Original Research Article

BIOREMEDIATION OF HEAVY METALS IN THE SOIL BY *PSEUDOMONAS AERUGINOSA* AND *TRICHODERMA HARZIANUM* USING *SOLANUM LYCOPERSICUM* AS TEST PLANT

ABSTRACT

This study determined the heavy metal concentrations of contaminated stream water and assessed the heavy metal contents of pre- and post-cropped sterilized soil. It also determined the the heavy metal uptake of the *S. lycopersicum* plant. This was with a view to assessing the potential of *Pseudomonas aeruginosa* and *Trichoderma harzianum* for degrading heavy metals in heavy metal contaminated stream water.Experimental pots containing 3000 g of sterilized soil was used for this experiment whereby 60 sample pots were used with various treatments in this study. *Solanum lycopersicum* seeds were raised in the nursery for a period of 3 weeks and treatments applied just before transplanting into the experimental pots. The plants were left for a week so as to be established properly and overcome transplanting shock before watering with the contaminated stream water. Heavy metal analysis using Atomic Absorption Spectroscopy (AAS) method was carried out on the contaminated stream water to determine the amount of heavy metal in the stream water before the commencement of the experiment. The contaminated stream water was applied to the pots in measured quantities; 0, 5 and 0%. Pre and post soil heavy metal analysis were carried out on the soil samples. At harvest, plant tissues were analysed for heavy metals using AAS method. The results showed that heavy metals were present in high concentration in the stream water sample. The values of the heavy metals in the stream water sample used for watering were Iron – 138.15 mg/L, Zinc – 68.4 mg/L, Lead – 7.89 mg/L and Copper – 8.98 mg/L. . Heavy metal analysis of the soil and all the treatments revealed that treatments with *P. aeruginosa* inoculation had the lowest level of Iron, Copper, Zinc and Lead followed by treatments inoculated with *T. harzianum*. The study concluded that the use of contaminated stream water for irrigation could be a potential source of heavy metals in tomato. However, inoculation of microorganisms for the treatment of the heavy metal contaminated sites was effective for increased health, growth and yield of tomato fruits.

KEWORDS: Bioremediation, *Pseudomonas aeruginosa*, T*richoderma harzianum*, *Solanum lycopersicum*

INTRODUCTION

Heavy metals represent a great environmental concern, because of their widespread use and distribution, and particularly their toxicity to human beings and the biosphere. However, they also include some elements that are essential for living organisms at low concentrations (Alloway, 1990). These elements are usually transition metals. They have high densities (>5 g cm-3) when compared with other materials (Baird and Cann, 2005). Human activities such as industrial production, mining, agriculture and transportation lead to release of high amount of heavy metals into the biosphere. The primary sources of metal pollution are the burning of fossil fuels, smelting of metal like ores, municipal wastes, fertilizers, pesticides and sewage (Nriagu, 1979, 1996; Pendias and Pendias, 1989; Rai, 2009). Heavy metal contamination may occur due to factors which could include irrigation with contaminated water, addition of fertilizers and metal based pesticides, industrial emissions, and transportation (Radwan and Salama, 2006; Tuzen and Soylak, 2007; Duran *et al*., 2007). Heavy metal pollution does not only affect the production and quality of crops, it also influences the quality of the atmosphere and water bodies. This threatens the health and life of animals as well as human beings by the way of food chain and most phenomenal is that, this kind of pollution is covert, long term and non-reversible (Zhang, 1999). Heavy metals are also one of the major contaminating agents in our food supply (Zaidi *et al.*, 2005; Khair, 2009). Bioremediation is a process that uses naturally occurring micro-organisms to transform harmful substances to nontoxic compounds, these processes which take advantage of microbial degradation of organic and inorganic substances can be defined as the use of micro-organisms to remove environmental pollutants of soils, water and sediments (Pala *et al*., 2006). Bioremediation involves the use of organisms for the treatment of polluted soils. These organisms which could be micro-organisms or green plants eliminate, attenuate or transform the harmful substances via biological processes to a less harmful substance (Mrayyana and Battikhi, 2005). Micro-organism breaks down organic molecules to carbondioxide, fattyacid and water in order to obtain energy and nutrients. Bioremediation occurs naturally (even though it could be enhanced by a number of processes), thus, it is widely accepted by the general public as a safe way of treating polluted soils. *Trichoderma harzianum* has potential in stimulating phytoremediation directly and indirectly and therefore, inoculation of plants with this fungus could be a feasible approach to enhance the degradation of hydrocarbons in polluted soil. *T. harzianum* also have the ability to solubilize metal ions and produce siderophores to chelate iron, making metal ions required for plant growth more available to the plant (Harman *et al*., 2004). The fungus is thought to colonize roots of annual plants for their entire lifetime by penetrating the outer layers of the roots (Harman *et al*., 2004). This makes the plants release more root exudates to the surrounding soil, thus, stimulating microbial degradation of pollutants. *Trichoderma harzianum* has been shown to induce the production of larger and deeper root systems, and plants inoculated with *Trichoderma harzianum* also produce greater plant biomass. Such plants are more resistant to abiotic stress and take up nutrients more effectively (Harman *et al*., 2004). Edwards *et al*., (2006) noted that various bacteria such as *Pseudomonas aeruginosa* produce surfactants that aid in the biodegradation. A recent study has found a *P. aeruginosa* strain that actually supports plant growth. This characteristic, along with the fact that *P. aeruginosa* can degrade polycyclic aromatic hydrocarbons, suggests the future uses of *P. aeruginosa* for environmental detoxification of synthetic chemicals and pesticides and for industrial purposes (Botzenhardt and Doring, 1993).

