sciendo

DOI: 10.2478/auoc-2020-0023

Ovidius University Annals of Chemistry Volume 31, Number 2, pp. 145 - 151, 2020

Quantitative and qualitative basement of microbial presence during phytoremediation of heavy metal polluted soil using *Chromolaena odorata*

Gloria Omorowa OMOREGIE*,[1,2] Abraham Goodness OGOFURE,[3] Beckley IKHAJIAGBE,[2] and Geoffrey Obinna ANOLIEFO[2]

[1]*Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Warri Delta State, Nigeria*
[2]*Environmental Biotechnology and Sustainability Research Group, Department of Plant Biology and Biotechnology, University of Benin, Nigeria*
[3]*Department of Microbiology, University of Benin, Nigeria*

Abstract. The presence and impact of bulk and rhizosphere microorganisms in contaminated soils can be huge, given that they have the ability to increase plants tolerance against abiotic stress, and also enhance plant growth, while supporting hastened remediation of disturbed soils. The present study quantitatively and qualitatively assessed presence of cultural fungi and bacteria during phytoremediation of heavy metal polluted soils using *Chromolaena odorata*. Stem cuttings of *C. odorata* were planted in soils polluted with Pb, Mn, Zn, Cd, and Cu at once (1ESC), thrice (3ESC) and five (5ESC) times their respective ecological screening concentrations (ESC). ESC of Pb, Mn and Zn is 50 mg/kg, Cd is 4 mg/kg, and Cu is 100 mg/kg. After 6 months, results showed that more than 10 species of bacteria and fungi were identified in the study, with *P. aeruginosa* and *Bacillus subtilis* being the most occurring bacteria while, *Penicillium* sp. and *Aspergillus niger* the most occurring fungi in both bulk and rhizospheric soils. The presence of known plant growth promoting rhizobacteria in plants rhizosphere including *Azotobacter* sp., *Bacillus subtilis*, *B. pumilus*, *Clostridium* sp., *P. aeruginosa*, and *Klebsiella* sp. was also reported.

Keywords: *Chromolaena odorata*; heavy metal; phytoremediation; pollution; microbes.

1. Introduction

Plants and animals require metals for metabolic functions; however, at elevated levels, these metals impede metabolic reactions in organisms. Metals like Pb, Cr, and Ni are not necessarily important to plants, possessing the ability to hinder plant growth through a number of biological processes including decreasing photosynthetic activities, plant mineral nutrition, and activity of important enzymes [1]. The toxicity of heavy metal increases the generation of reactive oxygen species thereby reducing the antioxidant systems which protect cells. Plants respond metal pollution in a number of ways as to enhance their survival capabilities through their complex system of antioxidant defenses. Some others have been reported to survive through direct or indirect interaction with soil and rhizospheric microorganisms. These microorganisms stimulate growth factors or enhance induced system responses in the plants with a view to promoting growth and development in the face of heavy metal (HM) pollution. Dimkpa *et al*. [2] and Weyens *et al*. [3] reported a root-mediated approach to survival under environmental stressed condition through the production of phytohormones with the ability to alter root morphology as an adaptation mechanism. These rhizospheric microorganisms can also induce a cascade of signaling molecules during systemic resistance. Plant growth promoting rhizobacteria (PGPRs for short) have been reported to change K/Na ratios as well as an alteration in the membrane phospholipid content, which invariably affects the permeability of substances [2].

Rhizobacteria can reduce the movement of HMs in polluted soils, thus resulting in HM precipitation as insoluble compounds in soil. Influence on soil pH and redox potentials have also been attributed to certain soil rhizobacteria which produce organic acids including malate, citrate and isocitrate [2-4]. Plant roots are also protected from direct exposure to HM through the development of soil sheaths around the plant root by bacterial exopolysaccharides [2]. It is difficult to separate plant-growth promoting soil microorganisms from plants in metal-polluted soils. A number of plants have been reported to have increased ability for survival in heavy metal-polluted soils, depending, however, on the severity of the contamination [5-10]. *Chromolaena odorata* (L) King and Robinson (Family Asteraceae) has been reported to have adaptive capacities for subsisting in HM-polluted soils [11, 12]. With an extremely fast growth rate, rapid seed production, and a perennial nature, the plant has been selected for remediation studies in both HM- and hydrocarbon-soils [7, 9, 11-16]. To what extent therefore is the composition of soil microorganisms that are directly or indirectly associated with the test plant?

