International Conference of Sciences, Engineering & Environmental Technology (ICONSEET), 5(21): 168 – 175, 2020
ISSN 0794-9650

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Phytoremediation Potential of *Solanum lycopersicum* (Tomato) in a heavy metal contaminated soil with the aid of *Pseudomonas aeruginosa*

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Abstract: This study monitor the physico-chemical parameters of heavy-metal polluted soil undergoing the process of bioremediation, by determining the concentration of heavy metals up took by the test plant through *P. aeruginosa* inoculated into the soil and thereby access the potential of the microbe in remediating heavy metal polluted soils. The ability of *S. lycopersicum* to grow and phytoextract Fe, Zn, Pb, No, Cr, As, Cu through the aid of *P. aeruginosa* to remediate a heavy-metal polluted soil was evaluated and analysed over a period of 8 weeks. The plant height, stem width, concentration of heavy metals in the soil and in the plants as well as isolates from soil sample around the plant were all measured, evaluated and analysed during and after the study. There was a gradual increase in the heights and the width of the tomato plant with increasing concentration of the inoculated *P. aeruginosa* as the week goes by, when compared with tomato plant with no inoculation (control). A significant difference in the concentration of all the heavy metals before and after the study was recorded. Post-cropped soil was found to have the lowest concentration of heavy metals remains after the study. The heavy metals up took by the tomato plant can be extracted for industrial use. This study investigated the remediation potential of *Pseudomonas aeruginosa* with the aid of *Solanum lycopersicum* in a heavy metals contaminated soils.

Key words: ***Pseudomonas aeruginosa, Solanum lycopersicum, Klebsiella pneumonia, Pseudomonas veronii, phytoextract, inoculation, postcropped.***

Study Background

Heavy metals can be described as elements with an atomic density greater than 6g/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 (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 (Liu *et al*, 2014). 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 rapid industrialization, urbanization and other anthropogenic sources. (Jern, 2006). The two main sources of heavy metals pollution are natural and human sources. The natural source include soil erosion, volcanic activities, urban runoff as well as aerosols particulate, while the human source of the pollution could come from metal finishing and electroplating processes, mining extraction and operations, through textile production as well as from nuclear power (Amarat *et al*, 2006). Some of the effects of heavy metals on plants include; lowered number of germinated seeds after planting, 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.(Gardey-Torresdey *et al*, 2005). Bioremediation a method to reduce or remove heavy metal pollution from the environment is considered as one of the natural ways to attenuate or transform harmful substances to a less harmful one, through the use of microorganisms or green plants. The micro-organisms produce surfactants which aid biodegradation and thus convert the heavy metals to nutrients and help to support plant growth, 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 (Yanchelismeh *et al*, 2011; De Bashan *et al.*, 2012). Some important factors to consider when choosing a plant as a phyto-remediator include; root system, above-the-ground biomass, toxic level of the pollutants, plant survival and its adaptability to prevailing environmental conditions, plant growth etc. (Lee, 2013). 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 (Miguel *et al*, 2013).Solanium lycopersicum (Tomato) has been reported to be found useful for bio-fortification or phytoremediation (Gerzberg *et al.*, 2014).

Crops harvested from the heavy metals contaminated soil are toxic and injurious to human health. 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), as well as to assess the remediating potential of *P. aeruginosa* in a contaminated soil. However, the aim of the study is to monitor the physicochemical parameters of the soil and to isolate and characterizethe rhizosphere bacteria from the root of a tomato plant.

MATERIALS AND METHOD

Sample source, collection, preparation and planting

Heavy metal polluted soil was collected from sasa market in Akure, the soil was sterilized at 118°C for 4 hrs. Seeds of *S. lycopersicum* collected at the Institute of Agricultural Research and Training (IAR&T), Apata, Ibadan, were planted in a big pot filled with the soil and five (5) replicates of the experimental pots were made.. Each of the replicates were inoculated with *Pseud. aeruginosa* at varying concentration from 0 ml (T-P), 5 ml (T+P5 ml), 10 ml (T+P10 ml), 15 ml (T+P15 ml), 20 ml (T+P20 ml) respectively. The ability of *S. Lycopersicum* to grow and phyto extract Fe, Zn, Pb, No, Cr, As, Cu through the aid of *P. aeruginosa* was assessed and evaluated over a period of 8 weeks. The plant height and stem width of the plant were measured weekly and the concentration of heavy metals was determined in the shoot of the plant before and after the treatments. The concentration of heavy metals in the soil were also compared at the beginning and at the end of the study, among the initial soil control, post cropped soil i.e. (Soil + T +P20 ml), as well as plant without P. aeruginosa. So also, isolates from soil sample around the tomato plant was also analysed for possible present of other bacteria at the end of the study.

