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RESEARCH ARTICLE

Heavy Metal Resistance by Endophytic Bacteria Isolated from Guava (*Psidium Guajava*) and Mango (*Mangifera Indica*) Leaves

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ABSTRACT

Heavy metal resistant bacteria are widespread in nature and their application in decontamination of polluted ecosystems is promising. In this study, ability of endophytic bacteria isolated from *Psidium guajava* (Guava) and *Mangifera indica* (Mango) for heavy metal resistance was assessed. Leaves samples form the two plants were collected and processed according to the standard laboratory practices. Heavy metals were analyzed using Atomic absorption spectrophotometer. Endophytic bacteria were isolated and identified using morphological and biochemical characteristics; heavy metal resistance was determined by plate dilution method. Heavy metal analysis revealed that the leaves samples contained considerable quantities of Manganese (Mn), Lead (Pb) and Cadmium (Cd) ranging from 1.21±1.6 mg/Kg (for Cd in Guava leaves) to 116.58±1.3 mg/Kg (for Mn in Mango leaves). A total of six bacterial species were isolated from both of the plants leaves (3 each). Guava endophytes were identified as *Streptococcus* sp, *Staphylococcus albus* and *Staphylococcus seiuri* whereas *Staphylococcus aureus, Staphylococcus xylulose* and *Staphylococcus intermedius* were from Mango leaves. The identified isolates were tested for ability to resist heavy metals *in-vitro* and were capable of showing different patterns of resistance to $MnCl_2$, $PbCl_2$ and $CdCl_2$. All the endophytes were highly resistant to $PbCl_2$ followed by $MnCl_2$ but susceptible to $CdCl_2$. The ability of plants and bacterial endophytes understudy to tolerate or resist heavy metals is a good indication of their phytoremediation potentials and thus, should be harnessed.

Keywords: Endophytes; Cadmium; Resistance; Manganese; Lead; Bioaccumulation

1 Introduction

Since the middle of the last century, human surge for economic fortunes and technological advancements has significantly affected ecosystems as a result of waste generation leading to environmental contamination. In the recent past, environmental contamination with negative effects on humans, animals and plants has become a global problem [1]. Currently, environmental pollution is invariably on the increase worldwide due to concomitant increase in natural and anthropogenic activities resulting to devastating effects on aquatic and terrestrial environments [2-4]. Large quantities of organic and inorganic waste-products are routinely released and some of the substances exert varying harmful effects on living organisms and once they enter an environment, they become part of the biological cycle that affects all forms of life. Most of the contaminations can be attributed to industrial, municipal and military waste management practices that promote disposal rather than treatment [5].

Heavy metal contamination is one of the present environmental challenges in both developed and developing economies which is principally associated to rapid industrialization and expansion of manufacturing power among nations [6]. Heavy metals are naturally occurring metals with relatively high densities (≥ 5g/cm^3), high atomic numbers (> 20) or high atomic weight [7]. Based on this definition, majority of the known elements in the Universe might be included, however, the persistence and toxicity of some of the metals even at very low concentration qualified them for inclusion into a group called toxic heavy metals which are environmentally and clinically more important [8]. However, some of the heavy metals are economically important and many others are indispensable for life in the biosphere even at trace level [7].

Environmental pollution due to heavy metals is one of the major health and environmental challenges; as it leads to toxicity, risk to human survival and disrupts ecological balance [9-10]. The presence of toxic heavy metals in the environment is said to be geogenic or anthropogenic with much resulting from smelting industry, residues from metalliferous mining, combustion of fossil fuel, and waste incineration in addition to naturally heavy metals-laden soils [11-13]. More so, many manufacturing industries like fertilizer and pesticide, metallurgy, iron and steel, electroplating, electrolysis, leather, photography, electric appliance, metal surface treating, aerospace and atomic energy installation contribute greatly to heavy metal contamination [14]. Indiscriminate manufacture and use of heavy metal containing compounds like fertilizers and discharge of untreated effluents especially from small-scale industries has led to many-fold increase in heavy metal concentration in ecosystems [4,15]. The most common heavy metals present in the environment include Mercury, Lead, Chromium, Cadmium, Zinc, Copper, Nickel, Arsenic, Cobalt and Tin and possess varying degree of toxicity to plants and animals [16,17].

