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Potentials of *Pseudomonas aeruginosa* and *Trichoderma harzianum* in the Growth of *Solanum lycopersicum* in Heavy Metal Contaminated Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKA managed the analyses of the study. Author BFA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study determined the growth and yield of *Solanum lycopersicum* in heavy metal contaminated soil and the heavy metal uptake of the harvested *S. lycopersicum* fruit. 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

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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%. Growth and yield data from the experiment were obtained and the results were subjected to ANOVA and descriptive analysis. 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. The effect of *P. aeruginosa* were found to be more pronounced among all the treatments as it enhanced the growth and yield of the plants when compared with other treatments. Also, tomato plants with *T. harzianum* inoculation had higher mean plant height of 66.7 ± 2.3 cm, higher number of leaves 78.0 ± 8.0 and leaf area of 77.5 ± 4.5 cm^2. Highest fruit yield was produced in tomato plants treated with *P. aeruginosa* at 5% and 10% level of contaminated stream water sample concentration. 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.

Keywords: Pseudomonas aeruginosa; bioremediation; heavy metal.

1. INTRODUCTION

Heavy or toxic metals are trace metals which are detrimental to human health and they have density that is at least five times that of water. Once released into the environment through the air, drinking water, food, or countless varieties of man-made chemicals and products, heavy metals are taken into the body via inhalation, ingestion and skin absorption [1]. Wastewaters contain toxic heavy metals that are introduced into the soil and aquatic system through various processes, prominent among them is irrigation [2]. Heavy metal pollution in the air increases through human activities [2]. 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 [3,4]. 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 [5]. Vegetables, especially leafy vegetables, accumulate higher amounts of heavy metals because these metals are absorbed in their leaves. Tomato (*Solanum lycopersicum*) is one of the most important vegetable and one of the most widely cultivated crops wordwide [6]. This is due to its acclimatization to a wide variety of environment as well as its nutritive value. They contribute to a healthy, well balanced diet and are rich in minerals, vitamins essential amino acids, sugars and dietary fibres [6]. *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 [7]. The fungus is thought to colonize roots of annual plants for their entire lifetime by penetrating the outer layers of the roots [8]. 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 [8]. [9] 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 [10]. Recent studies have established that the stream water flowing at the back of Ife Iron and Steel Company, Ile-Ife, is heavily contaminated with heavy metals. The stream water is primarily used for irrigation of vegetable farms. This could pose a serious health risk to vegetable consumers. This study intends to assess the potential of *Pseudomonas aeruginosa* and *Trichoderma harzianum* to degrade heavy metals in soils contaminated with heavy metal contaminated stream water.

2. MATERIALS AND METHODS

2.1 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 were 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. 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.

2.2 Culturing of Organisms

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.

2.3 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 [11]. 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.

Data on growth performance of the plants such as number of leaves, leaf area and vine length was measured weekly. Data on yield parameters such as number of fruits, weight of fruit and diameter of fruit was recorded at every harvest from 13 weeks after planting till the experiment was terminated at 15 weeks after planting. Data obtained was subjected to statistical analysis using descriptive and inferential methods.

2.4 Experiment (Treatment Layout)

Sterilized soils were polluted with contaminated stream water at a calculated percentage using the formula; Percentage soil contamination =

(Volume of Contaminated stream water/Volume of soil) x 100.

The layout of the experiment is as follows;

Treatment 1- sterilized soil + *S. lycopersicum*
Treatment1d- sterilized soil + *S. lycopersicum* (2)
Treatment 2- sterilized soil + *Trichoderma harzianum* + *S. lycopersicum*
Treatment 2d- sterilized soil + *Trichoderma harzianum* + *S. lycopersicum* (2)
Treatment 3- sterilized soil + *Pseudomonas aeruginosa* + *S. lycopersicum*
Treatment 3d- sterilized soil + *Pseudomonas aeruginosa* + *S. lycopersicum* (2)
Treatment 4- sterilized soil + *T. harzianum* + *P. aeruginosa* + *S. lycopersicum*
Treatment 4d- sterilized soil + *T. harzianum* + *P. aeruginosa* + *S. lycopersicum* (2)

Note: (2) and d means daily wetting of pots with contaminated water.