Materials and Instrument

The apparatus used in this experiment. Petri dishes, Mc Cartney bottles, weighing balance, paper tape, aluminium foil, spirit lamp, cotton wool, gloves, test tube rack, refrigerator etc.

Sterilization of equipments/glassware and Preparation of Nutrient Agar

All glass wares 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

28 grams of Nutrient agar powder was weighed with a weighing balance into a 1000 millilitres Erlenmeyer flask to dissolve the powder. The flask was corked and wrapped. The flask was placed inside an autoclave and sterilized at 121^0C for 15 minutes. After sterilization, the flask was brought out of the autoclave and allowed to cool to 45^0 C before used.

Enumeration of Total Heterotrophic Bacteria

Culturing of heterotrophic bacteria was done using nutrient agar prepared by serially diluting 0.1ml soil sample plated out in triplicate on the nutrient agar using pour plate method with un-inoculated plate serving as control. All plates were then incubated at 37^0c for 24 hours and then observed growth and colonies were counted after incubation.

Characterization and Identification of Bacteria Isolate

After incubation, the plates that were between 30 to 200 colonies were selected and used. Each bacterium colony type was subculture repeatedly into nutrient agar plates to obtain a pure culture. The isolates were characterized based on cultural characteristics such as shape, size, pigmentation, elevation and marginal characteristics, also morphological characteristics such as Cocci, Bacilli and Vibro were also observed. Lastly, biochemical reactions such as Gram staining, motility test, Catalase test, Triple sugar Iron (TSI) test, indole test, sugar fermentation respectively were carried out for proper identification.

RESULTS

There was an increase in the heights of the tomato plant with increasing concentration of the inoculated *P. aeruginosa* as the week goes by, as compared with tomato plant with no inoculation. The heights of the Tomato plant for all the different concentration at (4wks, 8wks) are as follows T-P (2.2 m, 4.6m), T+P5 ml (3.7m, 7.5 m), T+P10 ml (4.6 m, 8 m), T+P15 ml (6.4 m, 9.3 m), T+P20 ml(6.3 m, 9.4 m) respectively. In the same vein, the stem width of the tomato plant was found to follow similar pattern as the height. The width for all the concentration at (4wks, 8wks) were T-P (0.5cm, 0.8cm), T+P5ml (.3cm, 2.4cm), T+P10ml (1.3cm, 2.6cm), T+P15 ml (1.7cm, 2.8cm), T+P20 ml(1.8 cm, 3.0 cm) respectively. There was however slight difference in the width of the plant for the different concentrations of inoculates in the first two weeks with the lowest value recorded in T-P (0.2 cm) at 2 weeks.

