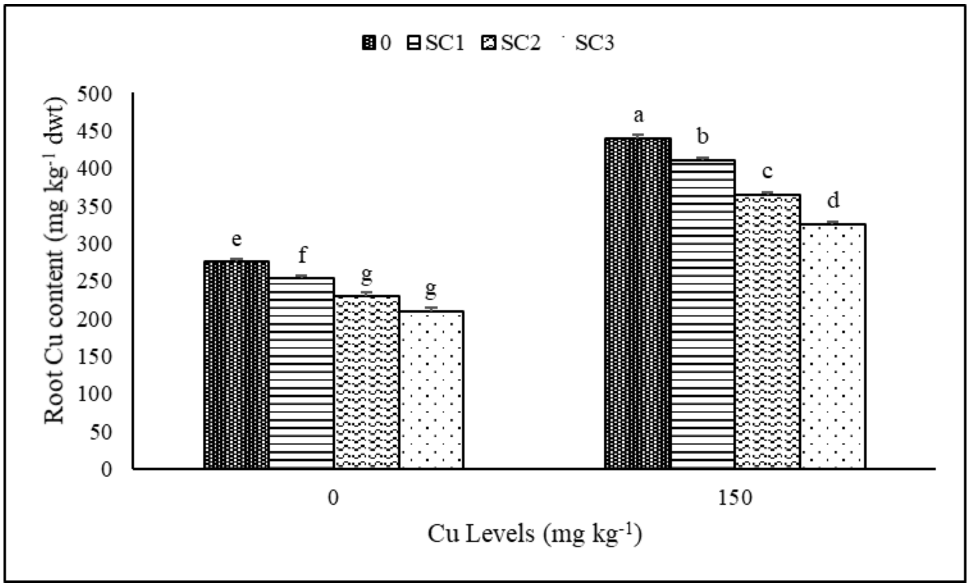

Fig 7. Root Cu content affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at p≤0.05 (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

Shoot Cu contents increased with adding the Cu concentration. Figure 8 shows the shoot Cu content affected by Cu stress and Cu-tolerant bacteria. Statistically significant differences among different Cu tolerant bacterial strains were reported ($p \leq 0.05$). When compared to the control, a Cu application at a rate of 150 mg P kg^{-1} of soil reduced shoot Cu content by 30%. Application of the Cu-tolerant bacterial strains SC1, SC2, and SC3 increased shoot Cu content by 7, 17, and 21%, respectively, compared to the control. When Cu was applied at the rate of 150 mg kg^{-1} of soil, Cu tolerant bacteria increased shoot Cu content by 7, 17, and 24%, respectively, compared to the application of Cu alone at the rate of 150 mg kg^{-1} of soil. Minimum shoot Cu content was observed when bacterial strain SC3 was used alone (110 mg kg^{-1} dwt) or at 150 mg Cu kg^{-1} of soil (152 mg kg^{-1} dwt).