Each of the layouts contaminated at 0, 5, and 10% (v/w) contaminated stream water concentration was replicated thrice. The experimental pots were watered regularly to ensure adequate moisture for proper growth of the test plant.

The following growth parameters of the plant were used to measure the effect of contamination and treatment in the experiment.

2.5 Number of Leaves after Treatment

The number of leaves on each plant was counted visually on weekly basis from the first day of pollution with contaminated water until the day of harvest.

2.6 Height of Plant after Treatment

The height of plant was taken from the terminal bud to the soil level, the measurement was taken weekly in centimeter using meter rule from the day of pollution to harvest time.

2.7 Area of Leaves after Treatment

The length (L) of leaves was measured from the node to the tip and the width (W) was measured from one end to the other on a weekly basis.

The Leaf area was determined using the formula; Leaf Area=L multiplied by W multiplied by 2.325.

The parameters are;

L = length of leaf
W = Width of leaf and 2.325 is a constant factor which is the correction factor.

The unit of Area is "cm^2"

3. 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.

3.1 Agronomic Data

The agronomic data of *S. lycopersicum* was taken weekly across all the treatments at different levels of contamination with contaminated stream water which showed different responses. The growth parameters showed reduction in its properties as the concentration of the contaminated stream water increased. These growth parameters include.

3.1.1 Number of leaves under different treatments

The number of leaves in treatment 1 without contaminated water pollution and with no inoculation of micro-organisms was significantly different from 3WAP to 4WAP. At 3WAP, treatment 3 with inoculation of *P. aeruginosa* and treatment 2 with dual inoculation of *T. harzianum* and *P. aeruginosa* were not significantly different from each other at p level ≤ 0.05. At 4WAP, treatment 2 with inoculation of *T. harzianum* had the highest value in number of leaves with 19.7 followed by treatment 4 with dual inoculation of *T. harzianum* and *P. aeruginosa* which had 17.7 number of leaves (Table 1). At 5WAP, the number of leaves in treatment 3 with inoculation of *P. aeruginosa* and treatment 4 with inoculation of *T. harzianum* and *P, aeruginosa* were not significantly different from each other. At 6WAP, the number of leaves of treatment 1 without contaminated water pollution and treatment 2 with inoculation of *T. harzianum* was not significantly different from each other at p level ≤ 0.05. Treatments 1, 2, 3 and 4 were not significantly different from 7WAP to 9WAP at p level ≤ 0.05. All the treatments showed decrease in their number of leaves from 9WAP to 12WAP when the plants began to fruit. At 12WAP, treatment 2 with inoculation of *T. harzianum* had the highest number of leaves of 46.3 followed by treatment 4 with dual inoculation of *T. harzianum* and *P. aeruginosa* with 39.0 number of leaves.

For 5% level of contaminated stream water concentration, at 3WAP, treatment 1 and treatment 1d (both with no inoculation of micro-organism) were not significantly different from each other but were significantly different from each other at 4WAP. Also treatment 2 and treatment 2d (both with inoculation of *T. harzianum*) were not significantly different from each other but were significantly different from each other at 4WAP (Table 2). Treatments 3 and 3d (both with inoculation of *P. aeruginosa*) were significantly different from each other at 3WAP and but were not significantly different from each other at 4WAP at p level ≤ 0.05. Treatment 3 with inoculation of *P. aeruginosa*, treatment 4 and treatment 4d (both with dual inoculation of *T. harzianum* and *P. aeruginosa*) were not significantly differently each other at 3WAP but were all significantly different at 5WAP. All the treatments were significantly different from each other from 6WAP to 9WAP at p level ≤ 0.05. At 8WAP, treatment 3 with inoculation of *P. aeruginosa* has the highest number of leaves of 89.0 followed by treatment 3d (also with inoculation of *P. aeruginosa*) with 72.3 but treatment 1d with no inoculation of micro-organisms had the lowest number of leaves of 40.3. From 9WAP to 12WAP, all the treatments showed decrease in their number of leaves when the plants started fruiting. At 11WAP, treatment 1d with no inoculation of micro-organisms and treatment 2d with inoculation of *T. harzianum* were not significantly different from each other. At 12WAP treatment 3 with inoculation with *P. aeruginosa* had the highest number of leaves of 61.0 with treatment 2 with inoculation of *T. harzianum* having the lowest number of leaves of 21.7.

