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Extent of Cadmium Stress on Plant Growth Promoting Microorganisms at the Rhizosphere Layers of *S. stenocarpa* and *V. unguiculata* accessions

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ABSTRACT: The importance of role microbes in nodulation of leguminous crops helps in the nutritional diet of the Nigerian populace. However, heavy metal residues from heavy fertilization is a major cause of concern to soil and crop production. The study aimed to isolate and characterize free living microbe (bacteria and fungi) from the soil polluted with cadmium at different ecological screening value (0ESV, 2.5ESV and 5ESV) cultivated with *Sphenostylis stenocarpa* and *Vigna unguiculata* compared with similar soil but without the plants. The microbial count was estimated using the Most Probable Number (MPN) method. Compared to the control, a decrease of rhizobia number and an increase of the metal concentration were observed. From the results, cadmium toxicity had little to no effect on the bacteria diversity of the bulk soil has the increased bacterial and fungi diversity was recorded in the 2.5 ESV and 5 ESV respectively. The rhizosphere layer of Tss93 in the Cd-2.5 ESV had a significantly increased microbial diversity compared to the other accessions with the lowest total heterotrophic bacteria count recorded in Tss92 irrespective of metal concentration. Cd toxicity resulted in an insignificant difference ($p>0.05$) in total heterotrophic fungi count irrespective of plant accession or metal concentration, however, the fungi diversity was heightened in the Tss93 (2.5 ESV) and Tss95 (2.5 ESV) respectively. Cd toxicity increased the rhizosphere THB and THF counts of *V. unguiculata* with the highest microbial diversity recorded in TVu91 and TVu95 sown in the Cd-2.5ESV and Cd-5ESV respectively. The presence of heavy metal degradable bacteria – *Pseudomonas aeruginosa* and Bacillus subtilis and fungi – *Aspergillus niger* and *penicillum sp.* indicates bioremediation capacities of both accessions. This suggest that the survival microbes in polluted soil reveal adaptation of some traits especially those involved in symbiosis. Though cadmium had a significant effect on the soil productive capabilities, the THBC and THFC of Tss91 and Tss95 (*S. stenocarpa*) and TVu93 and TVu94 (*V. unguiculata*) were significantly increased as the presence of plant growth promoting bacteria and fungi were reported.

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Soil metal contamination has increased significantly in recent times due to rapid industrialization and anthropogenic activities especially in Nigeria (Ohanmu *et al.*, 2018). Soil, air, organic and inorganic fertilizers are the main sources from which heavy metals are taken up by the root or leaves of plant (Ohanmu and Ikhajiagbe, 2018). Uptake of heavy metals by roots depends on microbes, soil and plant factors such as soil pH, organic matter, plant species and plant age. The surplus and high metal content in soils have serious environmental implications. Accelerated amount of heavy metal around plants roots is a major factor in plantation dieback in acidified stands (Deromeand Lindroos, 1998). Once metals increases in soil, it decreases accessible flow of essential elements in soils (Blaudez *et al.*, 2000), by impeding the mineralization process thereby increasing its significant uptake in plants. In Nigeria, the general practice of soil productivity enhancement with inorganic fertilizers adds more metal components to soil in form of salt and its derivatives (Ohanmu *et al.*, 2018). *S. stenocarpa*is an underutilized leguminous crop in the southern part of Nigeria while *V. unguiculata* is widely cultivated in most part of Nigeria especially in Northern part of the country. Nigeria, been the largest producer and consumer of V. unguiculatais faced with food security challenges to cater for the burgeoning population. The detrimental