MATERIALS AND METHOD

Collection of Contaminated water, seeds and microorganisms

Heavy metals contaminated stream water was obtained from a flowing stream. It is situated at 7°30’ Northern latitude and 4°28’ Eastern longitude. The sampling point was located at the back of the Ife Iron and Steel Nigeria Limited along Ife-Ibadan expressway. Surface water samples was collected at downstream into clean plastic kegs. The water samples were collected during the month of April, 2015. Seeds of *Solanum lycopersicum* cultivar (ROMA VF) were obtained from Institute of Agricultural Research and Training, Moor Plantation, Ibadan.

Culturing of Organisms

~~A culture of *Pseudomonas aeruginosa* was obtained from the Department of Microbiology, Obafemi Awolowo University (OAU), Ile-Ife. A culture of *Trichoderma harzianum* was also obtained from the Mycology unit of the Department of Crop Production and Protection, OAU, Ile-Ife.~~ A single colony of *P. aeruginosa* was subcultured by using nutrient agar in petri dishes and kept in the incubator for 48 hours at 37°C to a medium after which it was harvested by flooding with sterile distilled water. The bacterium inoculum was prepared by streaking a single colony of *P. aeruginosa* earlier isolated on plated nutrient agar plate and incubated at 37°C for 48 hours. Cells of *P. aeruginosa* were harvested from agar plates by flooding with sterile distilled water and standardized using a colorimeter to 10^8 CFU/ml. Spores of *Trichoderma harzianum* was subcultured by using potato dextrose agar in petri dishes and kept in the incubator for 7 days at 37°C to a medium after which it was harvested by flooding with sterile distilled water. The fungal spore solution was prepared by picking spores of *T. harzianum* earlier isolated on plated potato dextrose agar plate and incubated at 37°C for 7 days. Spores of *T. harzianum* were harvested from agar plates by flooding with sterile distilled water and standardized using a colorimeter to 10^7spores/ml.

Planting of seeds and contamination of experimental pots

Seedlings of *S. lycopersicum* were raised on nursery beds for a period of three weeks. Sixty pots, each containing three kilograms of soil from sterilized soil was used for this study. *Pseudomonas aeruginosa* inoculum solution (30 ml) was poured into a hole that was made in the middle of a set of 15 experimental pots containing sterized soil before *S. lycopersicum* seedlings are transplanted to it. *Trichoderma harzianum* spore solution (30 ml) was also poured into a hole that was made in the middle of another set of 15 experimental pots before *S. lycopersicum* seedlings are transplanted to them. The third set of 15 pots received dual inoculation of *Trichoderma harzianum* spore solution (15 ml) and *P. aeruginosa* innoculum before *S. lycopersicum* seedlings were transplanted into it; with the final set of 15 pots acting as control at various levels. Thereafter, pot preparation was arranged in a completely randomized design in the screenhouse.

Seedlings were left for a week to establish and overcome transplanting shock before wetting with the contaminated stream water at various concentrations of 0%, 5% and 10% v/v. Contaminated stream water was quantified using the formula: percentage soil contamination = (Volume of polluted stream water applied / Volume of soil) x 100. Each treatment of the experiment was replicated three times. Twenty four pots were watered with the contaminated stream water once during the experiment and another 24 pots watered daily with the contaminated stream water. The remaining 12 pots which served as the control experiment were watered daily with distilled water. Pots containing *S. lycopersicum* was watered regularly to ensure adequate moisture. Heavy metal analysis on the contaminated stream water was carried out using AAS (Atomic Absorption Spectrophotometer) for Iron, Copper, Lead, and Zinc pre experiment. Plant samples were also subjected to heavy metal analysis using AAS (Atomic Absorption Spectrophotometer) for Iron, Copper, Lead, and Zinc post experiment. Pre and post – soil tests were carried out to determine soil nutrients. Soil samples were also subjected to heavy metal analysis using AAS (Atomic Absorption Spectrophotometer) for Iron, Copper, Lead, and Zinc pre and post – soil tests. Data obtained was subjected to statistical analysis using descriptive and inferential methods.

RESULTS

The heavy metals analysis of the stream water showed that heavy metals (Iron, Zinc, Copper and Lead) were present in high concentration in the water. Iron (Fe) had the highest concentration of 138.15 mg/L followed by zinc (Zn) which had a concentration of 68.4 mg/L . The order of concentration was Fe>Zn>Cu>Pb.

After the soils were subjected to heavy metal analysis, it was observed that iron concentration of the soil increased as the contaminated stream water concentration increased in all the treatments without any inoculation of microorganism (Fig. 1). Treatments 3 and 3d inoculated with *P. aeruginosa* were lower in concentration of iron compared to treatments 2 and 2d which were inoculated with *T. harzianum*. Treatment 1d without any inoculation of microorganisms had highest iron concentration followed by treatment 1 also without any inoculation of microorganisms at 5% and 10% contaminated stream water concentration. The order of iron concentration across the treatments with 5% and 10% contaminated stream water concentration was 1d>1>4d>4>2d>2>3d>3 and 1d>1>4d>4>2d>2>3d>3 respectively.

Treatment 1d had the highest zinc concentration at 10% contaminated stream concentration followed by treatment 1 at the same 10% concentration. Treatment 3 at 0% concentration had the lowest iron concentration. Soil samples treated with single or both micro-organisms had the lowest value in zinc compared to soil polluted with contaminated stream water without any treatment with microorganisms (Fig 2). Treatment 3 had the lowest copper level of 2.46 part per million (ppm) at 5% contaminated stream water concentration while treatment 1d had the highest level of copper with 3.86 ppm at the same concentration (Fig 3). The order of copper concentration in 0% and 10% was treatment4>2>2>1>3and 1d>1>4d>4>2d>2>3d>3 respectively. Lead analyses in the soil indicated that the order of the concentration in 5% and 10% was 1d>1>4d>4>>2d>2>3d>3 and 1d>1>4d>4>>2d>2>3d>3 respectively, treatment 1d had the highest level of lead concentration followed by treatment 1 both at 10% contaminated stream water concentration while treatment 2 had the lowest at 0% (Fig. 4).