For 10% level of contaminated stream water concentration, treatment 1d with no inoculation of micro-organisms and treatment 2 with inoculation of *T. harzianum* were not significantly different from each other at 3WAP but were significantly different from each other at 4WAP at p level ≤ 0.05. Similarly, at 3WAP, treatment 3, 3d (both with inoculation of *P. aeruginosa*) and treatment 4d with dual inoculation of *T. hazianum* and *P. aeruginosa* were not significantly different from each other but were significantly different at 4WAP (Table 3). All the treatments were significantly different from 5WAP to 7WAP. At

Table 1. Mean number of leaves of test plant under different treatment with 0 % contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	11.0	13.7	22.7	42.7	45.0	53.0	42.3	39.3	36.3	32.3
2-SS + TH + TP	10.7	19.7	34.3	42.7	66.7	78.0	67.0	56.3	48.0	46.3
3-SS + PA + TP	10.0	15.3	22.0	30.0	40.0	49.0	43.0	36.7	33.3	35.0
4-SS+TH+PA+TP	10.0	17.7	22.0	36.7	54.0	63.0	51.7	45.3	40.7	39.0

Legend: SS: Sterilised soil; TP: Test plant; TH: Trichoderma harzianum; PA: Pseudomonas aeruginosa; Treatment 1 – Sterilised soil + Solanum lycopersicum; Treatment 2 – Sterilised soil + T. harzianum + Solanum lycopersicum; Treatment 3 – Sterilised soil + P.aeruginosa +Solanum lycopersicum; Treatment 4 – Sterilised soil + T. harzianum+ P. aeruginosa + Solanum lycopersicum

Table 2. Mean number of leaves of test plant under different treatment with 5 % contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	10.0	13.0	17.7	26.7	44.0	53.3	45.0	40.0	33.3	31.7
1d-SS + TP	10.0	10.7	14.7	20.3	31.3	40.3	34.0	24.0	25.7	22.3
2-SS + TH + TP	9.3	18.0	24.7	51.7	52.3	59.3	40.0	32.3	27.7	21.7
2d-SS+TH +TP	9.3	19.0	28.0	35.3	34.3	42.3	33.3	28.3	25.0	23.3
3- SS + PA + TP	10.3	16.7	32.3	60.0	80.0	89.0	79.3	75.3	68.0	61.0
3d-SS + PA + TP	11.0	17.0	25.7	46.3	63.3	72.3	63.0	59.7	56.0	52.0
4-SS+TH+PA+TP	11.0	16.0	26.0	39.7	45.0	54.3	45.0	37.0	33.3	33.3
4d-SS+TH+PA+TP	11.0	17.0	25.0	31.3	39.7	48.7	41.7	36.0	33.3	29.7

Table 3. Mean number of leaves of test plant under different treatment with 10% contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	9.3	12.7	13.7	23.3	32.3	42.3	34.0	28.0	25.7	23.3
1d-SS + TP	10.3	10.7	11.7	16.7	20.7	28.3	23.7	20.3	19.0	16.7
2-SS + TH + TP	10.0	18.0	23.0	29.0	41.7	50.3	44.0	37.7	36.7	33.3
2d-SS+TH +TP	10.7	16.0	21.3	27.3	30.7	37.0	32.7	27.3	25.7	24.3
3- SS + PA + TP	11.0	16.7	24.0	45.0	53.3	60.3	52.7	49.7	48.7	45.0
3d-SS + PA + TP	11.0	17.0	30.3	49.0	65.0	75.3	66.3	60.3	57.0	52.0
4-SS+TH+PA+TP	11.3	16.9	24.0	38.3	35.7	45.3	35.3	31.7	29.7	28.0
4d-SS+TH+PA+TP	11.0	17.0	25.0	36.7	45.0	55.0	45.7	40.3	36.7	33.3