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effects of cadmium are manifested in decreasing soil biological activities and plant metabolism and the inhibition of symbiotic N_2-fixation and nodulation by legumes (Ohanmu and Ikhajiagbe, 2018), thus reducing soil microbial population. The use of conventional methods, such as ion exchange, filtration, precipitation, oxidation, reverse osmosis, reduction and membrane separation, to reduce heavy metal toxicity in the environment to acceptable regulatory standards are often inadequate (Nanda and Abraham, 2011). Microorganisms play a vital part in controlling the speciation and cycling of metals in soil. Several bacterial strains contain genetic determinants of resistance to HMs (Hg, Ag, As, Cd, Cr, Ni, Pb, etc.) and discovered on plasmids and transposons which are exploited in bioremediation (Blaudez *et al.*, 2000; Ohanmu and Ikhajiagbe, 2018). The effectiveness of bioremediation is measured by the reduction in the toxic constituents caused by HM. Mueller et al (1996) defined bioremediation as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state or to levels below toxicity limits established by regulatory authorities. Cadmium toxicity is a major menace to the environment, plant sustainability and microbial populace. Studies on plants tissues like roots, shoots, trunks and leaves have shown cadmium to be a cumulative toxicant by most scientists (Stan *et al.*, 2011; Ikhajiagbe *et al.*, 2018; Ohanmu *et al.*, 2018). Phytoremediation is employed as an alternative method in the restoration and degradation of pollutant (McCutcheon *et al.*, 2003). Phytoremediation is the use of plants as filters for absorbing, immobilizing and transforming the pollutant to harmless form. The uptake of nutrients by plant is through the root system around the rhizosphere layer where microbes interacts with products from root exudates (Nanda and Abraham, 2011), that consists of a complex mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions, gaseous molecules, enzymes and root border cells (Dakoraand Phillips, 2002). The rate of exudation is increased by the presence of essential microorganisms in the rhizosphere and promoted by the uptake and assimilation of certain nutrients (Ikhajiagbe *et al.*, 2018). Taking into account the role of leguminous plants in animal and human feed, their associated symbiotic relationship with rhizobium bacteria as well as the resulting benefits for the environment, more attention should be drawn to understanding to what degree cadmium deform the rhizosphere layers of *S. stenocarpa* and *V. unguiculata* associated microbial populace. Been the first study on the rhizosphere layers of the stated plants to cadmium in Nigeria, will help in bridging a major gap on the subject matter and our understanding on environmental factors influencing diversity and structure of soil microbial communities; since biodiversity has been assumed to guarantee ecosystems stability, productivity and resilience towards disturbance (Mader *et al.*, 2002). Therefore, this study aimed to determine the extent at which cadmium stress affects plant growth promoting microorganisms at the rhizosphere layers of the *S. stenocarpa* and *V. unguiculata* accessions.

MATERIALS AND METHODS

Seed and Metal collection: The *S. stenocarpa*(TSs91, TSs92, TSs93, TSs94 and TSs95) and V. unguiculata(TVu91, TVu92, TVu93, TVu95 and TVu96) accessions used for the experiment were procured from the Genetic Resource Center, International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. Cadmium was procured as a solid crystal substance in the form of Cadmium Chloride from pyrex chemical laboratory, Benin City, Nigeria and dissolved in 2 liters of water base on the concentration of the cadmium used in the experiment (see Table 1).

Experimental setup: The potted experiment was carried out in the Botanical garden of plant biology and biotechnology, University of Benin, Benin city, Nigeria. Soil used for this study was collected at a depth of 0 – 30 cm using a soil auger from ten (10) different point within the botanic garden. The soil were pooled together and mixed to get a homogeneous sample, air-dried, grind to pass through a 2 mm sieve before subjecting to physicochemical analysis according to standard procedures. Fifteen kilograms (15 kg) of the soil was weighed into each of the ninety (90) polythene bags with a diameter of 30 cm and a height of 30cm. The soil has the following chemical characteristics: pH (5.97), electric conductivity (301.21 µs/cm), total organic carbon (0.49%), total nitrogen (0.18%) and potassium (1.48 meq/100 g soil). The bags were not perforated (to ensure that cadmium deposits and microbial populations in the soil do not escape from the perforated holes), watered and left for five days without planting. The seeds were sow on the sixth (6) day.

Experimental design: The experiment was laid out in a randomized block design (RBD) and replicated thrice (3). There were 3 treatments polluted with cadmium in the form of $CdCl_2$ using 0 ESV, 2.5 ESV and 5 ESV (Table 1) and poured into 15 kg polythene bag each without the plants. The 0 ESV is the unpolluted soil (control). The ESV is an acronym for Ecological screening valueESV – Ecological screening value (4 mg/kg) (Efroymson, 1997)..