Heavy metals are toxic to all living organisms ranging from microbes to higher plants and animals. The toxicity is however severer in microorganisms due to their smaller size and direct exposure to the environment [10,18]. Elevated levels of heavy metals can result in decreased microbial community size and structure; decreased organic matter mineralization and organic matter decomposition. The situation, however, tends to favor some species due to their ability to withstand, transform the toxic metals to environment-friendly state or use them as substrates [19,20]. Thus, some microbes may play a vital role in the biogeochemical cycling of toxic heavy metals and also in cleaning up metal contamination [21,22].

A lot of studies have explored the applicability of microorganisms in ameliorating heavy metal contamination and some were found to be exciting [23,26]. This led to the development of some biotechnological processes for remediation of polluted environments tagged as bioaccumulation and biosorption [26,27]. Bioaccumulation is a process that involves the uptake and removal of environmental pollutants (both organic and inorganic) by microorganisms. Bacteria and fungi are used as key actors to detoxify, reclaim and or immobilize pollutants [28]. These technologies are believed to be more effective than the conventional processes used in removal of heavy metals from industrial waste waters including chemical precipitation, oxi-reduction, filtration, electrochemical techniques and sophisticated separation processes using membranes; due to their cost expensiveness [23]. Biosorption on the other hand, is a process by which living and nonliving microbial cells as well as cellular products such as polysaccharides are used for heavy metal ions removal from aqueous solutions based on the principles of adsorption [29,30]. Biosorption as a bioremediation process has received increasing attention in recent years. Biosorption using the biomass of microorganisms is an effective and economic technology for the removal and recovery of heavy metal ions from waste waters. Biomass of various organisms such as bacteria, fungi, yeasts, and plants have been employed in investigating the effectiveness of biosorption process [31,32].

Despite the obvious advantages of the biotechnological processes, having the most competent and effective microbial candidates has remained a challenge to most researchers [33,34]. A number of microbes have so far been engineered genetically to meet the challenge but however, the consequences for accidental release of the modified organisms have restricted its widespread application. More so, autochthonous microbial species are considered more often due to competitive advantage of their environmental adaptations even if modification of their genome is desired [32,35]. As a result, search for these organisms have expanded to many places like contaminated soils, water, and sediments. Various metal-resistant bacteria have been previously reported to include *Bacillus* sp. [23], *Pseudomonas* sp. [24,36], *Stenotrophomonas maltophilia*, *Exiguobacterium* sp., *Pantoea* sp., *Aeromonas* sp. [37] and many fungal [38] and algal [39] species. Ability of bacterial endophytes to accumulate heavy metals is rarely been reported as a result of more attention being devoted to soil, wastewater or sediments; even though a lot of plants are known to flourish in heavy metal contaminated environments. It was against this backdrop that this study was designed with a view to providing information on the ability of bacterial endophytes of plants growing in an environment (the vicinity of a cement factory) that was hitherto reported to be containing heavy metals in high doses; to effectively resist heavy metals.

2 Materials and Methods

2.1 Sample Collection

Samples were collected from *Mangifera indica* (Mango) and *Psidium guajavum* (Guava) plants growing around the premises of a cement factory in Sokoto area (13°21'N and 5°5'E) Northwestern Nigeria. The plants were the most abundant plants in the area and previous studies by Inuwa [40] have shown that the area contained substantial quantities of heavy metals. Leaves and leafstalks (petioles) of the plants were cut using a sharp and sterile knife from different locations. The identity of the plant samples was confirmed at the Herbarium of Biological Sciences Department, Usmanu Danfodiyo University, Sokoto (UDUS). All the reagents used in the experiment are of analytical grade.

2.2 Sample Processing

The plant materials were processed according to the methods of Khan, *et al.* [41] and Fan *et al.* [42]. One gram (1g) of plant samples were washed under running tap water, surface-sterilized by dipping successively into 70% ethanol for 1 min, 3.5% sodium hypochlorite for 3 min, and finally rinsed three times in sterilized distilled water. After surface disinfection, the leaves and stalks were homogenized with 10ml 0.85% NaCl solution and quartz sand in a surface sterilized mortar. The homogenized material was agitated for 1hour at 30°C.