8WAP, treatment 3d with inoculation of *P. aeruginosa* had the highest number of leaves of 75.3 followed by treatment 3 also with inoculation of *P. aeruginosa* with 60.3 number of leaves while treatment 1d had the lowest number of leaves of 25.3. All the treatments showed decrease in their number of leaves from 9WAP to 12WAP when the plants began to fruit. All the treatments were significantly different from 9WAP to 12WAP at p level ≤ 0.05. At 12WAP, treatment 3d had the highest number of leaves of 52.0 while treatment 1d with no inoculation of micro-organism had the lowest number of leaves, having 16.7 number of leaves.

<u>3.1.2 Plant height under different treatments</u>

The plant height at p level ≤ 0.05 showed that at 0% level of contaminated stream water concentration, treatment 1 with no inoculation of micro-organism and treatment 3 with inoculation of *P. aeruginosa* had no significant difference from each other at 3WAP but significant difference at 4WAP. Also treatment 2 with inoculation of *T. harzianum* and treatment 3 with inoculation of *P. aeruginosa* showed no significant difference from each other at 4WAP (Table 4). All the treatments showed a significant difference from 5WAP to 12WAP. At 12WAP, treatment 2 had the highest value of plant height of 66.7 cm followed by treatment 4 with 56.7 cm plant height while treatment 1 had the lowest value of plant height of 47.7 cm.

For 5% level of contaminated stream water concentration, treatment 1d with no inoculation of micro-organism, treatment 3, treatment 3d (both with inoculation of *P. aeruginosa*) and treatment 4 with dual inoculation of *T. harzianum* and *P. aeruginosa* were not significantly different from each other at 3WAP at p level ≤ 0.05. At 4WAP treatment 1 and treatment 1d (both with no inoculation of micro-organism) were not significantly different (Table 5). There was an increase in plant height across all the treatments from 4WAP to 10WAP. There was also a significant difference in all the treatments from 5WAP to 10WAP. Treatment 1 with no inoculation of micro-organisms had stagnant plant height with no further increment or reduction from 10WAP to 12WA. At 12WAP, treatment 3 with inoculation of *P. aeruginosa* had the highest value of plant height with treatment 1d with no inoculation of micro-organisms having the lowest value of plant height.

For 10% level of contaminated stream water concentration, treatment 1 with no inoculation of micro-organisms, treatment 2d with inoculation of *T. harzianum* and treatment 4d with dual inoculation of *T. harzianum* and *P. aeruginosa* were not significantly different from each other at 3WAP at p level ≤ 0.05. Similarly, treatment 2 with inoculation of *T. harzianum* and treatment 3d with inoculation of *P. aeruginosa* were not significantly different from each other at 3WAP (Table 6). All the treatments showed a vast increase in height from 4WAP to 12WAP, all the treatments were also significantly different from 4WAP to 12WAP. At 12WAP, treatment 3d with inoculation of *P. aeruginosa* had the highest value of plant height of 61.3 cm followed by treatment 3, also with inoculation of *P. aeruginosa* with 61.0 cm value of plant height while treatment 1d with no inoculation of micro-organism had the lowest value with 30.3 cm.