* Corresponding author. *E-mail address:* omoregie.gloria@fupre.edu.ng (G. O. Omoregie)

2. Experimental

2.1. Plant materials and soil samples

Top-layered garden soil (0 – 10 cm) was collected from ten random spots in the Botanic Garden of the Ugbowo Campus of the University of Benin and pooled together to form composite soil sample. These were sun-dried to constant weight, and then 20 kg each was measured into 25 cm diameter and 36 cm long buckets already prepared for this study. The soils were polluted with Mn, Cd, Pb, Cu and Zn in their respective chloride forms. The reported ecological screening values (ESC) of the metals were 50, 4, 50, 100 and 50 mg/kg respectively [5]. The metals were therefore divided into 3 concentrations each on the basis of their reported ESC as once, thrice and 5 times their respective ESCs. Successful soil pollution with the respective metal concentrations were achieved by dissolving each measured quantity in distilled water and used to properly irrigate the soil up to its water holding capacity, which was earlier determined to be 190.3 ml/kg soil. The control soil was not amended with metal. The experimental buckets that held the soils were not perforated in other to ensure metals did not percolate further into the soil. Equal sized stem cuttings of *C. odorata* (2.0 - 2.3 cm in thickness) and length (30 cm) were thereafter planted vertically into the soil with 10 cm of stem cutting buried into the soil. Two stem cutting was planted per bucket.

2.2. Husbandry

Since the experimental buckets were exposed to prevailing weather condition, water requirements of the soil were augmented by wetting the soil in each bucket with 500 ml of tap water (pH 6.5-6.9). The setup was studied for 3 months after which antimicrobial determination was done since the aim of the experiment was to carry out a quantitative and qualitative assessment of microbial presence during phytoremediation of heavy metal polluted soil using *C. odorata*. The set up was kept in a well-ventilated screen house for 6 months.

2.3. Physicochemical parameters

Soil physicochemical parameters were analyzed [17-21] prior to soil contamination with the respective heavy metals. Soil pH was determined with a pH meter (Model 238 PHS-3C), whereas soil conductivity was studied through a hand-held conductivity meter (HI 70039P, Hanna Instruments). Determination of nitrogen in the soil was obtained by Kjeldahl digestion, and the resulting ammonium ion was colorimetric measured. Elements such as iron and manganese, which may interfere in the alkaline medium during colorimetric determination, were first complexed with sodium potassium tartrate. The ammonia was colorimetric determined as the indophenol blue complex by reaction with alkaline sodium phenate and sodium hypochlorite. Soil concentrations of Cd, Cu, Fe, Mn, Pb and Zn were determined using Atomic Absorption Spectrometry (Model: DW-AA4530F, China) using 228.8, 324.7, 248.3, 279.5, 283.3 and 213.9 nm respectively as wavelengths [21, 22]. The methods of Prichard and Barwick [21] and Mitra [22] were also adopted for validation of the precision and accuracy.

2.4. Heterotrophic bacterial and fungal counts

The pour plate method was employed in taking the heterotrophic bacteria counts. 1 ml of serially diluted portion of 10^4 of each soil sample was inoculated in triplicates onto nutrient (and anaerobe) agar plates for bacteria and potato dextrose agar plates for fungal counts. The plates were incubated at room temperature for 24 and 72 h respectively, for bacteria and fungi growth. After incubation colonies were then counted and the colony forming unit (cfu/g) of the soil samples determined. Bacterial colonies were counted using a colony counter and results were recorded. Enumeration of the isolates was done using the formula given in Equation 1 as described by Willey *et al*. [22]:

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum} \qquad (1)$$

2.5. Biochemical characterization and identification of selected isolates

Single or individual colonies on the agar plates were subcultured and purified before they were briefly subjected to biochemical testing after taking into account their cultural, morphological and staining properties/characteristics. They were thereafter cultured on different media such McConkey agar, *Bacillus cereus* agar, mannitol salt agar, *Pseudomonas cetrimide* agar, and anaerobe agar, to evaluate their characteristic growth. They were further subjected to biochemical tests viz: coagulase, catalase, indole, oxidase, citrate utilization, urease, nitrate reduction, motility, and triple sugar iron tests [23, 24]. All fungal plates were incubated at 30 °C for 5-7 days with daily monitoring of fungal growth. Fungal isolates were identified by cultural and microscopic features after wet mount stain. Key features such as nature of spores and mycelia were key during microscopic examination of the fungal isolates [22].