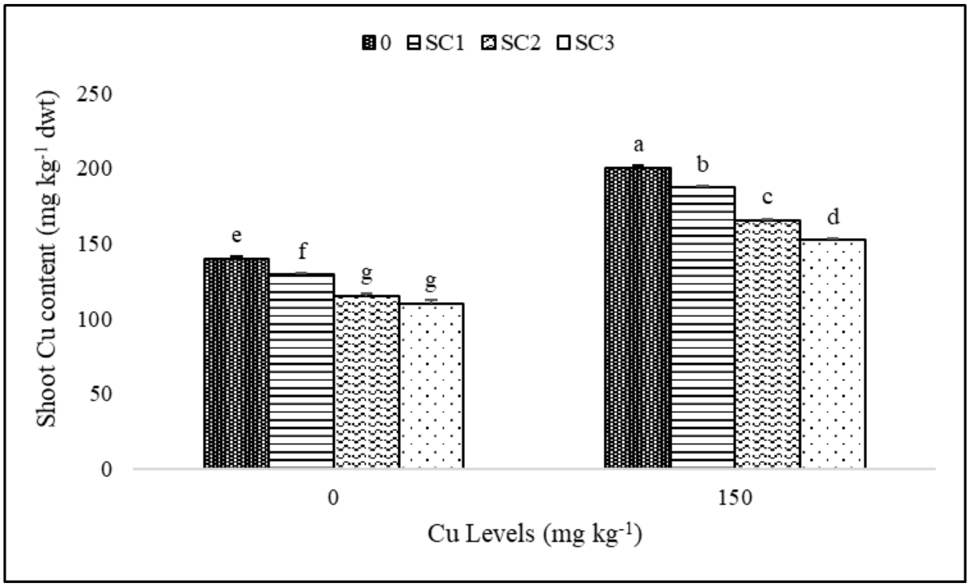

Fig 8. Shoot Cu content affected by Cu application and Cu tolerant bacteria. The error bar Shows the Standard error with 3 replications at p≤0.05 (n=3). SC1, SC2, and SC3 are the Cu tolerant bacterial strain. The same letter shows that there is no significant difference between treatments. Different letters show a significant difference.

DISCUSSION:

Soil contaminated with metals like Cu is one of the most important issues which causes the loss of agricultural soil and also enters in the food chain (Gall et al., 2015). In pot trial under Cu-contaminated soil (0, and 150 mg kg^{-1} soil) amended with bacterial strains SC1, SC2, and SC3 on physiology, and Cu concentration of mung bean and on earthworm activity was investigated. This study illuminated that Cu exogenous application strictly reduces physiological parameters of mung bean whereas amplified Cu concentration accumulation in plant tissues. Yet, the application of bacterial strains, improved physiology, and reduced Cu concentration in root and shoot of mung bean crops. Under Cu stress, various gas exchange characteristics, including net transpiration rate (A), stomatal conductance (gs), and sub stomatal conductance, significantly decreased. Ultimately, photosynthetic processes also decreased under Cu stress, indicating significant damage to the mung bean plants' photosystem. However, plants grown in soil inoculated with cu tolerant bacterial strains showed less of a decrease in various gas exchange attributes caused by Cu stress, demonstrating the beneficial effects of inoculation in Cu stress tolerance (Islam et al., 2015).

In this study, the chlorophyll contents and the photosynthetic rate was severely reduced under Cu stress. Cu-induced reductions in photosynthetic pigments may also be related to changes in the thylakoid membrane composition and chloroplast structure, as well as to changes in photophosphorylation of PS I and PS II, photoinhibition, and the photosynthetic electron transport chain (Sharma et al., 2017; Li et al., 2019). Similar reductions in these pigments have been observed in plants including maize, jute (Saleem et al., 2020; Parveen et al., 2020), cilantro (Zaouali et al., 2020), lentil (Hossain et al., 2020), spearmint (Chrysargyris et al., 2020), and cilantro (Zaouali et al., 20)**.** Total chlorophyll contents are crucial for photosynthesis and are extremely sensitive to abiotic environmental stresses. (John et al., 2009). Cu tolerant bacterial inoculation improved the chlorophyll content compared to the non inoculated Cu stressed soil. The same kind of results was reported by Islam et al. (2015) who reported that higher leaf chlorophyll content, number of pods, root nodulation, and 1000 seed weight were also higher in inoculated plants as compared with non-inoculated ones. Another study found that P. polymyxa and B. circulans inoculation increased the photosynthetic pigments of maize plants under both ideal and Cu stress conditions in each treatment group (Latef et al., 2020).

