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Enhanced Phytoremediation of Heavy Metal-contaminated Soil through *Solanum lycopersicum* and *Pseudomonas aeruginosa* Synergy

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Abstract- This study investigated the potential of tomato plant in phytoremediating a heavy-metal polluted soil through a comprehensive analysis of the physico-chemical parameters of the soiland determination of the concentration of heavy metal uptake by the plant, with the aid of P. aeruginosa inoculated into the soil. The ability of S. lycopersicum to grow and phyto-extract Zn, Cu, Fe Pb, Cr and as with the aid of P. aeruginosa to remediate a heavy-metal polluted soil was assessed over a period of 8 weeks. Plant height and stem width were measured weekly for 8 weeks, while heavy metal concentrations in both the soil and plant shoots were analyzed at the start and end of the experiment. The plant height and stem width exhibited a significant increase in response to increased concentrations of P. aeruginosa over time, compared to un-inoculated tomato plants (control). A significant difference in the concentration of all the heavy metals in the soil before and after the study was evaluated and recorded. Post-cropped soil was found to have the lowest concentration of heavy metals remaining in the soil after the study when compared with the pre-cropped soil and soil used as control. The tomato plants absorbed a significant amount of heavy metals from the soil even without added treatment. The microorganisms helped break down plant exudates into simpler substances that the plants could absorb, enhancing heavy metal uptake into the tomato plant's shoots. However, harmful metals like lead, arsenic, and cadmium remained mostly in the soil and did not accumulate significantly in the plant, suggesting that the tomato plant has a natural tolerance to prevent the buildup of toxic metals. Additionally, the microbes increased in number, likely feeding on the plant's exudates, showing a supportive interaction between the plant and microbes.

Keywords: Pseudomonas aeruginosa, Solanum lycopersicum, Phytoextract, inoculation, post-cropped soil.

INTRODUCTION

Heavy metals can be described as elements with an atomic density greater than 6 g/cm^{3}and they are commonly found as pollutant in waste waters. They could also be found in other contaminated terrestrial habitats. The most common metals that are usually toxic are arsenic,lead, mercury, cadmium, chromium, copper, nickel, silver and zinc (Marques *et al*., 2009:Adeyori, 2011). Heavy metals pollution in the environment could create a devastating health and environmental challenges to the people and other living organisms (Ogoyi, *et al*, 2011). Long term deposit of heavy metallic substance in crop production could result in harmful effect on human health through the consumption of such crops (Disit *et al*, 2015).

Heavy metals therefore pose a huge environmental concern, most importantly, because of their toxicity to human race as well as the biosphere even when they are at low concentration. Their occurrence and accumulation in the environment is as a result of direct or indirect human activities, such as rapidindustrialization, urbanization and other anthropogenic sources. (Jern, 2006: Cho-ruk *et al*.,(2006). The two main sources of heavy metals pollution are natural and human sources. The natural source includes soil erosion, volcanic activities, urban runoff as well as from aerosols particulate, while the human source of the pollution could come from metal finishing andelectroplating processes, mining extraction and operations, through textile production as well as from nuclear power (Burea *et al*, 2005: Duran *et al*., 2007).

Some of the effects of heavy metals on plants include: lowered number germination percentage, decreased lipid content, decreased enzyme activity, stunted plant growth, inhibition of photosynthesis, and reduction in chlorophyll production. The effects on animals include, organ failure and damage, carcinogenic diseases and at the extreme, death (Adegbenro and Babalola, 2017). Bioremediation, a method that is used to reduce or remove heavy metal contaminants from the environment, is considered as one of the naturalways to attenuate or transform harmful substances to a less harmful one, through the use of microorganisms or green plants or other organisms. The micro-organisms produce

surfactants which aid biodegradation and thus convert the heavy metals to nutrients and help to support plant growth. Prominent examples of such organisms are *Pseudomonas aeruginosa*, *Anthrobacter sp, Bacillus spp, Cupriavidus metallidurans, Enterobacter cloacae, Streptomyces sp, Zoogloearamigera* (Ramasamy *et al*., 2006).