2.7. Identification of fungal isolate

Potato dextrose (PDA) media was employed for the isolation of fungi by spread plate method using direct plating techniques. All the plates were incubated at 30 °C for 7 days. Fungal isolates were identified by cultural and microscopic features [22].

2.8. Statistical analysis

Data were analyzed using statistical package for social scientist (SPSS) version 21. Basic descriptive and one-way analysis of variance (using least significant difference) was employed for data analysis at 95 % confidence level.

3. Results and discussion

The physical and chemical properties of the soil before pollution have been presented on Table 1. Results showed that pH of the soil was 5.97, indicating slight acidity; whereas electrical conductivity was 301.21 µs/cm. Nitrogen content of the soil was 4.18% compared to 286.16 mg/kg of nitrate nitrogen. There was no evidence of Cd in the soil (< 0.001 mg/kg); however, the

soil was ferruginous (Fe, 1011.92 mg/kg). The concentration of the other test metals (Mn, Pb, Cu and Zn) were all below ecological screening concentration for the study.

Table 1. Physical and chemical properties of soil before pollution (mean ± S.E.M.)

Parameters	Mean (n = 5)
pH	5.97 ± 0.67
Electric conductivity (µs/cm)	301.21 ± 23.01
Total organic carbon (%)	0.49 ± 0.09
Total nitrogen (%)	4.18± 1.06
Exchangeable acidity (meq/100 g)	0.22 ± 0.08
Na (meq/100 g)	10.90 ± 2.11
K (meq/100 g)	1.48 ± 0.62
Ca (meq/100 g)	14.32 ± 3.10
Mg (meq/100 g)	12.01 ± 3.22
NO^{-}_{2} (mg/kg)	164.34 ± 23.03
NO^{-}_{3} (mg/kg)	286.16 ± 18.16
Soil texture	
Clay (%)	5.43 ± 0.88
Silt (%)	7.36 ± 1.74
Sand (%)	84.81 ± 12.12
Heavy metals	
Fe (mg/kg)	1011.92 ± 73.38
Cd (mg/kg)	< 0.001
Mn (mg/kg)	17.03 ±3.22
Pb (mg/kg)	0.03 ± 0.01
Cu (mg/kg)	3.93 ± 0.01
Zn (mg/kg)	30.12 ± 3.06

Table 2 shows the effects of treatment on below ground parameters of *C. odorata* at 6 months after sowing. Results showed that there were no significant changes in average length of main root in the control (73.14 cm) compared to the metal exposed plants (71.13 to 81.67 cm) however results showed significant decreases in number of primary root branches in the metal exposed plant when compared to the control. Whereas control plants have an average of 34 primary root branches, the Mn exposed plant had between 19 and 28 primary root branches, the Pb affected plants had between 17.11 and 24.21 as well as the Zn exposed plants which had a total of between 16.73 and 29.11 results general showed that as metal concentration increase the number of primary root branches for each of the metals decreased ($p < 0.05$).

Table 3 shows the quantitative composition of culturable bacteria and fungi within bulk soil region and rhizospheric soil of test plant at 6 months after exposure to metals. There was general reduction in composition of bulk and rhizospheric soil microorganisms. Although bacterial composition of bulk and rhizospheric soil differed, the comparative composition in HM-exposed soils was comparable. Bacterial composition ranges from 1.32 – 3.44 x 10^5 cfu/g in Cu, Cd, Pb and Mn-polluted soils, compared to 0.14 - 38 x 10^5 cfu/g in Zn-polluted soils.

Table 2. Effects of treatment on ground parameters of *Chromolaena odorata* at 6 months after sowing (mean ± S.E.M.)