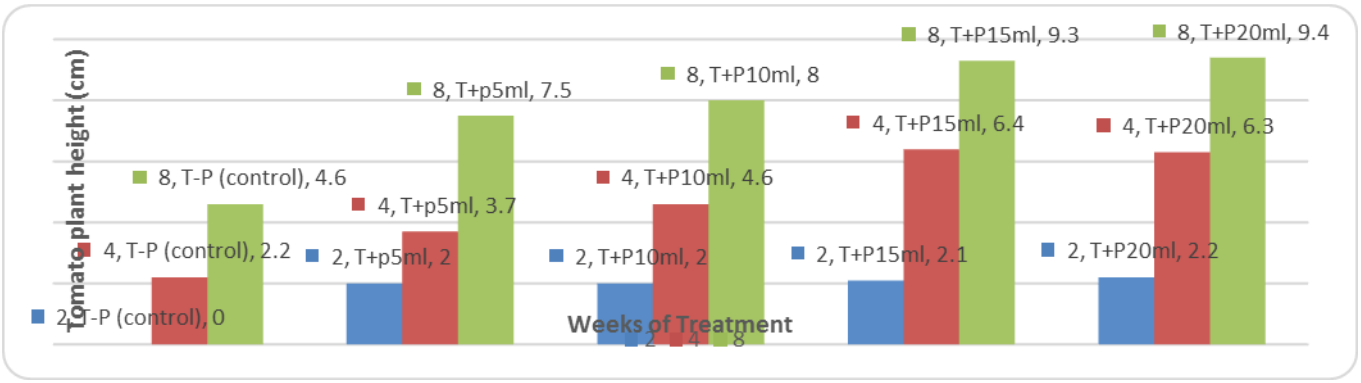

Figure 1: Tomato Plant Height Against number of weeks

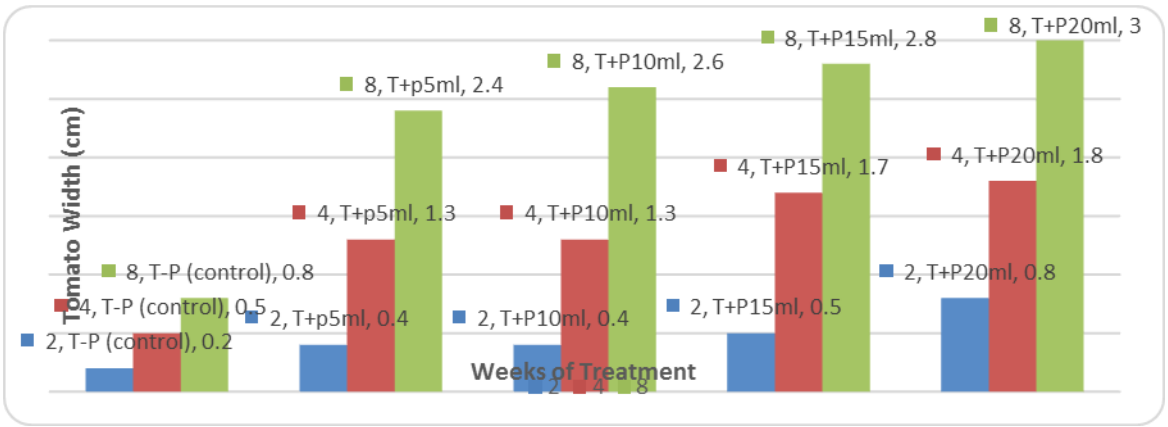

Figure 2: Width of Tomato plant

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

Treatment	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	2.5
T+P 5ml	67.5 6.5	62.0	5.5
T+P10ml	67.5 6.5	60.4	7.1
T+P15ml	67.5 6.5	58.5	9.0
T+P20ml	67.5 6.5	50.1	12.4

Generally there were significant differences in the concentration of Zn, Cu, Pb, Cd, Fe, and As in the polluted soil before and after the study, and this increases with increase in the concentration of the inoculation of *P. aeruginos*a with the difference ranging from (T- P) to (T+ P20 ml), in Zn (2.5 to 12.4) mg/kg, Cu (7.0 to 23.8) mg/kg, Pb (3.2 to 12.2) mg/kg, Cd (1.4 to 8.0) mg/kg, Fe (17.3 to 88.5) mg/kg and As (2.6 to 3.4) mg/kg respectively.

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

Treatment	Copper Conc in soil before study (mg/kg) S.D	Copper Conc in soil after 8 wks of study (mg/kgS.D	Differences (mg/kg)
T-P	56.3 5.2	49.3	7.0
T+P 5ml	56.3 5.2	44.7	11.6
T+P10ml	56.3 5.2	40.0	16.3
T+P15ml	56.3 5.2	37.6	18.7
T+P20ml	56.3 5.2	32.5	23.8

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

Treatment	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	3.2
T+P 5ml	32.8 3.1	24.7	8.1
T+P10ml	32.8 3.1	23	9.8
T+P15ml	32.8 3.1	23.4	9.4
T+P20ml	32.8 3.1	20.1	12.2