The photosynthetic rate, transpiration rate, stomatal conductance, and sub stomatal conductance of the plant were also reduced under Cu stress. Cu's function in photosystems and its inhibition of the secondary quinone acceptor QB are the main causes of the decline in photosynthetic and transpiration rates. Reduced photosynthetic and transpiration rates may have been caused by the chloroplast's grana, an essential location for carrying out photosynthetic processes (Baszynski et al., 1988). Reduced photosynthetic rate may be caused by plants' reduced ability to absorb light when stressed and by a blockage of the electron transport chain reaction, which also causes chlorosis (Gu et al., 2017). Our results are in line with Yagura et al. (2019), who observed reduced photosynthetic activity at higher Cu concentrations. Under Cu stress, the mung bean's stomatal and sub-stomatal conductance decreased. This reduction may be attributable to Cu's impact on the plant's leaf lamina, which thickened due to Cu toxicity, causing glandular and non-glandular hairs to grow in the stomata. It also decreased the amount of starch and chlorophyll in grain chloroplasts in mesophyll cells (Panou-Filotheou et al., 2001). The application of Cu tolerant bacterial strains increased the gas exchange parameters. Similar results were reported by Islam et al. (2015). They reported that a notable increase in different gas exchange characteristics such as photosynthetic rate, transpiration rate, stomatal conductance, substomatal conductance, as well as an increase in N and P accumulation was also recorded in inoculated plants as compared to un-inoculated Cu stressed plants.

The amount of available metal in the soil has an impact on the uptake and accumulation of Cu in plant parts, including both aerial and root-to-shoot transportation (Goncalves et al., 2009). In this study, Cu accumulation increased in both root and shoot but more accumulation was reported in roots compared to shoots (Fig, 7, 8) suggesting that there is less Cu translocation from root to shoot because the majority of heavy metals are sequestered in the vacuoles of root cells, rendering them non-toxic (Shanker et al., 2005). The fact that Cu is so readily available in soil when mung beans are planted, both in amended and unamended soils, may account for the higher Cu concentration in roots. With increasing exogenous application in soil, Cu accumulated in plant tissue and accumulated in a significantly higher proportion in plant roots. Such outcomes have previously been reported (Gupta, 2011). Additionally, the rate of transpiration and intercellular CO_2 accumulation affect the accumulation of Cu in plants. The current study demonstrated that when exposed to Cu under stress, the tested crop's stomatal, sub-stomatal, and transpiration machinery was severely reduced. This could be the cause of the high Cu concentration in plant tissue. Cu-tolerant bacteria reduced the Cu accumulation in both root and shoot. By stabilizing Cu in the soil and preventing its uptake or accumulation in plant parts, bacterial strains induced resistance in plants. According to the earlier study, P. vermicola helped Cu immobilize by releasing phosphorus from insoluble BP compounds, which reduced the amount of Cu uptake (Ma et al., 2013). Additionally, the lower concentration of Cu in the upper ground parts may be the result of the P. vermicola strain's ability to remove Cu through the process of adsorption and desorption (Islam et al., 2014a, b). In another study, the inoculation of maize plants with *Cu tolerant bacterial* induced a significant decrease in Cu content compared to control plants (Latef et al., 2020**).** Our findings are supported by the findings of Islam et al. (2015) who reported that bacterial inoculation decreased the Cu uptake in lentil plants grown in Cu-spiked soil as compared to the control.

Cu application decreased the Muscle Acetylcholinesterase Activity Level for the earthworm. This might be due to the toxicity of Cu to earthworms that cause most soft tissues to be unable to produce Cu-binding ligands when challenged with Cu (Morgan and Morgan 1990).

In an experiment Meregalli, (2017) reported that *Eisenia foetida* earthworms and *Eisenia hortensis* earthworms' Muscle Acetylcholinesterase Activity decreased at Cu level of 150 mg kg^{-1} soil compared to the control. A decrease in earthworm activity will reduce soil fertility and affect plant growth.