Concentration of contaminant in soil	Average plant height(cm)	Leaf Area (cm^2)	Average length of main root (cm)	Number of primary root branches
Control	132.2±1.00	18.87±0.51	73.14±11.88	34.41±9.68
Mn+1ESC	84.44±1.10	21.49±0.82	81.67±14.40	28.22±7.49
Mn+3ESC	100.1±1.40	21.96±2.40	68.72±2.21	19.01±6.94
Mn+5ESC	110.3±1.05	23.96±3.68	78.32±14.44	22.13±1.95
Cd+1ESC	112.2±1.00	21.92±1.45	71.13±11.36	29.41±9.28
Cd+3ESC	143.1±1.50	18.45±1.34	76.02±8.11	28.42±5.65
Cd+5ESC	110.3±1.20	17.71±1.11	76.82±5.17	14.34±6.01
Pb+1ESC	104.2±0.5	16.74±1.35	81.34±8.07	24.21±5.18
Pb+3ESC	79.12±1.52	17.93±2.40	73.81±19.01	17.11±6.19
Pb+5ESC	85.33±1.95	24.80±5.20	69.98±9.91	20.43±5.05
Cu+1ESC	98.43±1.01	20.41±3.4	79.65±12.8	32.32±3.61
Cu+3ESC	93.22±0.61	19.88±4.2	80.88±3.25	25.67±5.13
Cu+5ESC	87.43±0.88	16.21±3.4	76.92±5.33	22.31±1.53
Zn+1ESC	88.21±0.98	18.89±1.56	74.51±9.05	29.11±5.60
Zn+3ESC	111.4±0.91	23.32±1.30	79.03±8.58	16.73±5.82
Zn+5ESC	96.22±0.10	23.32±1.32	81.24±11.90	21.54±2.66
Significance	< 0.001	0.029	0.013	0.032
LSD (0.05)	16.3	2.46	12.38	11.6

Table 3. Quantitative composition of culturable bacteria and fungi within bulk soil region and rhizospheric soil of test plant at 6 months after exposure to metals (mean ± S.E.M)

Treatments	Bacteria (x 10^5 cfu/g)		Fungi (cfu/g x 10^4)	
	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	1.52±0.99	3.41±1.00	2.03±1.01	0.16±0.57
Cu+1ESC	1.91±0.99	0.64±1.08	0.13±0.51	0.37±0.51
Cu+3ESC	3.44±1.00	0.32±0.57	0.16±0.56	0.14±0.58
Cu+5EVS	2.61±0.95	0.03±0.57	0.37±0.67	0.22±0.58
Cd+1ESC	1.32±0.30	2.04±1.00	0.14±0.56	0.32±0.56
Cd+3ESC	1.63±1.00	2.02±1.00	0.22±0.57	0.35±0.56
Cd+5ESC	2.07±1.00	2.61±0.74	0.31±0.58	0.37±0.57
Mn+1ESC	2.81±1.01	0.34±0.57	0.35±0.58	0.13±0.58
Mn+3ESC	1.83±0.33	0.43±0.57	0.12±0.56	0.22±0.55
Mn+5ESC	1.61±0.32	0.92±0.16	0.14±0.57	0.23±0.61

Treatments	Bacteria (x 10^5 cfu/g)		Fungi (cfu/g x 10^4)	
Zn+1ESC	0.27±0.33	0.17±0.57	0.28±1.00	3.08±1.00
Zn+3ESC	0.38±1.00	0.27±0.61	2.62±0.21	0.28±0.57
Zn+5ESC	0.14±0.11	0.38±0.86	0.19±0.01	2.63±0.48
Pb+1ESC	1.51±0.35	0.11±0.11	0.12±0.11	0.19±0.11
Pb+3ESC	3.01±1.30	0.58±0.60	4.02±1.56	0.12±0.01
Pb+5ESC	2.92±1.00	0.53±1.00	0.45±0.92	0.41±0.24
Significance	< 0.001	< 0.001	0.003	0.025
LSD (0.05)	1.4	1.3	1.1	1.06

Generally, apart from Cd which showed statistically comparable rhizospheric bacterial composition (2.02 – 2.61 x 10^5 cfu/g), significant reduction in rhizospheric bacterial composition was reported in the other HM-treated soils compared to the control.