2.3 Isolation and identification of bacteria

From each of the homogenate, 1ml was serially diluted up to four folds and each dilution was separately spread on the surface of nutrient agar (NA; medium (Oxoid) in triplicate. Plates were incubated in darkness at 30°C for 7 days. Distinct colonies that developed on the media were picked at random and purified by streaking on separate freshly prepared NA plates. Pure isolates were subcultured on NA slants and stored at 4°C for further analysis. Isolates were identified based on cultural, morphological and biochemical characteristics according to the methods and schemes of Oyeleke and Manga [43] and Barrow and Feltham [44] respectively.

2.4 Detection of heavy metals in plant samples

From each of the plants' homogenate, 1g was weighed and digested as described by Ibrahim *et al.* [45]. Heavy metal analysis of plant samples was carried out using Atomic Absorption Spectrophotometer (Model 210 VGP, Norwalk) using direct air acetylene flame at suitable wavelength for each metal as described by Basset *et al.* [46]. Standards were prepared form 1000mg/kg stock solution of each of the metals.

2.5 Determination of heavy metal resistance by isolates

Metal resistance potential of each isolate was determined by the plate dilution method as adopted by Khan *et al.* [41]. Stock solutions of the heavy metal salts including $CdCl_2$, $PbCl_2$ and $MnCl_2$ were prepared in double distilled water and added to a sterilized nutrient agar in plates at 20 mg/l concentrations. In each test, 5μl of broth culture (McFarland No. 5) was applied onto duplicate agar plates containing the appropriate heavy metal salts and incubated at 30°C for 3 days. Absence of zones of inhibition on the agar plates indicated the ability of the isolates to resist heavy metals. Since there is no defining concentration of metal ions which can be used to distinguish metal resistant bacteria and metal sensitive bacteria, the concentration used in this study has been employed in similar studies reported on endophytic bacteria [47,48].

3 Results and Discussion

A total of six bacteria were isolated (3 each) from Guava and Mango plant samples. Morphological characteristics showed that all the isolates were Gram-positive cocci in chains and clusters. Cultural, morphological and biochemical characteristics showed that the bacterial isolates from Guava leaves were *Streptococcus* sp, *Staphylococcus albus* and *Staphylococcus seiuri* whereas those from Mango leaves were identified as *Staphylococcus aureus, Staphylococcus xylulose* and *Staphylococcus intermedius.* The presence of Gram-positive species in plant tissues have been reported severally and mostly attributed to plant tissue type, habitat, nature of the soil and its topography. Studies by Stępniewska and Kuźniar [49] have indicated the effects of aforesaid factors on the diversity of endophytes in different plants. Endophytic bacteria are found in virtually every plant on earth, including a wide range of agricultural and horticultural crops [50]. Occurrence of *Staphylococcus* species in this study is in consonance with the findings of Hung and Annapurna [51] who isolated among others *Staphylococcus* and *Deinococcus* species. Our results, however, were contrary to some other works in which, presence of Gram-negative bacteria in equal proportion [52] or in dominance [53] was reported. In a previous study [54] using *Cajnus cajan* and *Lablab purpereus,* isolated endophytes were identified to be the members of *Pseudomonas, Micrococcus, Bacillus, Rhodococcus* and *Flavobacterium.* Apparently, there is great diversity of endophytes with respect to their taxa and host plants. Hardoim *et al.* [55] proposed that the diversity is due to stochastic events, which are influenced by deterministic processes of colonization and microenvironment.