CONCLUSION:

It is concluded that Cu contaminated soil affects the physiological characteristics of crops and also affects the uptake of Cu to plant parts. Cu contamination also affected the earth worm activity that will deteriorate the soil's natural process of replenishing the nutrients. Cu application decreased the earth worm activity, gas exchange parameters and total chlorophyll content of mung bean crop while increased the Cu uptake in both roots and shoots. Cu tolerant bacteria proved effective in mitigating the adverse effects of Cu on total chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and sub stomatal conductance and reduced the Cu uptake by plant roots and shoots. From the results it is concluded that Cu tolerant bacteria could be an eco-friendly strategy to reduce the effects of Cu on crops in contaminated soils. Metals cannot be degraded, but their availability can be reduced. This study demonstrated the stabilization of Cu in amended soil, but ongoing monitoring is required to ensure Cu stabilization and risk assessment.

Acknowledgement

The resources were provided by Higher education commission under PhD fellowship program

Conflict of interest

Author's declare no conflict of interest

REFERENCES:

Abdel Latef, A.A.H., Zaid, A., Abo-Baker, A.B.A.E., Salem, W. and Abu Alhmad, M.F., 2020. Mitigation of copper stress in maize by inoculation with Paenibacillus polymyxa and Bacillus circulans. *Plants*, *9*(11), p.1513.

Baszynski, T., Tukendorf, A., Ruszkowska, M., Skorzynska, E. and Maksymieci, W., 1988. Characteristics of the photosynthetic apparatus of copper non-tolerant spinach exposed to excess copper. *Journal of plant physiology*, *132*(6), pp.708-713.

Bednarska, A.J., Choczyński, M., Laskowski, R. and Walczak, M., 2017. Combined effects of chlorpyriphos, copper and temperature on acetylcholinesterase activity and toxicokinetics of the chemicals in the earthworm Eisenia fetida. *Environmental Pollution*, *220*, pp.567-576.

Chrysargyris, A., Papakyriakou, E., Petropoulos, S.A. and Tzortzakis, N., 2019. The combined and single effect of salinity and copper stress on growth and quality of Mentha spicata plants. *Journal of hazardous materials*, *368*, pp.584-593.

Gall, J.E., Boyd, R.S. and Rajakaruna, N., 2015. Transfer of heavy metals through terrestrial food webs: a review. *Environmental monitoring and assessment*, *187*(4), pp.1-21.

Ghosh, M. and Singh, S.P., 2005. A review on phytoremediation of heavy metals and utilization of it's by products. *Asian J Energy Environ*, *6*(4), p.18.

Glick, B.R., Penrose, D.M., Li, J., 1998. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J. Theor. Biol. 190, 63–68.

Gonçalves, J.F., Antes, F.G., Maldaner, J., Pereira, L.B., Tabaldi, L.A., Rauber, R., Rossato, L.V., Bisognin, D.A., Dressler, V.L., de Moraes Flores, E.M. and Nicoloso, F.T., 2009. Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions. *Plant Physiology and Biochemistry*, *47*(9), pp.814-821.

Gonzalez-Mendoza, D., ESCOBOZA-GARCIA, F.E.R.N.A.N.D.O., SANTAMARÍA, J.M. and ZAPATA-PEREZ, O.M.A.R., 2013. Copper stress on photosynthesis of black mangle (Avicennia germinans). *Anais da Academia Brasileira de Ciências*, *85*, pp.665-670.

Gu, J., Zhou, Z., Li, Z., Chen, Y., Wang, Z. and Zhang, H., 2017. Rice (Oryza sativa L.) with reduced chlorophyll content exhibit higher photosynthetic rate and efficiency, improved canopy light distribution, and greater yields than normally pigmented plants. *Field Crops Research*, *200*, pp.58-70.

Gupta, D., 2011. Toxicity of copper and cadmium on germination and seedling growth of maize (Zea mays L.) seeds. *Indian Journal of Scientific Research*, *2*(3), pp.67-70.