Pseudomonas aeruginosa is ubiquitous in soil and is capable of metabolizing a wide range of organic and inorganic compound. It plays important roles in nutrient recycling and has the ability to quickly adapt to a contaminated environment. It helps in the remediation of heavy metals by acting as a bio-surfactant. Phytoremediation primarily depends on optimizing the remediation potentials of native plants growing in a polluted site (Sinha *et al*, 2004; Suc *et al.*, 2014). Some important factors to consider when choosing a plantas a phyto-remediator are the root system, above-the-ground biomass, toxic level of the pollutants, plant survival and its adaptability to prevailing environmental conditions, plant growth etc. (Soumitra *et al*., 2014: Ekperusi *et al*., 2015). In some contaminated environments, the process of contaminant removal by plant, involves uptake, which is largely by translocation from root to shoot, carried out through the xylem flow (Bolan *et al*, 2013). *S. lycopersicum* (Tomato) has been reported to be found useful for bio-fortification or phytoremediation (Gadd, (2010): Chibuike and Obiora , 2014). Soil contaminated with heavy metals are often abandoned for farming process, as crops produced from such are usually toxic and injurious to human health and can pose serious health challenges. The objectives of this study are to determine the concentration of heavy metals in a polluted soil, assess the heavy metals content of pre and post cropped soil, determine the heavy metal uptake by *Solanium lycopersicum*(Tomato) and assess the synergic efficacy of tomato plant and P. areginosa in rmediating heavy metal contaminated soil.

MATERIALS AND METHOD

Sample Collection

Heavy metal-polluted soil was collected from Sasa Market in Akure. To eliminate unwanted microorganisms, the soil was sterilized by heating it at 118°C for 4 hours. Seeds of *Solanum lycopersicum* (tomato) were obtained from the Institute of Agricultural Research and Training (IAR&T) in Apata, Ibadan.

Planting and Inoculation

The sterilized soil was used to fill large pots, and seeds of *S. lycopersicum* were planted. Five experimental pots were prepared as replicates. Each pot was then inoculated with different concentrations of *Pseudomonas aeruginosa* bacteria: one pot received no bacteria (T-P), while the others were given 5 ml (T+P5 ml), 10 ml (T+P10 ml), 15 ml (T+P15 ml), and 20 ml (T+P20 ml) of bacteria solution, respectively.

The study aimed to evaluate the tomato plant's growth and ability to extract heavy metals (Fe, Zn, Pb, Cr, As, Cu) from the soil, with the assistance of *P. aeruginosa*, over an 8-week period. Weekly measurements of plant height and stem width were taken, and heavy metal concentrations in the plant's shoots were analyzed before and after treatments. The initial and final heavy metal levels in the soil were also compared, including controls and pots with and without the bacteria. Additionally, soil samples around the tomato plants were examined to identify any other bacterial species present.

Sterilization of equipments/glassware

All glasswares (Petri dishes, Erlenmeyer flasks, Test tubes, Mccartney bottles, Graduated cylinders

Glass pipettes, Beakers, slides and cover slips, and glass rods) used in this experiment were sterilized in an autoclave at 121^0C for 15 minutes and were all allowed to cool before use

Preparation of Nutrient agar

Nutrient agar powder (28 g) was weighed into a 1000 millilitres Erlenmeyer flask and dissolved in 1000 cm^3 of distilled water. The flask was corked and wrapped. The nutrient agar solution was sterilized by autoclaving at 121^0C for 15 minutes followed by cooling to cool to 45^0 C prior to utiation.

Enumeration of Total Heterotrophic Bacteria

Heterotrophic bacteria were cultured on nutrient agar by serially diluting 0.1 ml soil samples, then plating in triplicate using pour plate method with an un-inoculated plate serving as the control. All plates were incubated at 37^0C for 24 hours, followed by observation and counting of colonies.