As provided on Table 4, microbial isolates in the bulk soil surrounding test plant at 6 months after exposure to metals have been presented. Culturable bacterial isolates in the control were *P. aeruginosa, S. aureus, Micrococcus varians,* and *Staphylococcus epidermidis.* In the Cd-exposed plants, bacterial isolates were generally *Micrococcus varians*, *Bacillus subtilis*, *P. aeruginosa, Staphylococcus epidermidis*. Culturable fungal species include *Aspergillus flavus, Trichoderma harzianum, Mucor mucedo, Fusarium solani, Penicillum* sp., *Aspergillus niger*. The most prevalent bacterial species was *B. subtilis,* whereas the most prominent fungi were *Mucor mucedo* and *A. niger.*

As presented also, Table 5 shows microbial isolates within rhizosphere of test plant at 6 months after exposure to metals. Culturable bacterial isolates in the control were *S. epidermidis*, *P. aeruginosa,* and *B. subtilis*, whereas fungal isolates included *A. niger*, *Penicillium* sp., *M. mucedo,* and *F. solani*. As observed, *A. niger* was found in Zn-polluted rhizospheric soil. *M. mucedo* was common to all soils. Similarly, the most common bacterial species, common to all soil treatments and control, was *B. subtilis.*

An attempt was made to separate the plant growth promoting rhizobacteria in the rhizospheric soil samples (Table 5). Very common to the study was *Azotobacter* sp., *B. subtilis*, *Clostridium* sp., *P. aeruginosa*, and *Klebsiella* sp.

Table 4. Microbial isolates in the bulk soil surrounding test plant at 6 months after exposure to metals

Treatments	Culturable bacterial isolates	Culturable fungal isolates
Control	*P. aeruginosa, S. aureus, S. epidermidis, M. varians,*	*A. flavus, T. harzianum, M. mucedo, F. solani, Penicillum sp., A. niger*
Cu+1ESC	*Proteus vulgaris, Bacillus subtilis, P. aeruginosa, Clostridium* sp., *Klebsiella* sp.	*M. mucedo, A. flavus., Penicillum sp., Aspergillus niger*
Cu+3ESC	*S. aureus, E. coli, M. varians, P. aeruginosa, B. subtilis*	*M. mucedo, A. flavus, Penicillum sp.*
Cu+5EVS	*S. aureus, E. coli, M. varians, B. pumilus, Proteus vulgaris, B. subtilis*	*T. harzianum, M. mucedo, Penicillum sp. A. niger*
Cd+1ESC	*M. varians, B. subtilis, P. aeruginosa*	*F. solani, M. mucedo, Penicillium sp.*
Cd+3ESC	*Proteus sp. Azotobacter* sp., *B. subtilis, S. epidermidis*	*M. mucedo, Trichoderma harzianum, A. flavus, Penicillium* sp.
Cd+5ESC	*Micrococcus varians, B. subtilis, P. aeruginosa, S. epidermidis*	*M. mucedo, T. harzianum*
Mn+1ESC	*Micrococcus varians, B. subtilis, P. aeruginosa*	*A. nidulans, M. mucedo, Penicillium* sp.
Mn+3ESC	*Azotobacter* sp., *B. subtilis, P. aeruginosa*	*M. mucedo, A. niger*
Mn+5ESC	*Micrococcus varians, B. subtilis, P. aeruginosa*	*M. mucedo, Rhizopus* sp.
Zn+1ESC	*S. epidermidis P. aeruginosa, B. subtilis*	*A. niger, Penicillium* sp., *M. mucedo, F. solani*
Zn+3ESC	*S. aureus, B. subtilis, S. epidermidis*	*A. niger, Microsporum* sp., *Penicillium* sp., *M. mucedo*
Zn+5ESC	*P. aeruginosa, B. subtilis, S. epidermidis*	*A. flavus, T. harzianum, M. mucedo, A. niger*
Pb+1ESC	*S. aureus, B. subtilis, S. epidermidis*	*A. flavus, T. harzianum, Penicillum sp., A. niger*
Pb+3ESC	*Azotobacter* sp., *B. subtilis, P. aeruginosa*	*M. mucedo, Rhizopus* sp., *A. niger*
Pb+5ESC	*Micrococcus varians, B. subtilis, P. aeruginosa*	*M. mucedo, F. solani, Penicillum* sp., *A. niger*

Table 5. Microbial isolates within rhizosphere of test plant at 6 months after exposure to metals