Table 1. Treatment designation without plant species or accession

Treatments	Explanation
0 ESV	Control (oil without cadmium or plant interference)
2.5 ESV	15kg soil polluted with cadmium at 2.5 times its ecological screening value
5 ESV	15kg oil polluted with cadmium at 5 times its ecological screening value

After the pollution of the soils, the various plant accession was sow into the experimental bags. The seeds of the various accessions of *S. stenocarpa*and *V. unguiculata* were sowed into the various bags containing each treatments and replicated thrice. Each treatments had separate control with the plants; *S. stenocarpa* (Table 2) and *V. unguiculata* (Table 3). The experiment spanned from late September, 2016 to March, 2017.

Table 2. Treatment designation with *S. stenocarpa* accession

Treatments	Description
Tss91 (0ESV)	Tss91 accessions sown in control soil
Tss91 (2.5ESV)	Tss91 accessions sown in 2.5ESV
TSs91 (5ESV)	Tss91 accessions sown in 5ESV
Tss92 (0ESV)	Tss92 accessions sown in control soil
Tss92 (2.5ESV)	Tss92 accessions sown in 2.5ESV
Tss92 (5ESV)	Tss92 accessions sown in 5ESV
Tss93 (0ESV)	Tss93 accessions sown in control soil
Tss93 (2.5ESV)	Tss93 accessions sown in 2.5ESV
Tss93 (5ESV)	Tss93 accessions sown in 5ESV
Tss94 (0ESV)	Tss94 accessions sown in control soil
Tss94 (2.5ESV)	Tss94 accessions sown in 2.5ESV
Tss94 (5ESV)	Tss94 accessions sown in 5ESV
Tss95 (0ESV)	Tss95 accessions sown in control soil
Tss95 (2.5ESV)	Tss95 accessions sown in 2.5ESV
Tss95 (5ESV)	Tss95 accessions sown in 5ESV

*Keys: African yam bean **Sample ID:** Tss92, African yam bean **Sample ID:** Tss91, African yam bean **Sample ID:** Tss93, African yam bean **Sample ID:** Tss94, African yam bean **Sample ID:** Tss95; ESV – Ecological Screening Value*

Table 3. Treatment designation with *V. unguiculata* accession

Treatments	Description
TVu91 (0ESV)	TVu91 accessions sown in control soil
TVu91 (2.5ESV)	TVu91 accessions sown in 2.5ESV
TVu91 (5ESV)	TVu91 accessions sown in 5ESV
TVu92 (0ESV)	TVu92 accessions sown in control soil
TVu92 (2.5ESV)	TVu92 accessions sown in 2.5ESV
TVu92 (5ESV)	TVu92 accessions sown in 5ESV
TVu93 (0ESV)	TVu93 accessions sown in control soil
TVu93 (2.5ESV)	TVu93 accessions sown in 2.5ESV
TVu93 (5ESV)	TVu93 accessions sown in 5ESV
TVu95 (0ESV)	TVu95 accessions sown in control soil
TVu95 (2.5ESV)	TVu95 accessions sown in 2.5ESV
TVu95 (5ESV)	TVu95 accessions sown in 5ESV
TVu96 (0ESV)	TVu96 accessions sown in control soil
TVu96 (2.5ESV)	TVu96 accessions sown in 2.5ESV
TVu96 (5ESV)	TVu96 accessions sown in 5ESV

*Keys: African yam bean **Sample ID:** Tss92, African yam bean **Sample ID:** Tss91, African yam bean **Sample ID:** Tss93, African yam bean **Sample ID:** Tss94, African yam bean **Sample ID:** Tss95; ESV – Ecological Screening Value*

Microbial Determination

Identification of soil microorganisms after the experiment: The isolation and characterization of bacterial and fungal were carried out (Cheesebrough, 2001). The soil samples were air-dried and sieved through a 2 mm mesh to remove undesirable material. The dilution series for the soil sample was done by transferring 1 gram of the soil to nine (9) millimetres (ml) of sterile distilled water in sterile glass containers as blank. The glass containers were shaken for 5 minutes and was taken as 10^{-1} dilution factor, 1 ml were then transferred from the 10^{-1} dilution into another 9 ml blank to obtain a 10^{-2} dilution and same process of transfer was repeated twice to obtain a dilution factor of 10^{-4} (Cheesebrough, 2001).