Hide, J.C., 1954. Diagnosis and Improvement of Saline and Alkali Soils. US Salinity Laboratory Staff; LA Richards, Ed. US Dept. of Agriculture, Washington, DC, rev. ed., 1954. vii+ 160 pp. Illus. $2.(Order from Supt. of Documents, GPO, Washington 25, DC). *Science*, *120*(3124), pp.800-800.

Hossain, M.S., Abdelrahman, M., Tran, C.D., Nguyen, K.H., Chu, H.D., Watanabe, Y., Hasanuzzaman, M., Mohsin, S.M., Fujita, M. and Tran, L.S.P., 2020. Insights into acetate-mediated copper homeostasis and antioxidant defense in lentil under excessive copper stress. *Environmental Pollution*, *258*, p.113544.

in heavily polluted soil and mine spoil. Appl. Soil Ecol. 28, 237–246.

Islam, F., Yasmeen, T., Ali, Q., Ali, S., Arif, M.S., Hussain, S. and Rizvi, H., 2014a. Influence of Pseudomonas aeruginosa as PGPR on oxidative stress tolerance in wheat under Zn stress. *Ecotoxicology and Environmental Safety*, *104*, pp.285-293.

Islam, F., Yasmeen, T., Ali, Q., Mubin, M., Ali, S., Arif, M.S., Hussain, S., Riaz, M. and Abbas, F., 2016. Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. *Environmental Science and Pollution Research*, *23*(1), pp.220-233.

Islam, F., Yasmeen, T., Riaz, M., Arif, M.S., Ali, S. and Raza, S.H., 2014b. Proteus mirabilis alleviates zinc toxicity by preventing oxidative stress in maize (Zea mays) plants. *Ecotoxicology and Environmental Safety*, *110*, pp.143-152.

Jackson, M.L., 2005. *Soil chemical analysis: advanced course*. UW-Madison Libraries parallel press.

John, R., Ahmad, P., Gadgil, K. and Sharma, S., 2009. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by Brassica juncea L.

Li, Q., Chen, H.H., Qi, Y.P., Ye, X., Yang, L.T., Huang, Z.R. and Chen, L.S., 2019. Excess copper effects on growth, uptake of water and nutrients, carbohydrates, and PSII photochemistry revealed by OJIP transients in Citrus seedlings. *Environmental Science and Pollution Research*, *26*(29), pp.30188-30205.

Liu, T., Shen, C., Wang, Y., Huang, C. and Shi, J., 2014. New insights into regulation of proteome and polysaccharide in cell wall of Elsholtzia splendens in response to copper stress. *PLoS One*, *9*(10), p.e109573.

Ma, Y., Rajkumar, M., Luo, Y. and Freitas, H., 2013. Phytoextraction of heavy metal polluted soils using Sedum plumbizincicola inoculated with metal mobilizing Phyllobacterium myrsinacearum RC6b. *Chemosphere*, *93*(7), pp.1386-1392.

Madhaiyan, M., Poonguzhali, S., Sa, T., 2007. Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (Lycopersicon esculentum L.). Chemosphere 69, 220–228.

Meregalli, T., 2017. The Effects of Soil Copper Contamination on Earthworm Cholinergic Transmission, Locomotion and Muscle Physiology.

Moodie, C.D., Smith, H.W. and McCreery, R.A., 1951. *Laboratory manual for soil fertility* (Vol. 71, No. 5, p. 400). LWW.

Ojha, R.B. and Devkota, D., 2014. Earthworms:'Soil and ecosystem engineers'–a review. *World Journal of Agricultural Research*, *2*(6), pp.257-260.

Panou-Filotheou, H., Bosabalidis, A.M. and Karataglis, S., 2001. Effects of copper toxicity on leaves of oregano (Origanum vulgare subsp. hirtum). *Annals of Botany*, *88*(2), pp.207-214.