<u>3.1.3 Leaf surface area under different treatments</u>

The leaf surface area (L.S.A) of *Solanum lycopersicum* plant at 0% level of contaminated stream water concentration showed that treatment 1 (with no inoculation of micro-organism) and treatment 3 (with inoculation of *P. aeruginosa*) at 3WAP were not significantly different from each other at p level ≤ 0.05. Also treatment 2 (with inoculation of *T. harzianum*) and treatment 4 (with dual inoculation of *T. harzianum* and *P. aeruginosa*) were not significantly different in their leaf surface area at 3WAP (Table 7). All the treatments showed drastic increase in their leaf surface area from 4WAP to 10WAP. Treatment 1 with no inoculation of micro-organism showed no further increment or reduction in its leaf surface area from 10WAP to 11WAP but there was a significant difference in its leaf surface area from 11WAP to 12WAP at p level ≤ 0.05. Treatment 2 with inoculation of *T. harzianum* showed no significant difference from 8WAP to 10WAP. Treatment 2 (with inoculation of *T. harzianum*), treatment 3 (with inoculation of *P. aeruginosa*), and treatment 4 (with dual inoculation of *T. harzianum* and *P. aeruginosa*) showed no increase in their leaf surface area from 11WAP to 12WAP. At 12WAP, treatment 2 with inoculation of *T. harzianum* had the highest leaf surface area while treatment 3 with inoculation of *P. aeruginosa* had the lowest leaf surface area.

For concentration of contaminated stream water at 5%, treatment 1d (with no inoculation of micro-organisms) and treatment 2d (with inoculation of *T. harzianum*) were not significantly different in their leaf surface area at 3WAP at p level ≤ 0.05. Also, treatment 3 (with inoculation of *P. aeruginosa*) and treatment 4d (with dual inoculation of *T. harzianum* and *P. aeruginosa*) were not significantly different from each other at 3WAP. There was an increase in the leaf surface area of all the treatments from 4WAP to 9WAP.

Table 4. Mean height of test plant (cm) under different treatment with 0 % contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	8.0	16.0	23.3	30.7	38.0	43.3	45.3	46.3	47.0	47.7
2-SS + TH + TP	8.3	18.3	30.7	41.0	52.7	60.0	63.3	64.0	65.3	66.7
3-SS + PA + TP	8.0	18.0	30.0	33.0	40.3	47.7	51.0	52.3	52.7	55.0
4-SS+TH+PA+TP	8.7	18.7	26.7	34.0	42.0	49.0	52.0	53.7	54.3	56.7

Table 5. Mean height of test plant (cm) under different treatment with 5% contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	8.0	13.0	18.3	26	34.7	40.7	42.7	44.0	44.0	44.0
1d-SS + TP	7.7	13.3	15.3	18.3	27.3	30.7	33.7	35.7	36.3	38.0
2-SS + TH + TP	8.0	19.7	28.7	43.3	51.7	58.3	60.7	62.7	63.3	45.7
2d-SS+TH +TP	8.0	18.3	25.3	36.0	41.7	48.0	51.0	52.0	52.7	54.7
3- SS + PA + TP	7.7	18.0	30.0	53.3	61.0	69.0	72.7	73.7	74.3	75.7
3d-SS + PA + TP	7.7	16.7	29.0	42.3	51.3	58.3	61.7	63.0	63.0	63.3
4-SS+TH+PA+TP	7.7	18.3	26.7	33.0	29.0	44.3	48.0	49.7	51.0	52.3
4d-SS+TH+PA+TP	8.3	19.0	26.0	31.7	38.3	43.7	47.3	49.3	50.0	55.0

Table 6. Mean height of test plant (cm) under different treatment with 10% contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	8.0	12.3	18.3	24.0	27.7	30.3	32.0	33.7	35.0	36.3
1d-SS + TP	7.3	13.3	15.0	18.3	23.3	25.7	27.7	29.0	29.7	30.3
2-SS + TH + TP	7.7	17.3	29.0	31.3	36.7	45.3	47.3	49.3	49.3	50.0
2d-SS+TH +TP	8.0	18.0	27.3	29.0	34.3	39.7	43.0	44.7	45.3	46.7
3- SS + PA + TP	8.3	17.7	31.0	39.3	47.7	55.0	58.3	59.7	60.3	61.0
3d-SS + PA + TP	7.7	19.0	29.7	43.3	50.0	55.7	59.3	60.7	61.0	61.3
4-SS+TH+PA+TP	8.7	19.0	21.0	26.0	32.7	38.0	41.0	42.3	42.3	42.3
4d-SS+TH+PA+TP	8.0	17.3	21.0	32.7	38.7	44.0	46.7	48.0	48.7	49.3

From 10WAP to 12WAP, here was no further increment or reduction in the leaf surface area of treatments 1, 1d, 2 and 4d (Table 8). At 12WAP, treatment 4d had the highest leaf surface area of 71.3 cm^2 followed by treatment 3 with 69.0 cm^2 leaf surface area while treatment 1d had the lowest leaf surface area of 47.2 cm^2.