Heterotrophic bacterial and fungal counts: Spread plate method was employed in taking the heterotrophic bacteria counts. One (1) ml of the serially diluted portion of 10^{-4} of each soil sample was inoculated onto nutrient agar (NA) plates for bacteria and potato dextrose agar (PDA) plates for fungal counts. The plates were inoculated at room temperature for 24 hours and 72 hours respectively, for bacteria and fungi growth. After incubation, the colonies were counted and the colony-forming unit (cfu/g) of the soil samples determined. The colony forming unit (cfu/g) of the soil samples was determined using the biochemical characterization according to Cheesebrough (2011).

Statistical analysis: The experimental setup was a randomized block design and a factorial analysis was adopted for data analyses. The SPSS-20® software was used employed in analyzing the data. Results were presented in bar chart (means and standard error, n= 3).

RESULTS AND DISCUSSION

Bulk soil: The effects of cadmium toxicity on total heterotrophic bacteria count (THBC) of the bulk soil at the end of the experiment is presented below (Figure 1). On cadmium exposure, the THBC of the bulk soil was heighten in the 2.5ESV compared to the control. However, the reduction in THBC observed in the 5 ESV is as a result of the increased concentration of cadmium present. Microbial diversity was found to reach its lowest in the 5 ESV treatment due to the increase in cadmium concentration. Hence a highly significant difference exists at $p<0.001$.

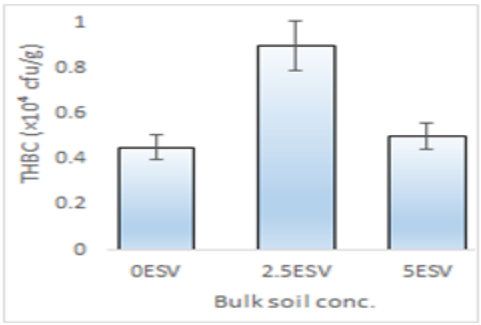

Fig 1. Total heterotrophic bacterial count of bulk soil (control) exposure to cadmium

The total heterotrophic fungi count (THFC) of the bulk soil exposed to cadmium at the end of the experiment was reported in Figure 2. Cadmium toxicity increased the THFC in the control compared to the 2.5 ESV. However, the rhizosphere layers of the 5 ESV treatment had an increased THFC in the 5 ESV treatment compared to the control.

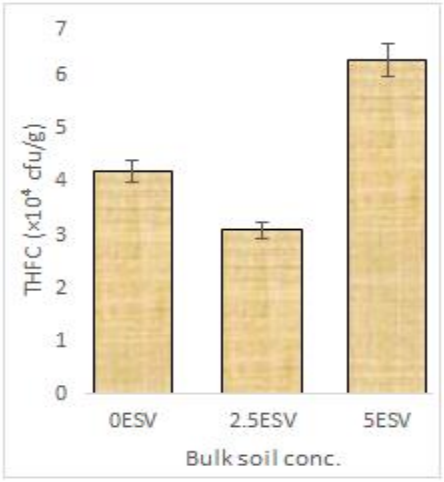

Fig 2. Total heterotrophic fungi count of bulk soil exposure to cadmium

Table 4 shows the identified microorganisms recorded in the bulk soil on exposure to cadmium toxicity. The presence of bacteria such as *Pseudomonas aeruginosa* and Bacillus subtilis indicates the degradation of cadmium in the soil (2.5 ESV and 5 ESV respectively). However, B. subtilis was also reported in the control. The presence of *Aspergillus niger* was reported in2.5 ESV and 5 ESV compared to the control. This are rhizosphere microbes that helps in the detoxification of heavy metals present in soils. *Rhizosphere total colony count of S. stenocarpa:* The THBC of *S. stenocarpa* accessions to cadmium pollution is presented below (Figure 3). Cadmium pollution resulted in the variation of THBC irrespective of accession type with the highest microbial diversity observed in the Cd-2.5ESV of Tss93. Tss92 had the lowest microbial diversity compared to the other treatments irrespective of concentration. The accession with the most bacteria diversity irrespective of concentration was Tss95 (0, 2.5, 5 ESV). The effects of cadmium toxicity on the rhizosphere THFC of *S. stenocarpa* accessions have been reported (Figure 4). Although cadmium toxicity resulted in an increased rhizosphere THFC in Tss95 between the 2.5 ESV and control, there was no significant difference at $p>0.05$. The effects of cadmium toxicity on the rhizosphere THBC of cowpea accessions has been reported (Figure 5). Cadmium toxicity resulted in an increased THBC in all treatment when compared to the control. TVu95 in the Cd-2.5 ESV and Cd-5 ESV had an increased THBC over the control. However, the THBC in Cd-2.5 ESV of the TVu91 was significantly ($p<0.05$) increased compared to the other accessions. The rhizosphere THFC of cowpea accessions exposed to cadmium toxicity is presented (Figure 6). Cadmium resulted in variation of fungi populace in the rhizosphere layer of the cowpea. Take for example, the highest difference in fungi population between the Cd-5ESV and control was recorded in TVu93 while TVu95 had the lowest difference.