Characterization and Identification of Bacteria Isolate

After incubation, plates with 30 to 200 colonies were selected for further analysis. Pure cultures were obtained through repeated subculturing of individual bacterial colonies on nutrient agar. The solates were characterized based on cultural characteristics such as shape, size,pigmentation, and morphological characteristics such as shape, size and arrangement. Biochemical reactions such as Gram staining, motility test, Catalase test, Triple sugar Iron (TSI) test, indole test, sugar fermentation were subsequently conducted to confirm identification.

RESULTS

In the study, tomato plants treated with varying concentrations of *Pseudomonas aeruginosa* exhibited different growth rates over 8 weeks, with higher bacterial concentrations generally promoting taller growth. The control group (no bacteria) reached a final height of 4.6 cm, while plants treated with 5 ml, 10 ml, and 15 ml of bacteria grew to 7.5 cm, 8 cm, and 9.3 cm, respectively. The tallest growth, 9.4 cm, was observed in the group receiving 20 ml of bacteria, indicating that higher bacterial concentrations may enhance tomato plant growth in heavy metal-polluted soil (Figure 1). The study also showed that tomato plant width increased with higher concentrations of *Pseudomonas aeruginosa* over 8 weeks. The control group, with no bacterial inoculation, reached a final width of 0.8 cm. In contrast, plants treated with 5 ml and 10 ml of bacteria grew to widths of 1.3 cm and 1.7 cm, respectively. The highest growth was observed in plants treated with 20 ml of *P. aeruginosa*, achieving a width of 2.8 cm, indicating that higher bacterial concentrations support increased plant width in metal-polluted soil (Figure 2).