Treatments	Culturable bacterial isolates	Culturable fungal isolates
Control	*S. epidermidis, P. aeruginosa, B. subtilis*	*A. niger, Penicillium sp., M. mucedo, F. solani*
Cu+1ESC	*Micrococcus varians, B. subtilis, P. aeruginosa*	*M. mucedo, Rhizopus* sp.
Cu+3ESC	*S. epidermidis P. aeruginosa. B. subtilis*	*A. niger, Penicillium* sp., *M. mucedo, F. solani.*
Cu+5EVS	*S. aureus, E. coli, Micrococcus varians, B. subtilis, S. epidermidis*	*A. niger, Microsporum* sp., *Penicillium* sp., *M. mucedo*
Cd+1ESC	*S. aureus, P. aeruginosa, B. subtilis, S. epidermidis*	*A. flavus, T. harzianum, M. mucedo, A. niger*
Cd+3ESC	*S. aureus, E. coli, P. vulgaris, B. subtilis, S. epidermidis*	*A. flavus, T. harzianum, A. niger*
Cd+5ESC	*Azotobacter* sp., *B. subtilis, P. aeruginosa*	*M. mucedo, Rhizopus* sp., *A. flavus*
Mn+1ESC	*Micrococcus varians, B. subtilis, P. aeruginosa*	*A. niger, Microsporum* sp., *Penicillium* sp., *M. mucedo*
Mn+3ESC	*S. aureus, M. varians, P. aeruginosa, S. epidermidis*	*A. flavus, T. harzianum, M. mucedo, A. niger*
Mn+5ESC	*Proteus vulgaris, B. subtilis, P. aeruginosa, Clostridium* sp., *Klebsiella* sp.	*T. harzianum, M. mucedo, F. solani*
Zn+1ESC	*S. aureus, E. coli, M. varians, Azotobacter* sp., *P. aeruginosa, B. subtilis*	*T. harzianum, F. solani, Penicillum* sp.
Zn+3ESC	*Azotobacter* sp., *S. aureus, E. coli, M. varians, P. vulgaris, B. subtilis*	*Rhizopus sp., T. harzianum, M. mucedo, F. solani, Penicillum* sp.
Zn+5ESC	*S. epidermidis, P. aeruginosa. B. subtilis*	*T. harzianum, M. mucedo, Rhizopus oryzea, A. flavus*
Pb+1ESC	*S. aureus, E. coli, M. varians, B. subtilis, S. epidermidis*	*A. niger, M. mucedo, Rhizopus* sp.

Treatments	Culturable bacterial isolates	Culturable fungal isolates
Pb+3ESC	*Azotobacter* sp., *S. aureus, P. aeruginosa, B. subtilis, S. epidermidis*	*A. flavus, T. harzianum, Penicillum* sp., *A. niger*
Pb+5ESC	*Azotobacter* sp., *S. aureus, E. coli, Proteus vulgaris*	*Penicillum* sp., *A. niger*

The survival of plants on HM-contaminated soils may be attributed to the presence of microorganisms in the root zones of plants (rhizosphere). Pierzynski *et al.* [30] noted over 90 times population of microorganisms in rhizosphere than that of non-rhizosphere soil. Plants have capacity to withstand relatively high concentrations of organic and inorganic chemicals without toxic effect and they can uptake and convert these contaminants quickly to less toxic metabolites. This is achieved by the release of root exudates, enzymes that stimulate activities of these soil microbes in assisted-remediation of contaminants. The microbial communities in the rhizosphere or near the root zone can breakdown the pollutant or make it more bioavailable, testing the plant capability when exposed to it [31]. This present study examines the microbial burden (qualitatively and quantitatively) on the rhizosphere of HM polluted plants. It was evident that the rhizosphere of the plant was home to tens of thousands of bacterial cells (Table 3) even after 6 months of HM pollution. A plethora of bacterial isolates which were isolated (Table 4) include *Bacillus* sp. (the most frequently occurring bacteria), *P. aeruginosa*, *Clostridium* and *Azotobacter* amongst others, while the fungal species include *Aspergillus, Trichoderma, Saccharomyces* and *Penicillum*. The results obtained in this study were in consonance with the reports obtained of Keister and Cregan [26], Egamberdiyeva and Islam [27] and Lugtenberg and Kamilova [28]. They opined that these bacteria which grows and proliferate in the rhizosphere are termed plant growth promoting rhizobacteria and part of their function(s) include plant growth enhancement and reduction of phytotoxicity of metals. Several other reports in literatures have implicated some of the bacteria reported in this study to significantly influence the supply of nutrients in plants by competing for mineral nutrients as well as by mediating the turnover and mineralization of organic compounds [33, 34]. Similar PGPR reported in literatures which were also isolated in this study include *Azotobacter* sp., *B. subtilis*, *B. pumilus, Clostridium* sp., *P. aeruginosa*, and *Klebsiella* sp. Therefore, these bacteria in the rhizosphere can induce and control the turnover of nutrients in the soil [33] as well as directly influence plant growth by releasing a variety of compounds, e.g., phytohormones or antimicrobial compounds [34]. Amongst the fungal species isolated in the rhizosphere of HM polluted soil of the plants include *Aspergillus* and *Penicillium* (being the most predominant). Other species of fungi isolated in the study include *Trichoderma*, *Rhizopus* and *Mucor*. These isolated have been known for their ability to withstand a plethora of conditions. Similar to the functions performed by the bacteria, the aforementioned fungi reported in this study have also been reported to play similar roles in plant growth promotion as well as nutrient turnover.