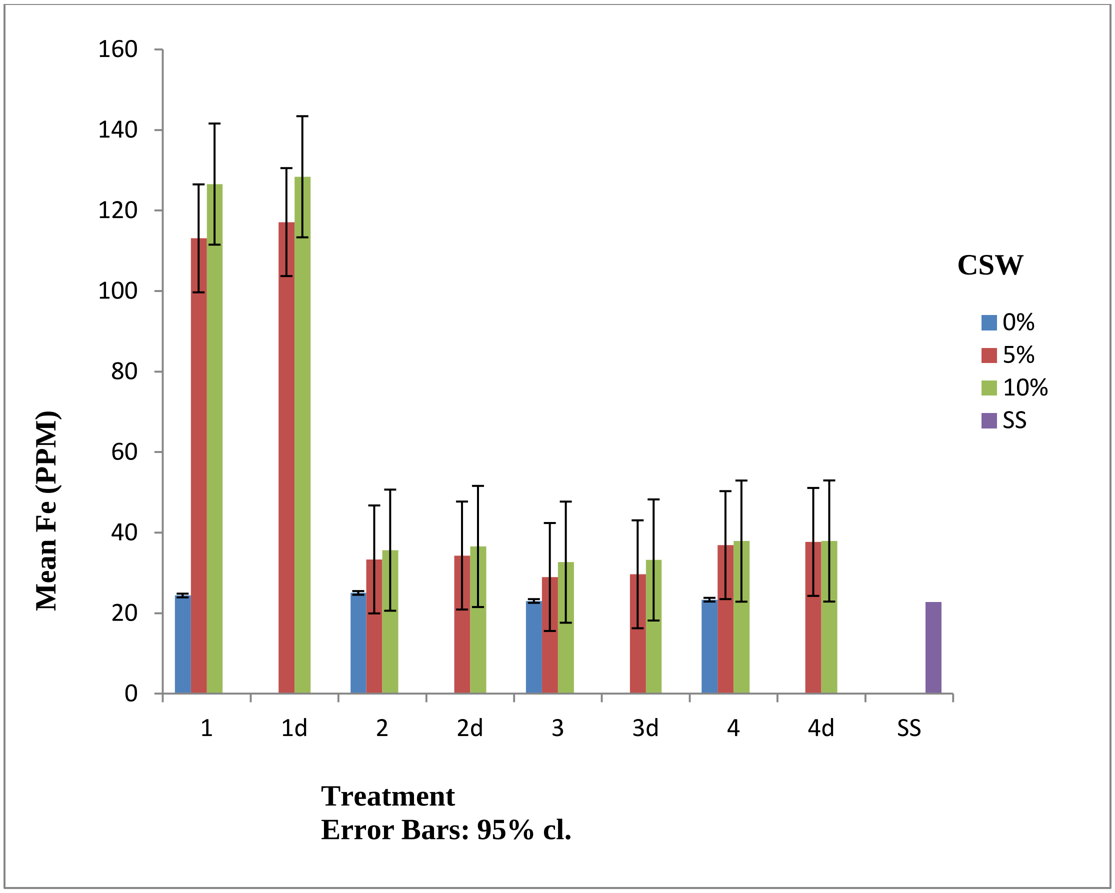

Figure 1: Iron (PPM) content of Pre and Post Planting Soil Samples

Legend

1-SS + TP
1d-SS + TP
2-SS + TH+ TP
2d-SS + TH + TP
3-SS + PA + TP
3d-SS + PA + TP
4-SS + TH + PA + TP
4d-SS + TH +PA +TP
SS - Sterilized soil before planting
d – Daily wetting of plants with contaminated stream water
Cl- Confidence level
TH – *T. harzianum*
PA - *P. aeruginosa*
TP - Test Plant
CSW- Contaminated Stream Water