Result of heavy metal contents of the plant samples is presented in Table 1. Manganese, Lead and Cadmium were detected averagely in large quantities. In Guava samples, Pb was the most abundant (90.44±1.2 mg/Kg) followed by Mn (61.49±1.6 mg/Kg) and Cd (1.21±1.6 mg/Kg). In Mango samples however, Mn was most abundant (116.58±1.3 mg/Kg) while Cd (10.89±0.93 mg/Kg) was the least also. The ability of Guava and Mango plants to harbor the amount of heavy metals observed in this study is a reflection of environmental contamination and their ability to withstand stress due to heavy metals. Some plants are generally known to tolerate and even detoxify heavy metals when growing or introduced into contaminated environment. A number of studies have reported heavy metal tolerant plants including Vassilev *et al.* [11], Khan *et al.* [41] and Jan *et al.* [56]. Although plants need some heavy metals as essential micronutrients, their excess in soil inhibits plant growth. The heavy metal tolerating capacity of plants mainly depends on plant species or genotype and the concentration of specific heavy metals in the environment [47,48]. Moreover, occurrence of theses metals in relatively high concentration is an indication of soil contamination due to heavy metal in the area which consequently led to metal accumulation. Previous studies from the area have indicated heavy metal contamination in soil which is mainly attributed to the industrial activities. Studies by Inuwa *et al.* [40] showed that the levels of some heavy metals within the study region reached 0.1μg/g Cd, 83.4 μg/g Cr, 486.0μg/g Pb and 22.9μg/g Ni. Dalhat *et al.* [57] also reported higher Pb, Zn, Cr and Cu concentrations in soil and *Lactuca sativa* grown in the area as against distant compared references. These findings are further supported by the work of Ibrahim *et al.* [45] who observed that the finished product of the industry (cement) contains appreciable amount of heavy metal that can reach up to 6.67 ppm Mn, 7.93 ppm Ni, 2.64 ppm Cr and 2.52 ppm Cd making it highest in Ni and Cr among the tested pairs.

Table 1: *Heavy metal content of plant samples*

Heavy metal	Guava Leaves (mg/kg)	Mango Leaves (mg/kg)
Mn	61.49 ±1.6	116.58 ±1.3
Pb	90.44 ±1.2	21.16 ±1.6
Cd	1.21 ±1.6	10.89 ±0.93

Table 2: *Resistance of Guava isolates to heavy metals*

Organisms	Resistance			
	Control	$MnCl_2$	$PbCl_2$	$CdCl_2$
Streptococcus sp.	+++	+++	+++	++
Staphylococcus albus	+++	++	+++	+
Staphylococcus seiuri	+++	++	+++	+

Table 3: *Resistance of Mango isolates to heavy metals*

Organisms	Resistance			
	Control	$MnCl_2$	$PbCl_2$	$CdCl_2$
Staphylococcus aureus	+++	++	+++	+
Staphylococcus xylulose	+++	++	+++	+
Staphylococcus intermedius	+++	++	+++	+

The differences observed between our study and that of Ibrahim *et al.* [45] was as a result of the plants ability to accumulate metals as compared to soil samples which are subjected to washing by running water or leaching; thereby decreasing metal concentration on the topmost soils. This agrees with the findings of Abdullahi *et al.* [58] who observed that top soils from the area had better chemical properties than the deep soil. The ability of the plants to flourish in the area is a good indication of its phytoremediation potentials. This is due to the fact that only heavy metal tolerant or accumulators are known to survive in high doses of toxic metals. According to Vassilev *et al.* [11] metal accumulating-plants are usually found on metalliferous soils and other metal rich soils. This ability may be a function of many factors among which genetic makeup and soil characteristics play a major role [59]. In addition, Ernst [60] expressed that natural exposure of plants to surplus of various metals has driven the evolution of metal hyperaccumulation as well as plant resistance to heavy metals.

Results for resistance of heavy metals by the isolates are presented in Tables 2 & 3. Among Guava isolates (Table 2), *Streptococcus* sp. was observed to be highly resistant to $MnCl_2$ and $PbCl_2$ as compared to $CdCl_2$ whereas *S. albus* and *S. seiuri* were most resistant to $PbCl_2$ but less to $CdCl_2$. Similarly, isolates from Mango plants were most resistant to Pb followed by Mn and Cd; with resistance to Pb been comparable only to that of the control (Table 3). These results have shown that the bacterial isolates were capable of resisting the effects of heavy metals on their cells and as such able to live in association with heavy metal-resistant plants. This might be due to their ability to adapt to different concentrations of heavy metals and become metal resistant. This is in accordance with the work of Goris *et al.* [61] who isolated thirty-one heavy metal resistant bacteria from industrial biotopes with considerable variations in activity. It might also be due to their inherent ability or presence of plasmids as opposed to selective pressure. Gupta *et al.* [62] have made similar assertions and Zolgharnein *et al.* [63] have reported that, the occurrence of plasmids in heavy metal resistant bacteria is more than that found in common bacteria. Resistance of endophytic *Staphylococcus* sp to Pb and Cd has been reported by Khan *et al.* [47] and thus supports our findings. Among heavy metals Zn, Cu and Pb are said to be less toxic, whereas Cd and Cr are highly toxic to microbes. This explains the kind of variation observed among the isolates with regards to heavy metal resistance in which Cd was the least to be resisted by isolates in this study. Heavy metals are arranged in the order of resistance Pb>Cu>Zn>Cd>Cr, although it might be influenced by adaptation of the organism to a stressed environment. Previous studies by Raja *et al.* [64] have made similar observations.