Table 4. Identified microorganisms of bulk soil.

Cd Conc.	Bacteria	Fungi
0 ESV	Staphylococcus epidymis, Bacillus subtilis, Staphylococcus aureus	Aspergillusflavus, Penicillumsp.,Microsporus sp.
2.5 ESV	Staphylococcus epidymis, Bacillus subtilis, *Pseudomonas aeruginosa*,	Aspergillusniger, Trichodermaharzianum, Rhizopus sp.
5 ESV	Staphylococcus epidymis, *Pseudomonas aeruginosa*, Bacillus subtilis, Staphylococcus aureus, Clostridium spp	Trichodermaharzianum, Microsporum sp., Mucormucedo, Penicillum sp. Aspergillusniger

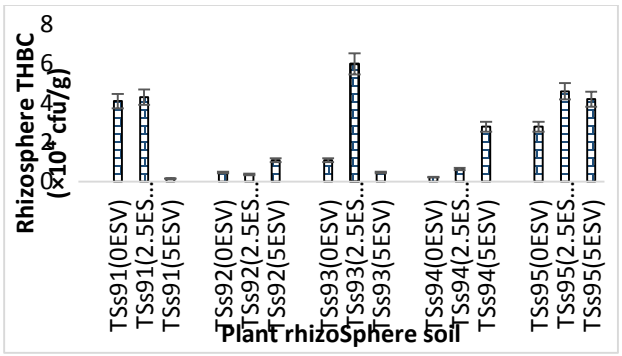

Fig 3. Rhizosphere total heterotrophic bacterial count of *S. stenocarpa*accessions exposed to cadmium

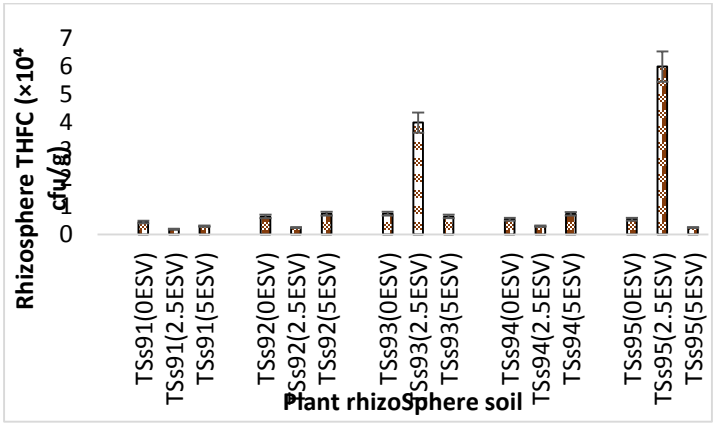

Fig 4. Rhizosphere total heterotrophic fungi count of *S. stenocarpa*accessions exposed to cadmium Rhizosphere total colony count of V. unguiculata

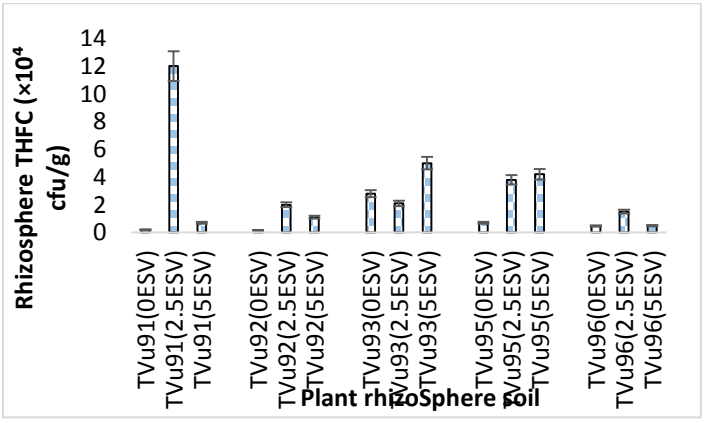

Fig 5. Rhizosphere total heterotrophic bacteria count of V. unguiculataaccessions exposed cadmium