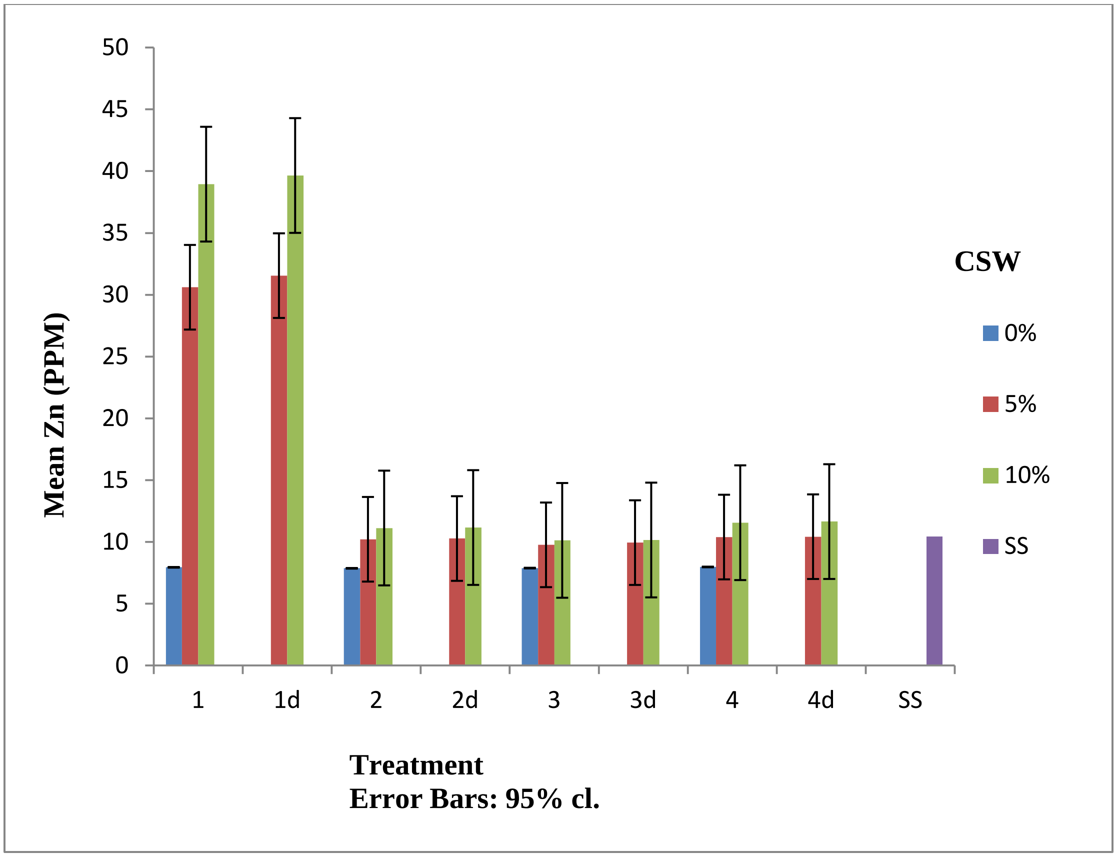

Figure 2: Zinc (PPM) content of Pre and Post Planting Soil Samples

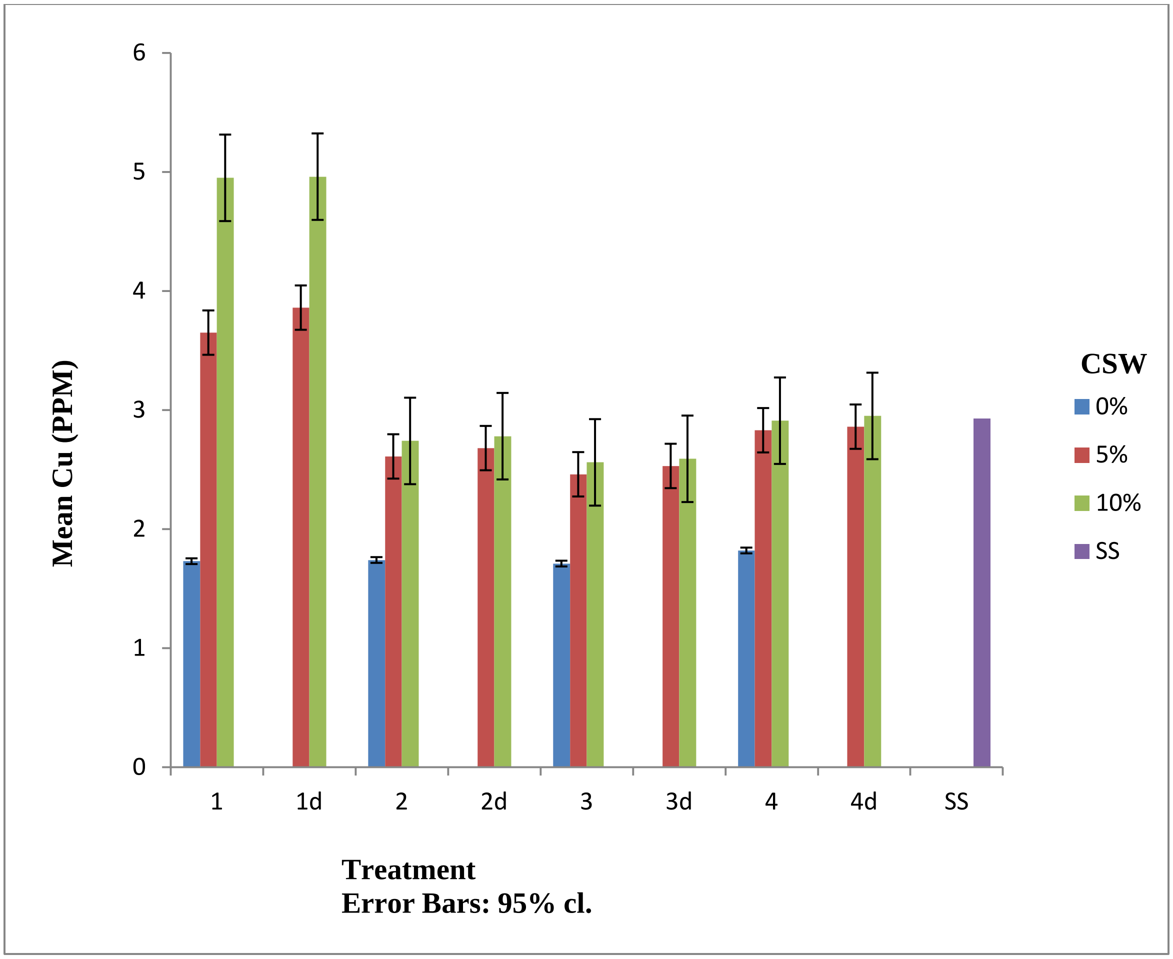

Figure 3: Copper (PPM) content of Pre and Post Planting Soil Samples

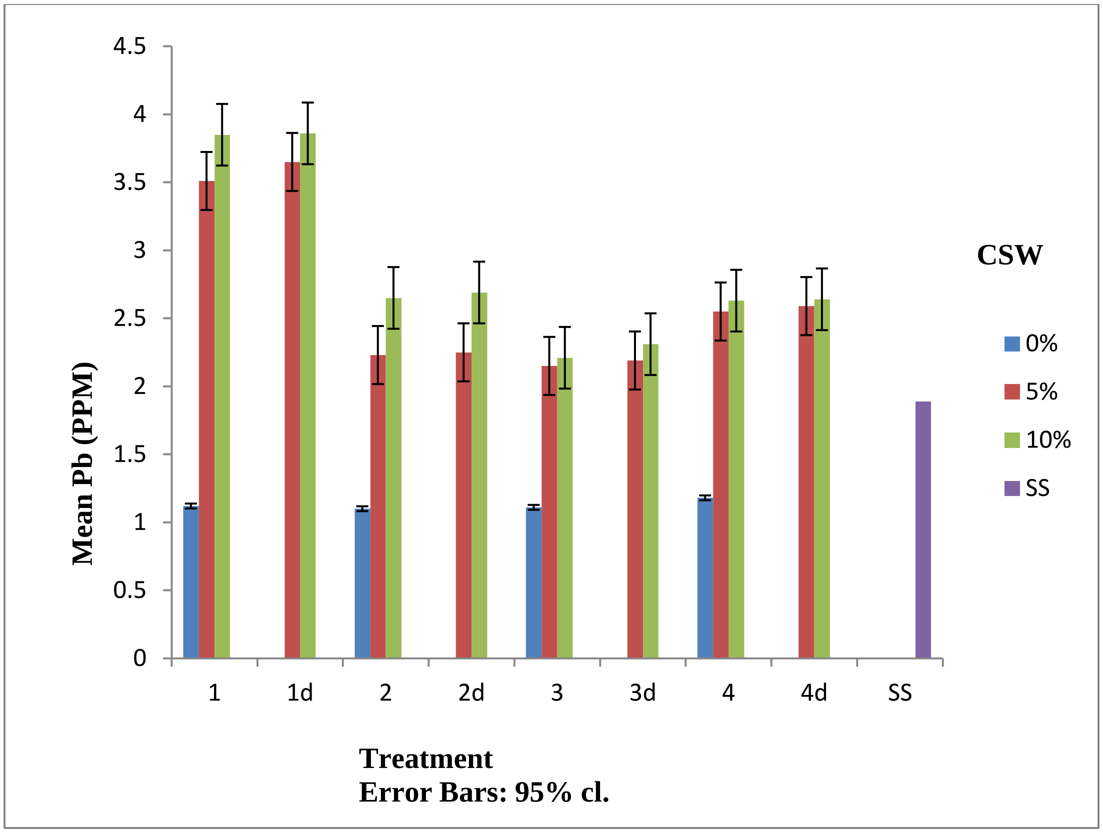

Figure 4: Lead (PPM) content of Pre and Post Planting Soil Samples

Heavy metal analysis carried out on plant samples showed that plants from soil samples without inoculation of micro-organisms had the highest heavy metal uptake as the concentration of contaminated stream water increased. For 5% contaminated stream water concentration, treatment 1 had the highest level of iron at 77.78 ppm followed by treatment1d with 77.71 ppm while treatment 3 had the lowest concentration of iron with 13.91 ppm (Fig 5).
The order of concentration in iron at 10% was sample 1>1d>4d>4>2d>2>3d>3. Zinc at 10% contaminated stream water concentration had the highest concentration in treatment 1d and the lowest at treatment 3 at same 10%. The order of zinc concentration at 5% was 1d>1>4d>4>2d>2>3d>3 while 10% was 1d>1>4d>2>2d>4>3d>3 (Fig. 6).
Copper in treatment 1d without any inoculation had the highest concentration at 5% and 10% followed by treatment 1 at same concentrations with treatment 3 inoculated with *P. aeruginosa* having the lowest value (Fig.7).
Lead content in the plant samples was highest in treatment 1d, followed by those from treatment 1 but lowest in treatment 3. Order of increase of lead is treatment 1d>1>4d>4>2d>2>3d>3 (Fig 8). Treatments 2 and 2d inoculated with *T. harzianum* had more of the heavy metal in plant tissue compared to treatments 3 and 3d treated with *P. aeruginosa*.