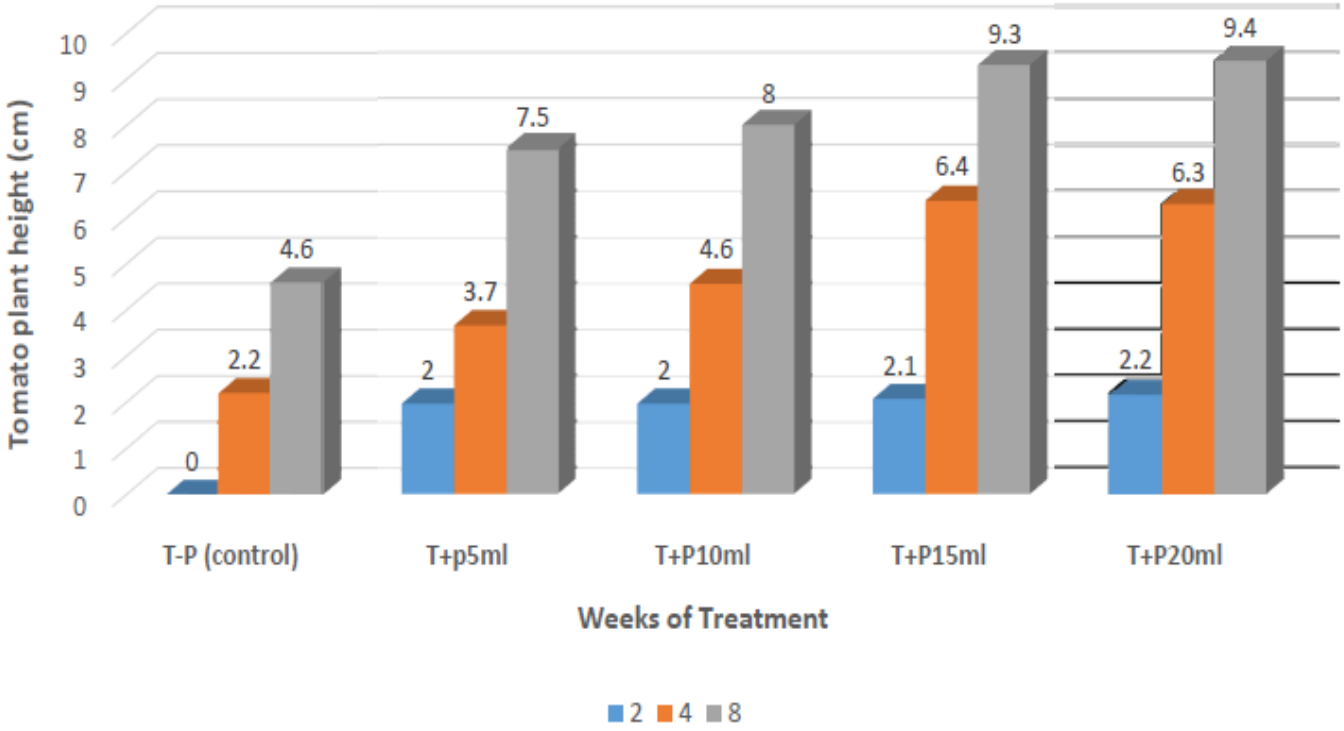

Figure 1: Effect of *Pseudomonas aeruginosa* concentration on Tomato Plant Height over 8 Weeks