4 Conclusion

In this study, it was evident that the plants understudies were harboring endophytic bacteria in their leaf's tissues. The bacterial species were all Gram positive of the genera *Staphylococcus* and *Streptococcus*. The plants' leaves were observed to contain appreciable amount of Mn, Pb and Cd. The bacterial species were resistant to all the heavy metals tested at various levels of resistance.

Therefore, the bacterial isolates and the corresponding plants are heavy metal resistant and thus, could potentially play a significant role in phytoremediation of heavy metal polluted soils.

5 Declarations

5.1 Acknowledgements

We acknowledge the support of laboratory staff in the Microbiology Research Laboratory, UDUS.

5.2 Competing Interests

The authors declare that there is no conflict of interest exist in this article.

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References

[1] P. Lazo, E. Steinnes, F. Qarri, S. Allajbeu, S. Kane, T. Stafilov, M. V. Frontasyeva, H. Harmens. “Origin and spatial distribution of metals in moss samples in Albania: A hotspot of heavy metal contamination in Europe” Chemosphere, 9:135, 2017.

[2] P. Patel, N. Janardhana Raju, B. C. Sundara Raja Reddy. U. Suresh . D. B. Sankar . T. V. K. Reddy. “Heavy metal contamination in river water and sediments of the Swarnamukhi River Basin, India: risk assessment and environmental implications” *Environ Geochem Health*; 2017.

[3] J. N. Ihedioha . P. O. Ukoha . N. R. Ekere. “Ecological and human health risk assessment of heavy metal contamination in soil of a municipal solid waste dump in Uyo, Nigeria” *Environ Geochem Health*; 2016.

[4] S. Dash, S. S. Borah, A. Kalamdhad. “A modified indexing approach for assessment of heavy metal contamination in Deepor Beel, India” *Ecological Indicators 106:105444;* 2019.

[5] J. Heritage, Evans, E. G. V. and Killington, R. A. “The microbiology of soil and nutrient recycling” Inc. *Microbiology in Action*. Cambridge University Press, UK; 1999.

[6] S. Mishra, R. N. Bharagava, N. More, A. Yadav, S. Zainith, S. Mani, P. Chowdhary, “Heavy Metal Contamination: An Alarming Threat to Environment and Human Health” In: R. C. Sobti et al. (eds.), *Environmental Biotechnology: For Sustainable Future*, 2019.

[7] M. Koller, H. M. Saleh, “Introductory chapter: introducing heavy metals” In: M. H. Saleh, R. F. Aglan, (eds) “heavy metals“ IntechOpen, 2018.

[8] J. O. Duruibe, M. O. C. Ogwuegbu, J. N. Egwurugwu. “Heavy metal pollution and human biotoxic effects” International Journal of physical sciences; 2(5):112-118, 2007.

[9] WHO “Health risks of heavy metals from long-range transboundary air pollution” *Joint WHO/Convention Task Force on the Health Aspects of Air Pollution*. WHO, Regional Office, Europe; 2007.

[10] L-M. Cai, Q. Wang, J. Luo , L-G. Chen, R-L. Zhu, S. Wang, C. Tang, “Heavy metal contamination and health risk assessment for children near a large Cu-smelter in central China” *Science of the Total Environment* ;650: 725–733; 2019.

[11] A. Vassilev, Schwitzguébel, J., Thewys, T., Lelie, D. V. and Vangronsveld, J. “The use of plants for remediation of metal- contaminated soils” *The Scientific World Journal* 4, 9–34, 2004.

[12] R. Xiao, S. Wang, R. Li, J. J. Wang, Z. Zhang, “Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China” *Ecotoxicology and Environmental Safety*, 141:17–24; 2017.

[13] V. Masindi, and K. L. Muedi, ‘’Environmental contamination by heavy metals” In: H. F. Saleh, and R. F. Aglan (eds) *Heavy metals*. Intechopen, 2018.