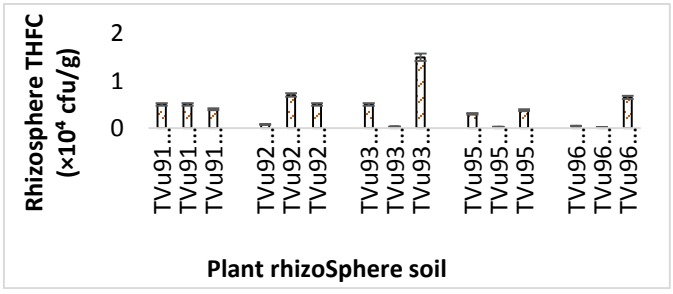

Fig 6. Rhizosphere total heterotrophic fungi count of V. unguiculataaccessions exposed cadmium

***Culturable rhizosphere bacterial and fungi isolates*:** When metal destroys soil textures, it becomes unsuitable for microorganisms to inhabit and affects crops productivity. Table 5 and 6 shows the culturable bacteria and fungi isolates identified from the rhizosphere layer of *S. stenocarpa*accessions exposed to different cadmium toxicity. Some HM-degradable bacteria such as *Pseudomonas aeruginosa* and Bacillus subtilis were presence in the cadmium polluted soil (2.5 ESV and 5 ESV respectively) and control irrespective of accession type. The culturablerhizosphere bacteria and fungi of V. unguiculatais presented below (Table 7 and 8). Heavy metal degrading bacteria and fungi were observed in both cadmium treatment and control. There are a lot of factors that affects the microbial diversity and quantity of the soil. They include but not limited to moisture, pH, temperature, oxygen, organic matter, conductivity, micronutrients and micronutrients, etc. The highest THBC and THFC recorded was in 2.5 ESV and 5 ESV. This is due to certain additive effects or interactions exhibited by soil microorganisms in adverse conditions (Chaperon and Sauve, 2008). This may be because heavy metal polluted soils display a large heterogeneity of metal distribution among soil constituents (Nanda and Abraham, 2011). Microbial diversity plays a vital role in legumes nodulation and survival of the root system in plants which is the first point of contact with the soil.

Table 5. Culturablerhizosphere bacterial of *S. stenocarpa* accessions

Plant rhizosphere soil	Bacterial isolates
TSs91(0ESV)	Micrococcus letus Staphylococcus epidymis, *Pseudomonas aeruginosa*, Staphylococcus aureus
TSs91(2.5ESV)	*Pseudomonas aeruginosa*, Bacillus subtilis, Micrococcus letus
TSs91(5ESV)	*Pseudomonas aeruginosa*, Staphylococcus aureus
TSs92(0ESV)	Staphylococcus epidymis, Bacillus subtilis, Staphylococcus aureus
TSs92(2.5ESV)	Bacillus subtilis, Micrococcus letus, Staphylococcus aureus
TSs92(5ESV)	Staphylococcus epidymis, *Pseudomonas aeruginosa*,
TSs93(0ESV)	Bacillus subtilis, Escherichia coli, Staphylococcus aureus
TSs93(2.5ESV)	Staphylococcus epidymis, Micrococcus letus
TSs93(5ESV)	Staphylococcus epidymis, *Pseudomonas aeruginosa*, Staphylococcus aureus
TSs94(0ESV)	Staphylococcus epidymis, Bacillus subtilis,
TSs94(2.5ESV)	Staphylococcus epidymis, Bacillus subtilis, Staphylococcus aureus
TSs94(5ESV)	*Pseudomonas aeruginosa*, Micrococcus letus, Staphylococcus aureus
TSs95(0ESV)	Staphylococcus epidymis, Staphylococcus aureus, *Pseudomonas aeruginosa*, Staphylococcus aureus, Clostridium spp
TSs95(2.5ESV)	Staphylococcus epidymis, Bacillus subtilis, *Pseudomonas aeruginosa*, Escherichia coli
TSs95(5ESV)	Staphylococcus epidymis, Staphylococcus aureus, *Pseudomonas aeruginosa*, Bacillus substilis