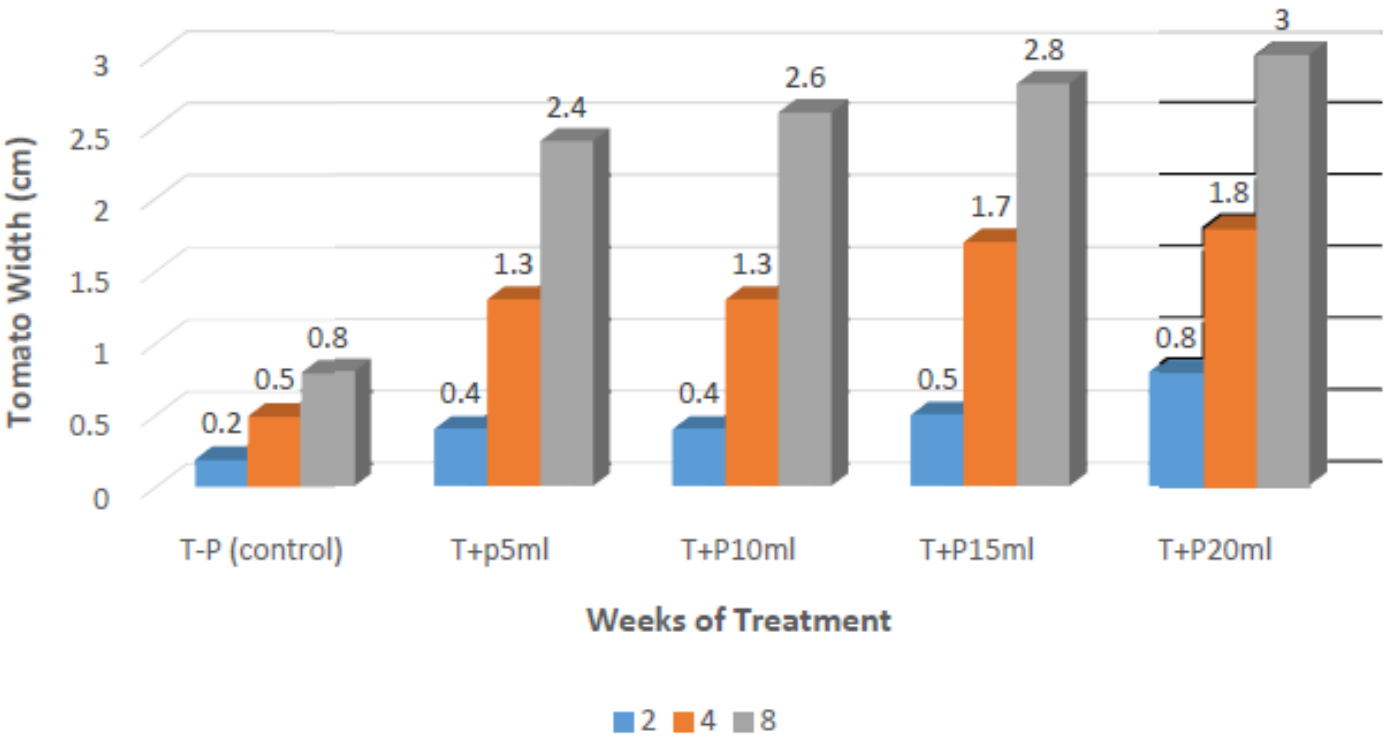

Figure 2: Effect of Pseudomonas auruginosa concentrations on Tomato stem width over 8 weeks

Generally, the concentrations of Zn, Cu, Pb, Cd, Fe, and As in the polluted soil varied before and after the study, with higher levels observed as the concentration of *P. aeruginosa* inoculation increased. The difference ranging from (T- P) to (T+ P20 ml) in Zn (2.5mg/kg to 12.4 mg/kg) (Table 1), Cu (7.0mg/kg to 23.8 mg/kg) (Table 2), Pb (3.2mg/kg to 12.2 mg/kg) (Table 3), Cd (1,4 mg/kg to 8.0 mg/kg) (Table 4), Fe (17.3 mg/kg to 88.5 mg/kg) (Table 5), Ar (0.6 mg/kg to 3.4 mg/kg) (Table 6).

Table 1: Concentration of Zinc in soil before and after eight weeks of study

Treatments	Zinc conc in soil before study (mg/kg)+ S.D	Zinc conc in soil after 8 wks of study (mg/kg) + S.D	Differences (mg/Kg)
T-P	67.5 ±6.5	65.0 ±6.1	2.5
T+P 5ml	67.5 ±6.5	62.0±5.9	5.5
T+P10ml	67.5 ±6.5	60.4±5.6	7.1
T+P15ml	67.5 ±6.5	58.5±5.4	9.0
T+P20ml	67.5 ±6.5	50.1±4.9	12.4

Table 2: Concentration of Copper in soil before and after eight weeks of study

Treatments	Copper Conc in soilbefore study (mg/kg) + S.D	Copper Conc in soil after 8 wks of study (mg/kg) + S.D	Differences (mg/kg)
T-P	56.3 ±5.2	49.3 ±4.3	7.0
T+P 5ml	56.3 ±5.2	44.7±4.1	11.6
T+P10ml	56.3 ±5.2	40.0±3.9	16.3
T+P15ml	56.3 ±5.2	37.6±3.7	18.7
T+P20ml	56.3 ±5.2	32.5±3.1	23.8

Table 3: Concentration of Lead in soil before and after eight weeks of study

Treatments	Lead Conc. in soil before study (mg/kg)+S.D	Lead Conc. in soil after 8 wks of study (mg/kg) + S.D	Differences (mg/kg)
T-P	32.8 ±3.1	29.6±2.9	3.2
T+P 5ml	32.8 ±3.1	24.7±2.6	8.1
T+P10ml	32.8 ±3.1	23. 0 ± 2.4	9.8
T+P15ml	32.8 ±3.1	23.4±2.5	9.4
T+P20ml	32.8 ±3.1	20.1±2.1	12.2

Table 4: Concentration of Cadmium in soil before and after eight weeks of study

Treatments	Cadmium Conc. in soil before study (mg/kg) + S.D	Cadmium Conc. in soil after 8 wks of study (mg/kg) + S.D	Differences (mg/kg)
T-P	18.1±4.2	16.7±4.1	1.4
T+P 5ml	18.1±4.2	14.2±3.2	3.9
T+P10ml	18.1±4.2	13.6±3.0	4.5
T+P15ml	18.1±4.2	11.1±2.1	7.1
T+P20ml	18.1±4.2	10.1±1.8	8.0