For 10% level of contaminated stream water contamination, treatment 2, treatment 2d and treatment 3 were insignificantly different from each other in their leaf surface area at 3WAP. Also, treatment 3d and treatment 4d were insignificantly different from each other at 3WAP (Table 9). All the treatments showed a significant difference in their leaf surface area from 4WAP to 8WAP. There was no significant difference in treatments 1, 3 and 4d from 8WAP to 10WAP. Treatment 3 showed no further increment or reduction in its leaf surface area from 8WAP till the experiment was terminated. At twelve weeks after planting, treatment 3 had the largest leaf surface area of 71.3 cm^2 followed by treatment 2d with 70.5 cm^2 of leaf surface area with treatment 1 having the lowest leaf surface area of 48.8 cm^2.

4. DISCUSSION

Solanum lycopersicum cultivated in sterilized soil inoculated with *Pseudomonas aeruginosa* with contaminated stream water had the highest plant height (74.3 cm) in comparison with other samples. Treatments inoculated with *Trichoderma harzianum* and treatments with dual inoculation of *T. harzianum* and *P. aeruginosa*

Table 7. Mean leaf surface area (cm^2) under different treatment with 0% contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	6.6	15.7	22.2	25.7	35.6	51.1	54.2	56.6	56.6	58.2
2-SS + TH + TP	6.0	20.9	27.1	46.1	52.7	74.4	74.4	74.4	77.5	77.5
3-SS + PA + TP	6.6	17.4	21.7	27.5	23.2	46.5	51.9	54.2	57.3	57.3
4-SS+TH+PA+TP	6.0	19.3	22.4	34.9	31.7	55.8	58.1	58.1	60.4	60.4

Table 8. Mean leaf surface area (cm^2) under different treatment with 5% contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	6.8	12.6	20.1	25.2	37.1	65.0	65.1	68.2	68.2	68.2
1d-SS + TP	6.4	9.7	14.9	23.4	31.3	39.5	47.2	47.2	47.2	47.2
2-SS + TH + TP	5.4	19.3	26.1	28.6	29.4	52.7	52.7	55.0	55.0	55.0
2d-SS+TH +TP	6.4	16.4	18.7	28.4	26.3	49.2	51.5	51.5	54.2	54.1
3- SS + PA + TP	5.8	19.9	19.3	48.1	51.5	69.7	65.9	65.9	65.9	69.0
3d-SS + PA + TP	6.6	19.9	16.6	33.7	35.6	60.4	60.4	60.4	65.9	65.9
4-SS+TH+PA+TP	6.0	17.4	21.5	27.7	30.4	52.7	57.3	57.3	57.3	58.9
4d-SS+TH+PA+TP	6.6	19.3	16.0	25.2	45.3	68.2	70.9	71.3	71.3	71.3

Table 9. Mean leaf surface area (cm^2) under different treatment with 10% contaminated stream water concentration

	Weeks after planting									
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-SS + TP	6.4	12.6	15.1	23.4	35.2	41.8	41.8	41.8	44.1	48.8
1d-SS + TP	7.2	11.6	17.4	15.1	21.7	44.5	46.5	51.9	54.2	54.2
2-SS + TH + TP	5.8	17.4	18.8	19.9	23.2	44.9	47.2	49.6	49.6	51.9
2d-SS+TH +TP	5.8	15.5	17.0	20.9	29.4	66.6	66.6	70.5	70.5	70.5
3- SS + PA + TP	5.8	16.4	20.1	29.4	40.3	71.3	71.3	71.3	71.3	71.3
3d-SS + PA + TP	6.2	15.5	29.9	37.2	48.8	63.5	63.5	65.9	68.2	68.2
4-SS+TH+PA+TP	6.6	20.9	14.3	21.3	25.1	53.0	55.8	56.9	59.7	59.7
4d-SS+TH+PA+TP	6.2	15.5	16.2	20.9	34.1	51.7	51.7	51.7	55.8	61.2