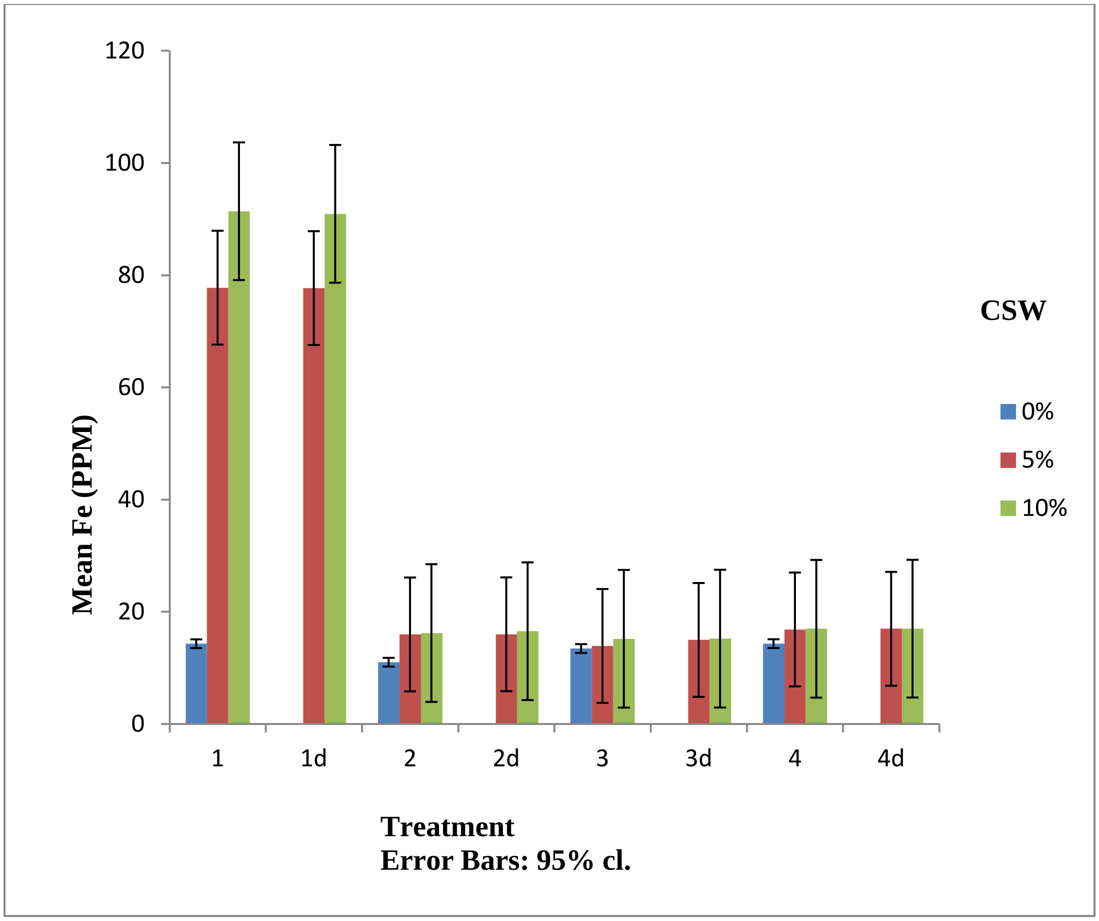

Figure 5: Iron (PPM) content of *S. lycopersicum* across all the treatments

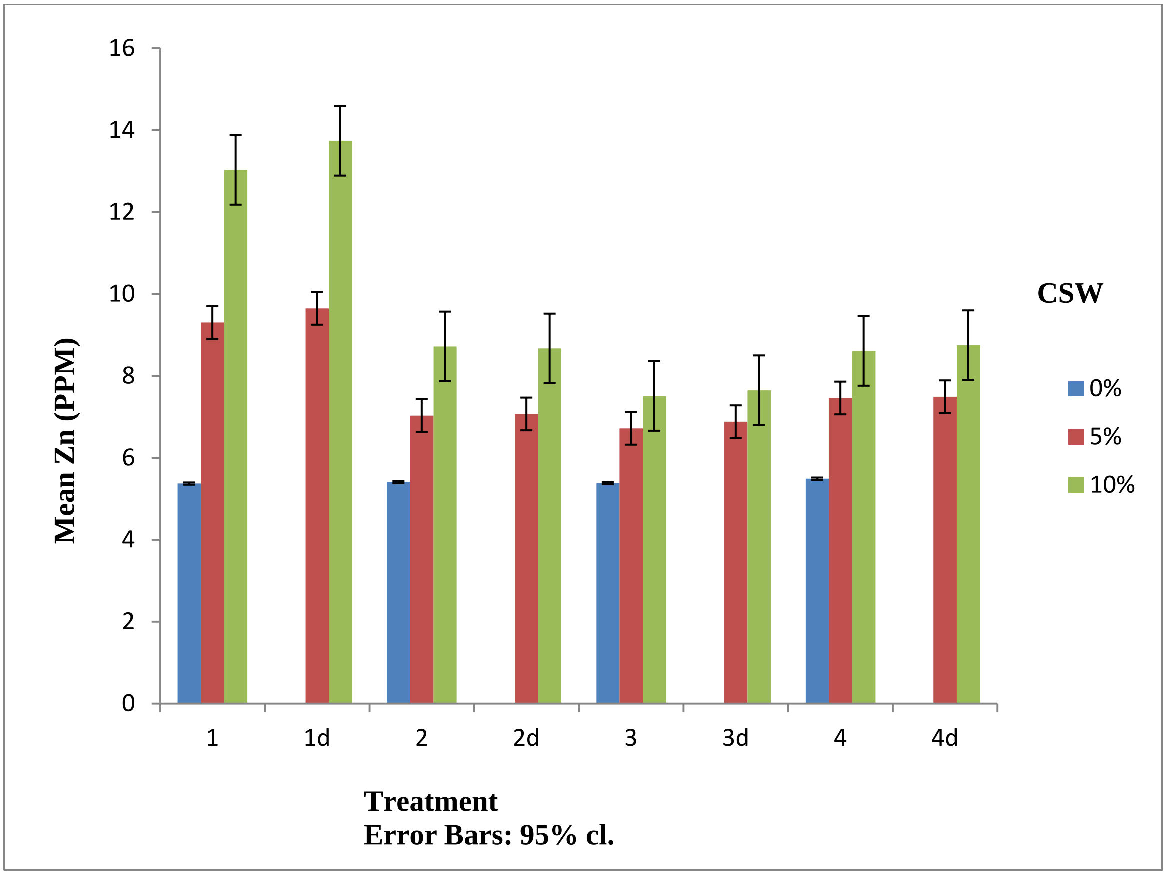

Figure 6: Zinc (PPM) content of *S. lycopersicum* across all the treatments

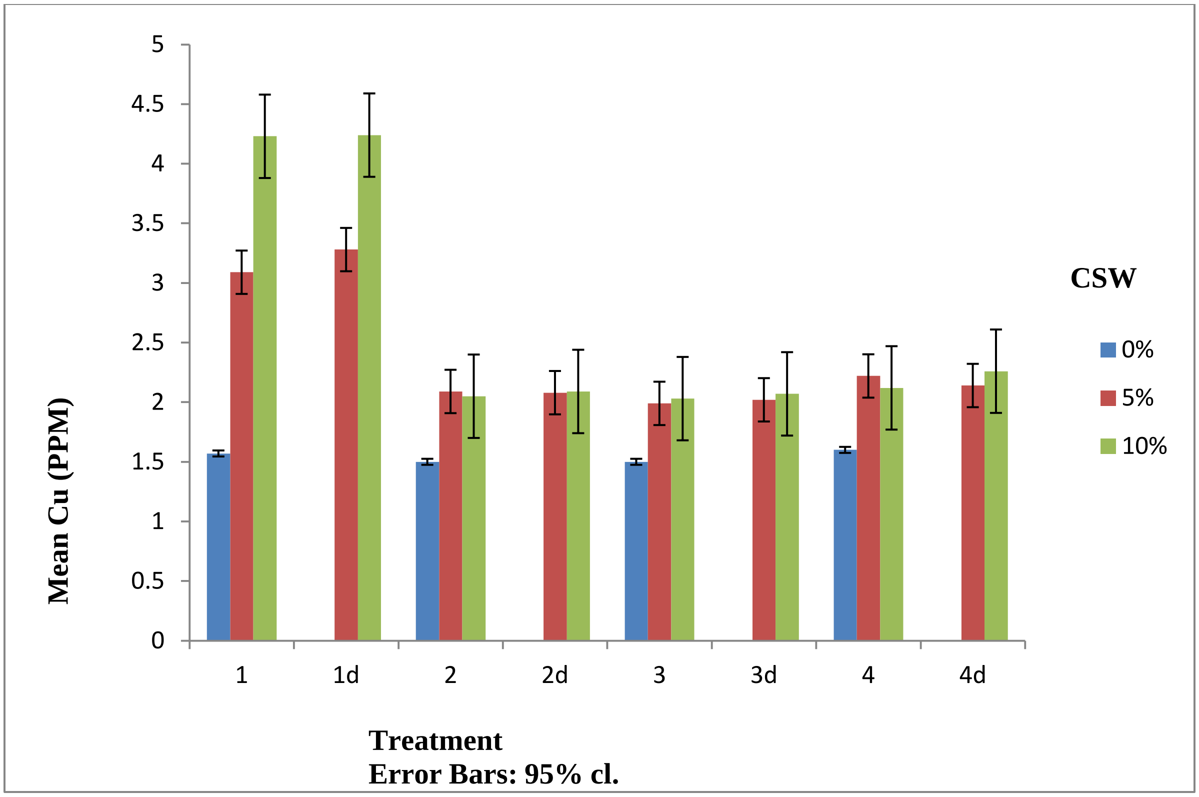

Figure 7: Copper (PPM) content of *S. lycopersicum* across all the treatments

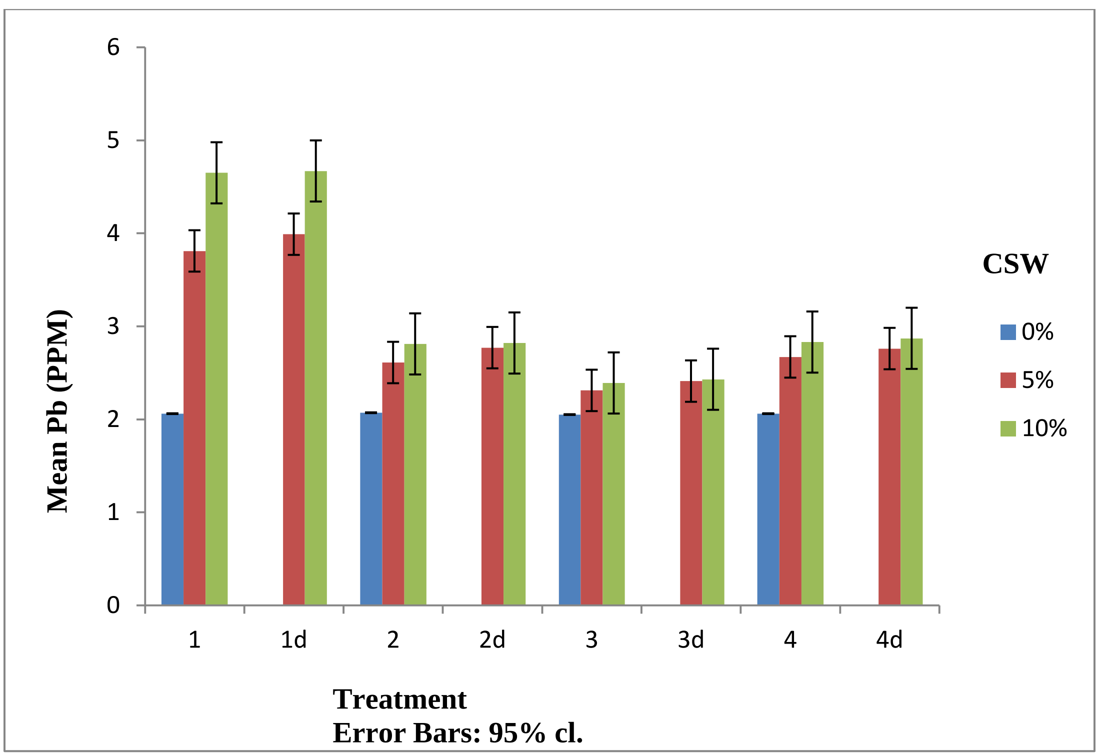

Figure 8: Lead (PPM) content of *S. lycopersicum* across all the treatments

DISCUSSION

Heavy metals are elements that exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity (Opaoluwa, 2010). They are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20. Industrial effluents are usually considered as undesirable for arable soil, plants, animals and human health. This is due to the contained heavy and trace metals like Cr, Mn, Fe, Cu, Co, Zn, Ni, As, Cd and Pb that are discharged continuously into water source (streams/ nullahs, canals and rivers). These are allowed to spread on agricultural lands. The unplanned disposal of these effluents has increased the threat of environmental pollution (Gulfraz *et al.*, 2003). Soils, whether in urban or agricultural areas represent a major sink for metals released into the environment from a wide variety of anthropogenic sources (Niragu, 1991).