PGPR are usually in contact with the root surface, and improve growth of plants by several mechanisms, e.g., enhanced mineral nutrition, phytohormone production, disease suppression [35]. Two groups of PGPR were described: one group is involved in the nutrient cycling and plant growth stimulation (biofertilizers) [36] and the second group is involved in the biological control of plant pathogens (biopesticides) [37]. Coincidentally, the results in this study, corresponds with the above description of PGPR as some of the isolated bacteria and fungi have been found to exhibit both functions of PGPR in plants. *Bacillus* and *Trichoderma* have been widely used as biological control agents while others have been found to be amongst bacteria present in substances used as biofertilizers. Among PGPRs selected in the study, *Azotobacter* sp. was selected because it was reported that stimulated plant growth during Zn and Pb toxicity was key in *C. odorata* survival, and as such, the researcher suggested possible link with the bacterium [25]. Similarly, *B. subtilis* which is a PGPR, has the capacity for enhanced plant growth because of reduced metal phytotoxicity by sequestration (Table 6). In the study, HM sequestration was majorly reported (i.e. presence of HM in organic forms). Although the mechanism for which this sequestration occurred was not investigated, it is suspected that rhizobacteria like *B. subtilis, Clostridium* sp., and *P. aeruginosa* may have played a number of roles [26-28].

Table 6. Presence of plant growth-promoting rhizobacteria in the test plant

Known PGPR associated with plant rhizosphere discovered in this study	Plant associated response as reported in literature	Reference
Azotobacter sp.	Stimulates plant growth during Zn and Pb toxicity	[25]
B. subtilis	Enhances plant growth because of reduced metal phytotoxicity by sequestration	[26, 27, 28]
Clostridium sp.	Enhances plant growth because of reduced metal phytotoxicity by sequestration	[26, 27, 28]
P. aeruginosa	Enhances plant growth because of reduced metal phytotoxicity by sequestration	[26, 27, 28]
Klebsiella sp.	Enhances root and shoot growth under metal toxicity	[26, 29]
P. aeruginosa	Enhances root and shoot growth	[27]

4. Conclusions

The presence of soil microorganisms have been reported to enhance plant growth and survival capabilities of *Chromolaena odorata* even under severe environmental stress conditions such as in HM pollution. This is evident from the morphological parameters of the test plant (below ground) even after 6 months of planting in

an HM polluted soil. More so, a plausible reason for the increased growth of the test plant in the HM contaminated soil could be attributed to the presence of a repertoire of bacteria and fungi (the biodiversity of microbial presence) in the root zone of the test plant. The study also reveals the rich diversity of soil microorganisms associated with the root zone of the test plant. The ubiquity of microorganisms (bacteria and fungi) in the environment irrespective of environmental factors makes possible a plethora of processes which man can harness to make the environment safe, cleaner and better as with the case of phytoremediation of HM contaminated soil using *Chromolaena odorata.*

Conflict of interest

The authors declare that there is no conflict of interest regarding this research article.

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Received: 24.07.2020
Received in revised form: 18.10.2020
Accepted: 22.10.2020