had lower plant height than treatment inoculated with *P. aeruginosa*. Also treatments with dual inoculation had a higher plant height than treatment with single inoculation of *T. harzianum*. This result implies the synergistic relationship between the two organisms although *P. aeruginosa* functioned better on its own than the combination of both organisms. This confirms the report of [12] who reported *P. aeruginosa* as a plant rhizobacterium which aggressively colonizes plant roots and induces plant growth. The effect of *P. aeruginosa* were more articulated among the contaminated stream water polluted treatments as it was investigated to enhance the growth and development of the plants when compared with other treatments and control.Test plant cultivated in soil treated with *P. aeruginosa* at various level of contamination (5% and 10%) had better and improved growth parameters (i.e plant height, leaf surface area and number of leaves) when compared with inoculation of *T. harzianum*, treatments with dual inoculation of *T. harzianum* and *P. aeruginosa* and the treatment which serves as the control. Soil without inoculation of microorganisms were found to have the lowest growth parameters at 10% concentration. This result established that the plants solely depended on the available nutrients present in the soil. This could also be as a result of the heavy metals from the contaminated stream water. This correlates with the report of [13]. This report observed that growth reduction in plants is as a result of changes in physiological and biochemical processes in plants growing on heavy metals polluted soil [13,14,15]. This also confirms the reports of [16,17,18] that it could be as a result of contaminated soil through irrigation with contaminated water bodies (streams canals and rivers). Farmers use wastewaters to irrigate their crops. This wastewater contains large amount of organic materials and some substantial amount of inorganic materials and toxic heavy metals [16,17,18]. This is especially true as heavy metals does not play any beneficial role towards the development of plants [19]. *Solanum lycopersicum* grown in soil without contaminated stream water were found to grow better than those from contaminated soil. This shows that the contaminated stream water hinders the development and growth of the plant. Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress [20,21,22]. These toxic effects (both direct and indirect) could lead to a decline in plant growth which sometimes results in the death of plant [23]. It further denotes that soil polluted with contaminated stream water are low in fertility and nutrient hence, does not support adequate plant growth and development. It was observed from this study that the values of the growth parameters decreased as the contaminated stream water concentration increased. This is due to changes in the soil condition as a result of the increased contaminated stream water. This toxicological and physiological stress is as a result of the heavy metals present in the contaminated water which the plants are exposed. Most of the reduction in growth parameters of plants growing on polluted soils can be attributed to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes as reported by [24]. It was also observed that daily watering of the plants at various level of contamination had reduced growth parameters when compared to the plants that were watered with the contaminated stream water once during the experiment. Higher concentration of contaminated stream water could be linked with the interference of the soil physical and chemical properties and subsequently the transpiration and photosynthetic processes in the plant, thereby resulting in decrease in growth parameters. The reduction of plant growth observed could be due to reduction of essential nutrient and mineral content with increasing contaminated stream water concentration in the soil [24]. These elevated 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.

5. CONCLUSION

Atmospheric pollution and effluent discharge from industries have adverse side effects on soil and water bodies. These contaminations result in elevated heavy metal concentrations of soil and water. Due to the complexity involved in the conventional methods for remediation of soil, the use of microbes has arisen as a time-saver for bioremediation. Biological decomposition of persistent organic pollutants and heavy metals is one of the most effective and important ways to remove these compounds from the environment. It is evident from the result of this study that biodegradation of heavy metals is an environmental friendly and easy approach to transform heavy metals in polluted soils.

Pseudomonas aeruginosa used in this study was found to have a greater ability to increase plant growth in heavy metal polluted soil and absorb nutrients from the soil. *Pseudomonas aeruginosa* also showed a higher ability in transforming the heavy metals in the soil than *Trichoderma harzianum.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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