Table 5: Concentration of Iron in soil before and after eight weeks of study

Treatments	Iron Conc. in soil before study (mg/kg) + S.D	Iron Conc. in soil after 8 wks of study (mg/kg) + S.D	Differences (mg/kg)
T-P	189.3±23.9	172.0±22.7	17.3
T+P 5ml	189.3±23.9	161.0±22.1	28.3
T+P10ml	189.3±23.9	141.6±20.3	47.7
T+P15ml	189.3±23.9	120.4±19.2	68.9
T+P20ml	189.3±23.9	100.8±12.1	88.5

Table 6: Concentration of Arsenic in soil before and after eight weeks of study

Treatments	Arsenic Conc. in soil before study (mg/kg) + S.D	Arsenic Conc. in soil after 8 wks of study (mg/kg) + S.D	Differences (mg/kg)
T-P	8.6±1.7	8.0±1.5	0.6
T+P 5ml	8.6±1.7	6.7±1.3	1.9
T+P10ml	8.6±1.7	6.1±1.1	2.5
T+P15ml	8.6±1.7	5.8±1.0	2.8
T+P20ml	8.6±1.7	5.2±0.8	3.4

The bar chart (Figure 3) illustrates the concentration of various heavy metals in soil samples taken at three stages: pre-cropped, post-cropped, and soil without *Pseudomonas aeruginosa* treatment. Generally, the concentrations of Zn, Cu, Pb, Cd, and Fe decreased after cropping, indicating possible uptake by plants or microbial activity reducing these metal levels in the soil. For example, Fe, initially the most abundant at around 180 mg/kg in the pre-cropped soil, showed a notable reduction in the post-cropped soil. Soil without *P. aeruginosa* displayed relatively stable levels of most metals, with slightly lower values than pre-cropped soil but higher than post-cropped, suggesting that *P. aeruginosa* could contribute to the reduction of heavy metals in soil during the cropping process.