[14] B. Volesky, “Biosorption and Biosorbents”. In: *Biosorption of heavy metals*. B. Volesky (ed.) CRC Press, Boca Raton, Florida) 3-5, 1990.

[15] M. L. Riskuwa-Shehu, H. Y. Ismail, M. Suleiman, “Biosorption of Heavy Metals by *Oscillatoria* Species” *Microbiology Research Journal International;* 27(6): 1-8, 2019.

[16] A. K. Bhattacharya, Manda, S. N. and Das, S. K. “Heavy metals accumulation in water sediment and tissues of different edible fishes in upper stretch of gangetic west Bengal” *Trends in Applied Science Research* 3, 61-68, 2008.

[17] E. M. Ogbo, and J. A. Okhuoya, “Bio-absorption of some heavy metals by *Pleurotus tuber-regium* Fr. Singer (an Edible Mushroom) from crude oil polluted soils amended with fertilizers and cellulosic wastes” *International Journal of Soil science*; 6, 34-48, 2011.

[18] P. C. Patel, Goulhen, F., Boothman, C., Gault, A. G., Charnock, J. M., Kalia, K. and J. R. Lloyd, “Arsenate detoxification in a Pseudomonad hypertolerant to arsenic. Archives of Microbiology, 187: 171–183, 2007.

[19] S. Banerjee, Gothalwal, R., Sah, P. K. and S. Sao, “Microbial observation in bioaccumulation of heavy metals from the ash dyke of thermal power plants of chhattisgarh, India” *Adv. in Bioscience and Biotech.* 6, 131-138, 2015.

[20] X. Li, D. Meng, J. Li, H. Yin, H. Liu, X. Liu, C. Cheng, Y. Xiao, Z. Liu, M. Yan, “Response of soil microbial communities and microbial interactions to long-term heavy metal contamination” *Environmental Pollution* 231: 908e917, 2017.

[21] K. Issazadeh, Pahlaviani, M. K. and A. Massiha, Bioremediation of toxic heavy metals pollutants by *Bacillus* spp. isolated from Guilan Bay sediments, North of Iran. International Conference on Biotechnology and Environment Management *ICBEE*, 18, 67-71, 2011.

[22] J. Tang, J. Zhang, L. Ren, Y. Zhou, J. Gao, L. Luoa, Y. Yang, Q. Peng, H. Huang, A. Chen, “Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution” *Journal of Environmental Management* 242:121–130, 2019.

[23] A. A. C. Da Costa, and P. F. Duta, "Bioaccumulation of Copper, Zinc, Cadmium and Lead by *Bacillus* sp., *Bacillus cereus*, *Bacillus sphaerecus* and *Bacillus subtilis" Brazilian Journal of Microbiology* 32, 1-5, 2001.

[24] R. Pardo, Herguedas, M. and E. Barrado, “Biosorption of cadmium, copper, lead and zinc by inactive biomass of

Pseudomonas putida" Analytical and Bioanalytical Chemistry, 376: 26-32, 2003.

[25] E. Sevgi, Coral, G., Gizir, A. M. and A. Sangun, "Investigation of heavy metal resistance in some bacterial strains isolated from industrial soils" *Turkish Journal of Biology*, 34: 423-43, 2010.

[26] O. A. Oyewole, S. S. L. Zobeashi, E. O. Oladoja, R. O. Raji, E. E. Odiniya, A. M. Musa. "Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil" *SN Applied Sciences*, 1:857; 2019.

[27] B. Volesky, and Z. R. Holan, "Biosorption of heavy metals. *Biotechnology Progress*, 11:235–250, 1995.

[28] A. M. Sulaimon, Odeyemi, A. T., Ogunjobi, A. A. and I. O. Ibrahim, "Bioaccumulation of heavy metals using selected heavy metal tolerant organisms isolated from dumpsite leachate" *Nature and Science*, 12(10):101-106, 2014.

[29] S. N. Azizi, Colagar, A. H. and S. M. Hafeziyan, "Removal of Cd (II) from Aquatic System Using *Oscillatoria* sp. Biosorbent" *The Scientific World Journal*, 1-7, 2012.

[30] M. U. Mustapha, N. Halimoon, "Microorganisms and biosorption of heavy metals in the environment: a review Paper" *J Microb Biochem Technol* 7:253–256, 2015.