Parveen, A., Saleem, M.H., Kamran, M., Haider, M.Z., Chen, J.T., Malik, Z., Rana, M.S., Hassan, A., Hur, G., Javed, M.T. and Azeem, M., 2020. Effect of citric acid on growth, ecophysiology, chloroplast ultrastructure, and phytoremediation potential of jute (Corchorus capsularis L.) seedlings exposed to copper stress. *Biomolecules*, *10*(4), p.592.

Piotrowska-Seget, Z., Cycon´ , M., Kozdro´ j, J., 2005. Metal-tolerant bacteria occurring

Saleem, M.H., Rehman, M., Kamran, M., Afzal, J., Noushahi, H.A. and Liu, L., 2020. Investigating the potential of different jute varieties for phytoremediation of copper-contaminated soil. *Environmental Science and Pollution Research*, *27*(24), pp.30367-30377.

Samal, B. and Kotiyal, P.B., 2013. Bioremediation of copper contaminated soil using bacteria. *Octa Journal of Environmental Research*, *1*(1).

Sarker, U. and Oba, S., 2021. Color attributes, betacyanin, and carotenoid profiles, bioactive components, and radical quenching capacity in selected Amaranthus gangeticus leafy vegetables. *Scientific Reports*, *11*(1), pp.1-14.

Shanker, A.K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S., 2005. Chromium toxicity in plants. *Environment international*, *31*(5), pp.739-753.

Sharma, N., Bakshi, A., Sharma, A., Kaur, I., & Nagpal, A. K. (2021, November). Health Risk Associated with Copper Intake through Vegetables in Different Countries. In *IOP Conference Series: Earth and Environmental Science* (Vol. 889, No. 1, p. 012071). IOP Publishing.

Sharma, P., Sirhindi, G., Singh, A.K., Kaur, H. and Mushtaq, R., 2017. Consequences of copper treatment on pigeon pea photosynthesis, osmolytes and antioxidants defense. *Physiology and Molecular Biology of Plants*, *23*(4), pp.809-816.

Tariq, F.S., 2021. Heavy metals concentration in vegetables irrigated with municipal wastewater and their human daily intake in Erbil city. *Environmental Nanotechnology, Monitoring & Management*, *16*, p.100475.

Van Groenigen, J.W., Lubbers, I.M., Vos, H.M., Brown, G.G., De Deyn, G.B. and Van Groenigen, K.J., 2014. Earthworms increase plant production: a meta-analysis. *Scientific reports*, *4*(1), pp.1-7.

Wang, L., Yang, X., Ren, Z., Hu, X. and Wang, X., 2015. Alleviation of photosynthetic inhibition in copper-stressed tomatoes through rebalance of ion content by exogenous nitric oxide. *Turkish Journal of Botany*, *39*(1), pp.10-22.

Watanabe, F.S. and Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. *Soil Science Society of America Journal*, *29*(6), pp.677-678.

Wu, Y., Li, X., Tan, F., Zhou, X., Mu, J. and Zhao, X., 2021. Lactobacillus fermentum CQPC07 attenuates obesity, inflammation and dyslipidemia by modulating the antioxidant capacity and lipid metabolism in high-fat diet induced obese mice. *Journal of Inflammation*, *18*(1), pp.1-11.

Yagura, R., Imanishi, J. and Shibata, S., 2019. Effects of copper ions on the growth and photosynthetic activity of Scopelophila cataractae. *Lindbergia*, *2019*(1), pp.1-7.

Zaouali, W., Mahmoudi, H., Salah, I.B., Mejri, F., Casabianca, H., Hosni, K. and Ouerghi, Z., 2020. Copper-induced changes in growth, photosynthesis, antioxidative system activities and lipid metabolism of cilantro (Coriandrum sativum L.). *Biologia*, *75*(3), pp.367-380.

Zhang, L.L., He, X.J., Chen, M., An, R.D., An, X.L. and Li, J., 2014. Responses of nitrogen metabolism to copper stress in Luffa cylindrica roots. *Journal of soil science and plant nutrition*, *14*(3), pp.616-624.