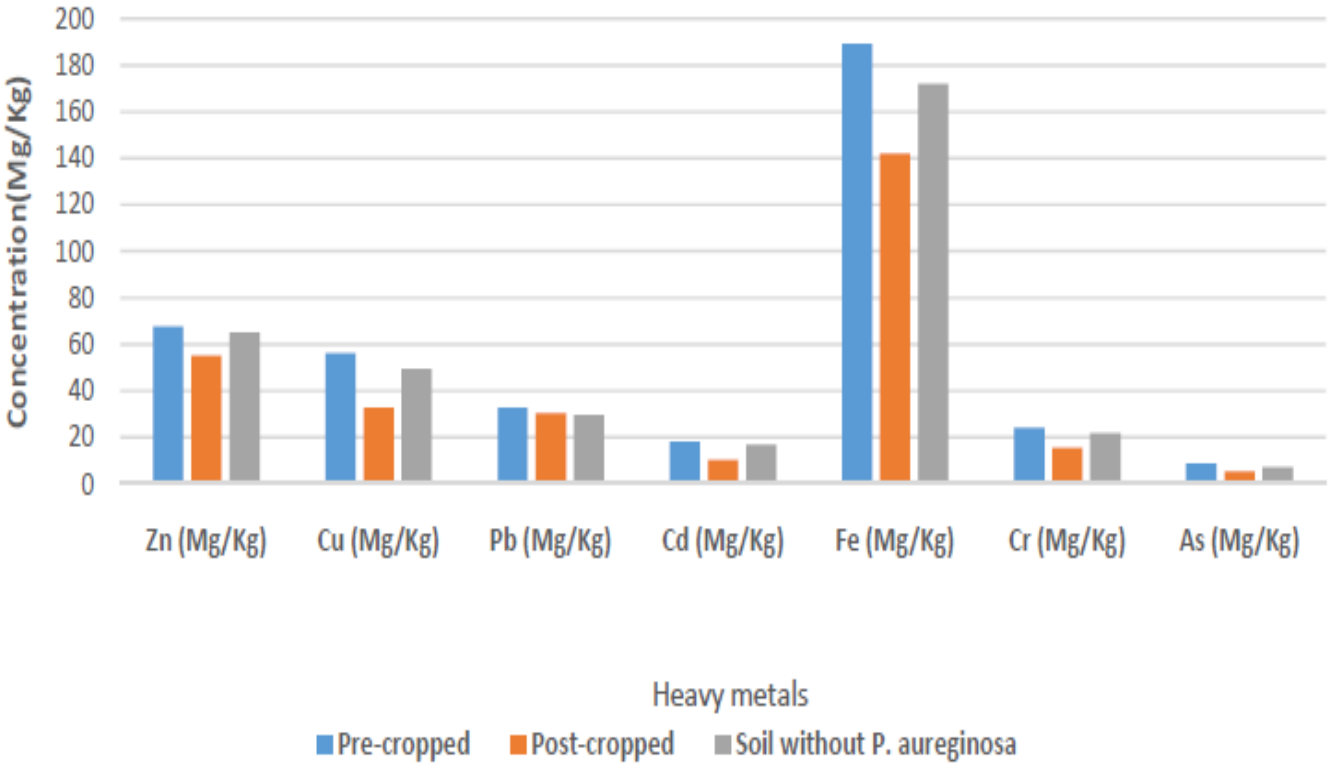

Figure 3: Concentration of heavy metals for pre-cropped, post-cropped and soil without *P.aeruginosa*

Figure 4: Heavy metals concentration in the shoot of Tomato

Results (Figure 4) indicated an increase in heavy metal concentration in the plant's shoot as the treatment concentration increased, suggesting that the organism used in this treatment enhances metal uptake into the plant, which can be valuable for industrial extraction. The control (T-P) exhibited minimal heavy metal uptake, highlighting the potential of tomato plants as effective phytoremediators. Furthermore, analyses of the plant and soil samples before and after the eight-week study showed that Iron, Copper, and Zinc accumulated more in the shoot than in the roots across all treatments. This is in agreement with findings of Mohammad and Moheman (2010). They reported that increasing Cd and Zn concentrations in soil result in an increase in the accumulation of Cd or Zn in the plant tissues. Plants have developed mechanisms to extract certain heavy metals into their metabolism while blocking harmful ones. Notably, this study found that hazardous metals like arsenic and lead were present in higher concentrations in the soil than in any part of the plant. In contrast, metals essential for plant metabolism, such as those involved in photosynthesis and functioning as antioxidant enzyme cofactors, accumulated more in the plant itself. Plants require trace amounts of certain heavy metals like cobalt, copper, manganese, molybdenum, and zinc for growth and metabolic functions. These metals are absorbed and utilized efficiently, while non-essential and toxic metals like arsenic and lead are restricted or detoxified. This is in agreement with (Hall, 2002; Kushwaha *et al.*, 2015; Ghori *et al.*, 2019; Manoj *et al.*, 2020).