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

Treatment	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	18.14.2	16.7	1.4
T+P 5ml	18.14.2	14.2	3.9
T+P10ml	18.14.2	13.6	4.5
T+P15ml	18.14.2	11.1	7.1
T+P20ml	18.14.2	10.1	8.0

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

Treatment	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	189.323.9	172.022.7	17.3
T+P 5ml	189.323.9	161.022.1	28.3
T+P10ml	189.323.9	141.620.3	47.7
T+P15ml	189.323.9	120.419.2	68.9
T+P20ml	189.323.9	100.812.1	88.5

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

Treatment	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	8.61.7	8.01.5	0.6
T+P 5ml	8.61.7	6.71.3	1.9
T+P10ml	8.61.7	6.11.1	2.5
T+P15ml	8.61.7	5.81.0	2.8
T+P20ml	8.61.7	5.20.8	3.4

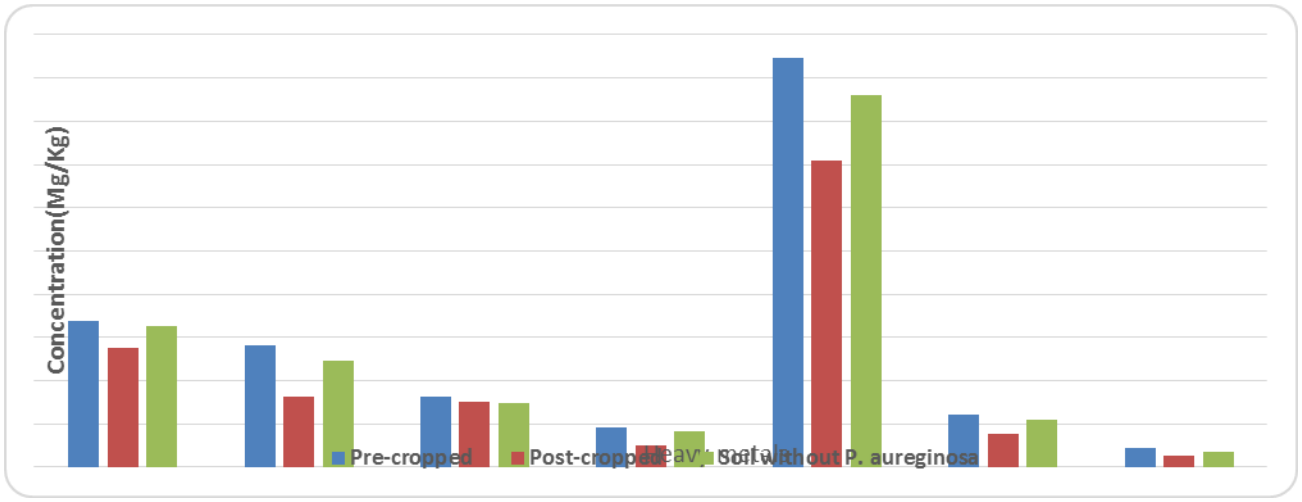

Figure 9: Concentration of heavy metals for pre-cropped, post-cropped and soil without *P.aeruginosa*

The results above showed that post-cropped soil (Soil + *P. aureginosa* + Plant) had the lowest value of concentration of heavy metals remaining in the soil after the study when compared with the pre-cropped soil(Initial soil control) and soil without *P. aureginosa* (Soil without *P. aureginosa*) for all the heavy metals. It was also recorded that apart from *P. aeruginosa*, other strain of organisms found in the soil isolate samples were *Klebsiella pneumonia* and *Pseudomonas veronii*.