Table 6. Culturablerhizosphere fungal of *S. stenocarpa* accessions

Plant rhizosphere soil	Fungal isolates
TSs91(0ESV)	Penicilium sp., Aspergillusflavus, Mucormucedo, Aspergillusniger
TSs91(2.5ESV)	Mucormucedo, Trichodermaharzianum, Aspergillusniger
TSs91(5ESV)	Mucormucedo, Penicilium sp., Aspergillusflavus
TSs92(0ESV)	Fusariumsolani, Mucormucedo, Penicilium sp.
TSs92(2.5ESV)	Trichodermaharzianum, Aspergillusflavus, Mucormucedo, Penicilium sp.
TSs92(5ESV)	Mucormucedo, Fusariumsolani, Trichodermaharzianum, Penicilium sp.
TSs93(0ESV)	Aspergillusflavus, Aspergillusniger, Penicillumsp.,Microsporus sp.
TSs93(2.5ESV)	Mucormucedo, Trichodermaharzianum, Aspergillusflavus, Penicilium sp.
TSs93(5ESV)	Aspergillusflavus, Trichodermaharzianum, Mucormucedo, Aspergillusniger
TSs94(0ESV)	Aspergillusniger, Trichodermaharzianum, Rhizopus sp.
TSs94(2.5ESV)	Microsporum sp., Penicillium sp., Aspergillusniger, Mucormucedo
TSs94(5ESV)	Aspergillusflavus, Trichodermaharzianum, Microsporum sp., Mucormucedo, Aspergillusniger
TSs95(0ESV)	Fusariumsolani, Trichodermaharzianum, Mucormucedo, Penicilium sp.
TSs95(2.5ESV)	Aspergillusflavus,Mucormucedo, Trichodermaharzianum, Penicilium sp.
TSs95(5ESV)	Penicilium sp., Mucormucedo, Aspergillusniger

Table 7. *Culturablerhizosphere bacterial of V. unguiculata accessions*

Plant rhizosphere soil	Bacterial isolates
TVu91(0ESV)	*Pseudomonas aeruginosa*, Staphylococcus aureus
TVu91(2.5ESV)	Bacillus subtilis, *Pseudomonas aeruginosa*, Staphylococcus aureus
TVu91(5ESV)	Bacillus subtilis, *Pseudomonas aeruginosa*, Micrococcus letus,
TVu92(0ESV)	*Pseudomonas aeruginosa*, Staphylococcus aureus, Clostridium spp
TVu92(2.5ESV)	Bacillus subtilis, E. coli, Staphylococcus aureus
TVu92(5ESV)	Staphylococcus epidymis, Bacillus subtilis,
TVu93(0ESV)	Bacillus subtilis, *Pseudomonas aeruginosa*, Micrococcus letus, Staphylococcus aureus
TVu93(2.5ESV)	Staphylococcus epidymis, Micrococcus letus, Clostridium sp
TVu93(5ESV)	Staphylococcus epidymis, Bacillus subtilis, Staphylococcus aureus
TVu95(0ESV)	Bacillus subtilis, Staphylococcus aureus
TVu95(2.5ESV)	Staphylococcus epidymis *Pseudomonas aeruginosa*, Staphylococcus aureus, Proteus mirabilis
TVu954(5ESV)	Bacillus subtilis, Staphylococcus epidymis, *Pseudomonas aeruginosa*, Staphylococcus aureus
TVu96(0ESV)	Staphylococcus epidymis, *Pseudomonas aeruginosa*,
TVu96(2.5ESV)	Micrococcus letus, Staphylococcus epidymis, Bacillus subtilis, *Pseudomonas aeruginosa*,
TVu96(5ESV)	Bacillus subtilis, Micrococcus letus, Staphylococcus aureus