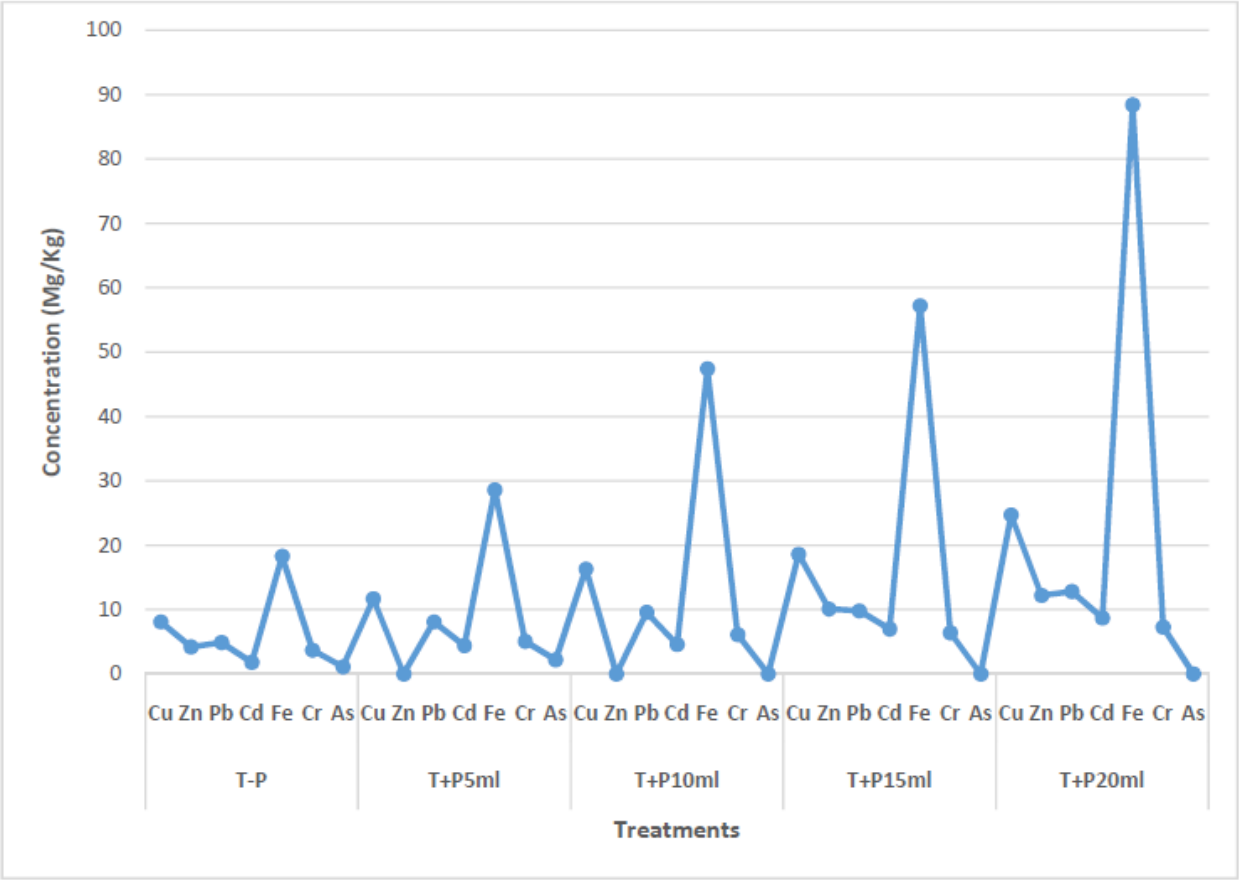

Figure 4: Heavy metals concentration in the shoot of Tomato

CONCLUSION

This study therefore highlighted the complementary roles of phytoremediation and bioremediation in the uptake and management of heavy metals by tomato plants. The findings demonstrate that tomato plants were able to absorb significant amounts of heavy metals from the soil even without microbial treatment, while the microorganisms facilitated the biodegradation of plant exudates into inorganic, absorbable forms. Together, these processes enhanced the uptake of heavy metals into the shoots of the tomato plants. Importantly, the accumulation of hazardous metals such as lead, arsenic, and cadmium, known for their toxicity, was restricted primarily to the soil, with minimal levels detected in the plant's roots and shoots. This indicates a possible tolerance mechanism within the plant to limit the uptake of these harmful metals. Additionally, the observed increase in microbial populations after the experiment suggests that the tomato plants may release exudates that support microbial growth, further promoting bioremediation.

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