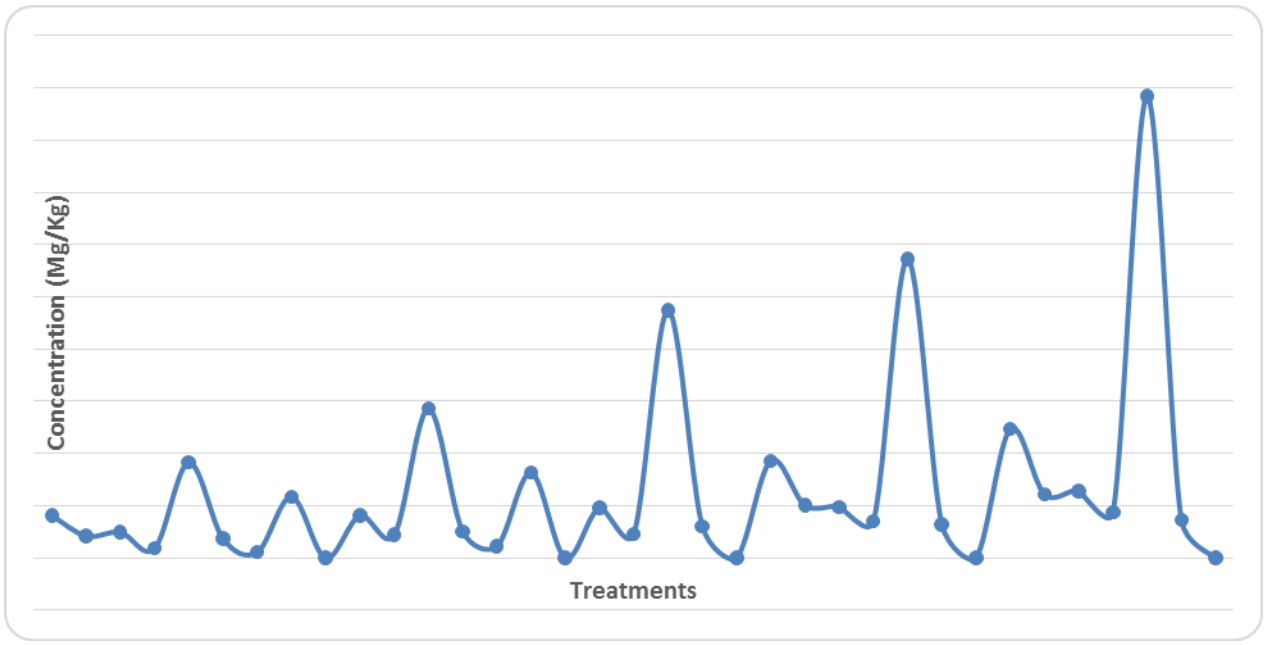

Figure 10: Heavy metals concentration in the shoot of Tomato

From the above results, the concentration of heavy metals in the shoot of the plant increases as the concentration of treatment increases, which clearly shows that the organism used in this treatment enhances the uptake of these metals into the various parts of the plant where they can be further extracted for industrial use. The control (T-P) which was found to have a little uptake of the heavy metals suggests the efficacy of the tomato plant alone to act as a phyto-remediator. Moreover, results of analyses carried out on the soil before and after eight weeks of study, confirmed that both iron and zinc accumulated in higher amount in the shoot than below the ground part of the plant in all the treatments. This is in agreement with (Adk *et al*, 2013, Wu *et al*, 2013). It is certain that

plants have developed a certain mechanism which help them to phyto-extract some heavy metals into their metabolism and also for blocking the presence of those ones they found injurious. Interesting finding of this study reveals that the concentrations of hazardous heavy metals such as arsenic and lead, were higher in soil than in any part of the plant, while other metals were found to be more in concentrations in the plant body, as they are found to be necessary for the plant metabolism, mainly useful in photosynthesis and as an enzyme anti-oxidant cofactor. This is in line with what was found by Farzad *et al.,* 2017).

CONCLUSIONS

Both phytoremediation and bioremediation play an important role in this study. This is due to the fact that the tomato plants were able to absorb handful amount of the heavy metals from the soil even without the treatment, while the microorganisms assisted in biodegrading the exudates from the plant to an inorganic absorbable substances.. Furthermore, accumulation of some of the heavy metals such as Lead, Arsenic and Cadmium which are considered to be injurious to the well-being of the plants were limited to below the soil level and were not found in appreciable amount in the shoot and the root of the plant, indicating that the plant also possess a tolerance mechanism to limit the accumulation of hazardous heavy metals. The strains of organisms seen after the experiment increased in number which further suggested that the Tomato plant produces exudates which the organism could feed upon.

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