Table 8. Culturablerhizosphere fungal of V. unguiculataaccessions

Plant rhizosphere soil	Fungal isolates
TVu91(0ESV)	Aspergillusflavus, Trichodermaharzianum, Mucormucedo, Aspergillusniger
TVu91(2.5ESV)	Aspergillusniger., Mucormucedo, Rhizopus sp.
TVu91(5ESV)	Aspergillusniger, Aspergillusflavus, Rhizopus sp.
TVu92(0ESV)	Aspergillusniger, Microsporum sp., Penicillium sp., Mucormucedo
TVu92(2.5ESV)	Mucormucedo, Trichodermaharzianum, Aspergillusflavus, Penicilium sp.
TVu92(5ESV)	Aspergillusniger, Microsporum sp., Penicilliumsp.,Mucormucedo
TVu93(0ESV)	Microsporum sp., Mucormucedo, Aspergillusniger
TVu93(2.5ESV)	Mucormucedo, Trichodermaharzianum, Aspergillusniger
TVu93(5ESV)	Fusariumsolani, Trichodermaharzianum, Mucormucedo, Penicilium sp.
TVu95(0ESV)	Trichodermaharzianum, Penicillum sp. Aspergillusniger
TVu95(2.5ESV)	Aspergillusflavus, Trichodermaharzianum, Penicillum sp., Aspergillusniger
TVu954(5ESV)	Aspergillusflavus, Trichodermaharzianum, Microsporum sp., Mucormucedo, Penicillum sp.
TVu96(0ESV)	Trichodermaharzianum, Microsporum sp., Mucormucedo, Penicillum sp. Aspergillusniger
TVu96(2.5ESV)	Mucormucedo, Penicillum sp., Trichodermaharzianum, Aspergillusniger
TVu96(5ESV)	Aspergillusflavus, Trichodermaharzianum, Aspergillusniger

In this study, there was high number of THBC in the Tss92 (2.5 ESV) which increased with cadmium concentration. The interaction between microorganisms and plant roots might have a greater impact on both the increase of nutrient uptake and migration of metal uptake. However, Tss95 accessions irrespective of concentration had a high significant microbial population compared to the other accessions and increased with the metal.

This is because elevated metal concentrations can exert a selective pressure on the microbial communities increasing the numbers of metal tolerant or resistant strains in such soils (Gil-Sotres, 2005). This study has reported the low bacteria populace in Tss91 (5 ESV) soil maybe due to the cadmium stress ability to convert the viable bacterial cells to non-culturable form (Paton *et al.,* 1997). However, this is not always the case as plant accessions influence the microbial species and number present in a polluted environment as observed in the THFC of *S. stenocarpa*in this study. Plant growth promoting bacteria and fungi were also recorded irrespective of accession type or cadmium concentration. The presence of rhizosphere microorganisms was also in the degradation of cadmium. Braud *et al.* (2009) in the screening of 16 different metals including cadmium revealed that pyoverdinesiderophores produced by *Pseudomonas aeruginosa* are able to chelate all these metals. Aspergillusniger, an organic acid producing fungi was able to mobilize large amounts of Pb and P from pyromorphite indicated the presence of organic acid in dissolution of minerals (Sheng *et al.,* 2008). However, the absence of some bacteria species in the cadmium polluted soil maybe as a result of the destruction of soil by the metal (Robinson *et al.,* 2001; Lei *et al.,* 2011), thereby making it uninhabitable for microbial communities. For example, numerous metals (e.g. Cu, Ni. Zn, Cd, As) have been reported to inhibit the growth, morphology and activities of various groups of microorganisms (Khan and Scullion, 2002; Shi *et al.,* 2002; Bondarenko *et al.,* 2010) including symbiotic N2 fixers (Santamarıa *et al.,* 2003; Stan *et al.,* 2011). Cadmium as a metal has no biologically significant relevance rather than toxic effect. Thereby disturbing enzyme activities, inhibition of DNA-mediated transformation in microorganisms, reduced symbiosis between microbes and plants and increased plant predisposition to fungal invasion (Kabata-Pendias andPendias, 2001; Mohanupuria *et al.,* 2007).

Conclusion: To sum, cadmium concentration and plant species have significant effect on soil microbial diversity. The ability of leguminous plants to undergo symbiotic relationship with rhizobium microbes and their ability to form nodules makes them a good bioremediation factor in metal mitigation.

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