Su *et al.* (2014) reported that low concentration of heavy metals could stimulate microbial growth and increase microbial biomass, while high concentration could decrease soil microbial biomass significantly. The microorganisms used in this study (*T. harzianum* and *P. aeruginosa*) were highly effective in biodegrading heavy metals. The bio-sorption potential of the organisms used in this study showed that *T. harzianum* and *P. aeruginosa* posses effective heavy metal absorption capacity. It was discovered in this study that at higher concentrations of these metals, there were reductions in plant growth. This may be due to the decrease in growth parameters of *S. lycopersicum* as the contaminated stream water concentration increased in this study. Heavy metals of soil in all the soil samples showed an increase as the contaminated stream water increased in concentration. Treatments inoculated with *P. aeruginosa* were found to have lower concentration of heavy metals (Fe, Zn, Cu and Pb) followed by treatments inoculated with *T. harzianum*. Due to a change in their oxidation state, heavy metals can be transformed to become either less toxic, easily volatilized, more water soluble (and thus can be removed through leaching), less water soluble (which allows them to precipitate and become easily removed from the environment) or less bioavailable (Marques *et. al.*, 2009).

The biodegrading ability of *P. aeruginosa* which showed the most efficient heavy metal uptake from the soil is in agreement with report of Lewis *et al.* (2002) and Odeyemi *et al.* (2011) which stated that *Psedomonas* spp have a high biodegrading ability. Report from Jankiewicz *et al.* (2000) also support the findings from this study which noted that *P. aeruginosa* cells grown in biofilms accumulate higher amounts of heavy metals. Also, many species of soil fungi including *Trichoderma* are able to dissolve through the release of chelating compounds of organic acids. The fungus releasing organic acids causes acidification of the environment, which helps increase the mobility of heavy metals (Barea *et al.*, 2005; Ledin, 2000; Wang and Chen, 2009). This study confirms this reports. Treatments inoculated with dual inoculation of *T. harzianum* and *P. aeruginosa* were found to have slightly higher concentration of heavy metals than treatments inoculated with *P. aeruginosa* or *T. harzianum*. However treatments with no inoculation of one or two microorganisms showed very high concentration of heavy metals in the soil in comparison with treatments with dual microorganisms. This confirms that the microorganisms used in this study biodegraded the heavy metals in the soil. This also revealed that there is positive and productive interaction between *T. harzianum* and *P. aeruginosa* in bioremediation of heavy metals heavy metals polluted soil.

Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils. Some metals with unknown biological function (Cd, Fe, Zn, Cu, Cr, Pb, Co, Ag, Se, Hg) can also be accumulated (Cho-Ruk *et al.*, 2006). Contaminant uptake by plants and its mechanisms have been being explored by several researchers. It could be used to optimize the factors to improve the performance of plant uptake. According to Sinha *et al.* (2008), the plants act both as “accumulators” and “excluders”. Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or biotransform the contaminants into inert forms in their tissues. The excluders restrict contaminant uptake into their biomass. Plant has a lot of consequences from heavy metal pollution in soil (Liao 1993, Su *et al.*, 2014, Wu *et al.*, 1998), plants were also seen to be polluted by heavy metals (Yin *et al*., 1999), which consequently threatens the health of animals and human beings via the food chain (Wang *et al*., 2001).

Heavy metals such as cadmium and lead are non-essential elements for plants. Microbial populations are generally higher in the rhizosphere than in the root-free soil. This is due to a symbiotic relationship between soil microorganisms and plants. This symbiotic relationship can enhance some bioremediation processes. Plant roots also may provide surfaces for sorption or precipitation of metal contaminants (Sas-Nowosielska *et al.*, 2008) .This study were found to show reduction in growth parameters as heavy metals increased which is brought by increase in contaminated stream water concentration. Iron, Zinc, Copper and Lead level were higher in plant tissues from soil samples containing no inoculation of microorganisms at 5% and 10% contaminated stream water concentration. This was discovered to affect the growth of the plants. Su *et al.*, (2014) reported that dicots, leafy vegetable crops are sensitive to Zn toxicity, especially spinach and beet; because of their inherent high Zn uptake capacity. However soil samples containing *P. aeruginosa* was generally the lowest in plant heavy metal uptake of iron, zinc, copper and lead followed by samples containing *T. harzianum*. This may be an indication that the heavy metals in the soil had been degraded by the microorganisms used which also showed there is low amount of heavy metals in soil left for the plant to absorb. This result was found to be consistent with the work of Soumitra *et al.* (2014) which demonstrated that *P. aeruginosa* reduced heavy metal uptake in *Oryza sativa* L. and increase its growth. Also *Trichoderma* spp. produces organic acids such as gluconic acid, fumaric acid, and citric acid, which can decrease the pH of the soil and allow for the dissolution of phosphate, as well as macro- and micronutrients such as iron, manganese, and magnesium, which are necessary for plant metabolism (Ociepa, 2011; Cao *et al.*, 2008) Treatments inoculated with a combination of *T. harzianum and P. aeruginosa* in this study had lower concentration of heavy metals in their plant tissue compared to treatments without inoculation of microorganisms. This may insinuate that there is positive and effective interaction between *T. harzianum* and *P. aeruginosa* in the reduction of heavy metals build up in plant cultivated on heavy metals polluted soil. concentrations of metals were attributed to the contaminated stream water irrigation. The results from this study indicates that there is a serious potential health risk associated with heavy metals in tomato by using contaminated water for irrigation by farmers for tomato production.

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