[31] J. Wang, and C. Chen, "Biosorbents for heavy metals removal and their future" *Biotechnology Advances*, 27(2):195–226, 2009.

[32] P. Diep, R. Mahadevan, A. F. Yakunin, "Heavy Metal Removal by Bioaccumulation Using Genetically Engineered Microorganisms" *Frontiers in Bioengineering and Biotechnology*, 6(157):1-20; 2018.

[33] M. Ahmed, and A. Malik, "Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater" *Bacteriology Journal*, 2, 12-21, 2011.

[34] S. E. Elsilk, Elshanshoury, A. R. and P. S. Ateya, "Accumulation of heavy metal resistant avirulent *Bacillus anthracis* PS2010 isolated from Egypt" *African Journal of Microbiology Research*, 8(12):1266-1276, 2014.

[35] Mishra, A., and Malik, A. Recent advances in microbial metal bioaccumulation. Crit. Rev. Environ. Sci. Technol. 43, 1162–1222, 2013.

[36] S. K. Singh, P. P. Singh, A. Gupta, A. K. Singh, J. Keshri, "Tolerance of Heavy Metal Toxicity Using PGPR Strains of *Pseudomonas* Species" In: PGPR Amelioration in Sustainable Agriculture, 2019.

[37] M. Z. Alam and S. Ahmad, "Chromium removal through biosorption and bioaccumulation by bacteria from tannery effluents contaminated soil" *Clean-Soil Air Water*, 39: 226-237, 2011.

[38] S. Congeevaram, Dhanarani, S., Park, J., M. Dexilin, and K. Thamaraiselvi, "Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates" *J. of Hazardous Materials*, 146: 270-277. 2007.

[39] M. Usmani, and H. Sultana, "Use of microalgae for the removal of environmental pollutants" *International Journal of Scientific World*, 3 (1): 1-11, 2015.

[40] M. Inuwa, Abdulrahman, F. W., Birnin-Yauri, U. A. and S. A. Ibrahim, "Analytical Assessment of some Trace Metals in Soils around Industrial Areas of North West Nigeria" *Trends in Applied Science Research*, 2(6):515-521, 2007.

[41] Khan, S. Z., Doty, L., Brian, O., Gang, X., Jun, W. K., Glenda, S., Azra, V. and James, T. S. "Diazotrophic endophytes of native black cottonwood and willow" *Symbiosis* 47(1):23-33, 2005.

[42] M. Fan, Z. Liu, L. Nan, E. Wang, W. Chen, Y. Lin, G. Wei, "Isolation, characterization, and selection of heavy metal-resistant and plant growth-promoting endophytic bacteria from root nodules of *Robinia pseudoacacia* in a Pb/Zn mining area" *Microbiological Research*, 2018.

[43] S. B. Oyeleke, and S. B. Manga, "Essentials of Laboratory Practicals in Microbiology" Tobest Publisher Minna, Nigeria, 2008.

[44] G. I. Barrow, and K. A. Feltham, "Cowan and Steel's manual for identification of medical bacteria" 33rd edition. London, Cambridge University Press, 1993.

[45] H. H. Ibrahim, Birnin-Yauri, U. A., Muhammad, C. and A. Umar, "Assessment of pollution potentialities of some portland cement" *Nigerian Journal of Basic and Applied Science* 20(2): 182-184, 2012.

[46] J. Basset, Denny, R. C., Jeffery, G. H. and J. Mendham, "Vogel's Textbook of quantitative inorganic analysis including elementary instrumental analysis" 4th edition, Longman Publishing Co. Inc. London PP 35 – 40, 1997.

[47] M. U. Khan, Angela, S., Muhammad, H., Kaneez, F., AsmaImran, M. A., Ghulam, S., Qaiser, M. K. and A. Muhammad, "Cr-resistant rhizo- and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils" *Frontiers in Plant Science* 5(755):1-10, 2015.

[48] C. Liu, H. Lin, Y. Dong, B. Li, L. Wang, "Identification and characterization of plant growth–promoting endophyte RE02 from Trifolium repens L. in mining smelter" *Environmental Science and Pollution Research*, 26:17236–17247; 2019.

[49] Z. Stępniewska, and A. Kuźniar, "Endophytic microorganisms— promising applications in bioremediation of greenhouse gases" *Appl Microbiol Biotechnol*. 97 (22):9589–96, 2013.

[50] A. V. Sturtz, Christie, B. R. and J. Nowak, "Bacterial endophytes: potential role in developing sustainable systems of crop production" *Journal of Critical Revolution on Plant Science* 19: 1–30, 2000.

[51] P. Q. Hung, and K. Annapurna, "Isolation and characterization of endophytic bacteria in soybean (Glycine SP.)" *Omonrice* 12: 92-101, 2004.

[52] D. K. Zinniel, Lambrecht, P., Harris, N. B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C. A., Arunakumari, A., Barletta, R. G. and A. K. Vidaver, "Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants" *Appl. Environ. Microbiol*. 68:2198-2208, 2002.

[53] A. K. Elbeltagy, Nishioka, H. S., Sato, T., Sato, Y. I., Morisaki, H., H. Mitsui, and K. Minamisawa, "Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties" *Soil Sci. Plant Nutr*. 46:617-629, 2000.

[54] M. L. Riskuwa-Shehu, and H. Y. Ismail, "Isolation of endophytic bacteria and phytoremediation of soil contaminated with polycyclic aromatic hydrocarbons using *Cajanus cajan* and *Lablab purpereus" Bioremediation Science and Technology Research*; 6(1):26-30, 2018.

[55] P. R. Hardoim, Van Overbeek, L. S. and J. D. Elsas, "Properties of bacterial endophytes and their proposed role in plant growth". *Trends Microbiol.* 16(10):463–71, 2008.

[56] R. Jan, Khan, M.A.; Asaf, S.; Lubna; Lee, I.-J.; K. M. Kim, "Metal Resistant Endophytic Bacteria Reduces Cadmium, Nickel Toxicity, and Enhances Expression of Metal Stress Related Genes with Improved Growth of

Oryza Sativa, via Regulating Its Antioxidant Machinery and Endogenous Hormones" *Plants* , 8:363, 2019.

[57] M. H. Dalhat, A. R. Amale, M. Maimuna, I. Bashiru, K. Sirajo, "Comparative Study of Mineral and Phytochemical Analysis of Soil and *Lactuca sativa* Grown in the Vicinity of Cement Company of Northern Nigeria (Sokoto Cement) and Usmanu Danfodiyo University Sokoto (Kwalkwalawa)" *Asian Journal of Research in Biochemistry*, 1(1):1-10, 2017.

[58] A. Abdullahi, Usman, Y., Noma, S. S., Audu, M., Danmoma, N. M. and R. S. Shuaibu, "Fertility status of fadama soils in gantsare village, wamakko local government, sokoto state as affected by cement dust" *Nig. J. Basic & Applied Sci.* 18(1):58-64, 2010.

[59] S. Abdelkrim, S. H. Jebara, O. Saadani, Heavy metal accumulation in Lathyrus sativus growing Heavy metal accumulation in Lathyrus sativus growing Arch Microbiol. 201: 107. 2019.

[60] W. H. O. Ernst, Evolution of metal hyperaccumulation and phytoremediation hype. *New Phytology*, 146, 357–358, 2000.

[61] J. Goris, De Vos, P., Coenye, T., Hoste, B., Janssens, D., Brim, H., Diels, L., Mergeay, M., Kersters, K. and P. Vandamme, "Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp. nov., *Ralstonia metallidurans* sp. nov. and *Ralstonia basilensis" Int. J. of Syst. and Evol. Microbiol.* 51:1773-1782, 2001.

[62] A. Gupta, Phung, L., Chakravarty, L. and S. Silver, "Mercury resistance in *Bacillus cereus* RC607: Transcriptional organization and two new open reading frames" *Journal of Bacteriology*, 181: 7080-7086, 1999.

[63] H. Zolgharnein, Azmi, M. L., Saad, M. Z., Mutalib, A. R. and C. A. R. Mohamed, "Detection of plasmids in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas" *Iranian Journal of biotechnology*, 5:232-239, 2007.

[64] C. E. Raja, Anbazhagan, K. and Selvam, G. S. "Isolation and characterization of a metal-resistant Pseudomonas aeruginosa strain". *World Journal of Microbiology & Biotechnology*, 22: 577–585, 2006.