Webology (ISSN: 1735-188X)
Volume 19, Number 5, 2022

In-Vitro Evaluation Of Siderophore Production By Bacteria In The Presence Of Heavy Metal

Alexander Perez-Cordero[1*], Donicer Montes –Vergara[2] and Yelitza Aguas –Mendoza[3]

[1]Grupo Bioprospección Agropecuaria, Laboratorio de investigaciones microbiológicas, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Sincelejo, Sucre Colombia.

[2]Departamento de Zootecnia, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Sincelejo, Sucre-Colombia.

[3]Grupo de Investigación Gestión Integral de Procesos, Medio Ambiente y Calidad, Facultad de Ingeniería, Universidad de Sucre, Sincelejo, Sucre- Colombia.

* Correspondence: Author: Alexander Perez-Cordero[1]

ABSTRACT

Heavy metal contamination of soils is becoming widespread and causing serious environmental problems. There is evidence of the synthesis of siderophores by bacteria when they grow in environments with the presence of toxic heavy metals; therefore, the aim of this study was to evaluate in-vitro the production of siderophores at different concentrations of $Pb(NO_3)_2$. Three strains of endophytic bacteria were selected from the genomic bank of the microbiological research laboratory of the University of Sucre, for endophytic bacteria isolated from mercury-contaminated environments. Lead tolerance tests were carried out at different concentrations (100, 200, 300, 400 and 500 mg/L), and the capacity of these bacteria to produce siderophores was evaluated. The results obtained indicate that the endophytic strains evaluated produced siderophores at different concentrations of the metal lead. The results indicate that these endophytic bacteria could contribute to the phytoremediation of plant species adapted to different environments contaminated with lead in the Colombian Caribbean.

Keywords: Bioremediation, Siderophores, Lead, Bulkhorderia cepaceae, Bacillus cereus.

INTRODUCTION

Iron (Fe) is an essential element for virtually all living things in which it is necessary for important cellular functions such as DNA synthesis, respiration and free radical detoxification. In nature, it is mainly found in the Fe3+ form as part of salts and hydroxides of very low solubility, chemical forms that make it impossible for some living organisms to use it [1]. This metal is essential for cellular metabolism as a cofactor for numerous enzymes, as well as fulfilling various functions in essential

biological processes, such as oxygen transport, DNA synthesis, nitrogen fixation, respiration and photosynthesis.

There are two major types of ligands produced by living organisms that form chelates with iron; a) those produced by microorganisms, known as microbial siderophores or simply siderophores, and b) those produced by plants, known as phytosiderophores. For the acquisition of Fe3+, microorganisms have developed mechanisms to solubilize it and make its uptake efficient. One of the mechanisms employed by bacteria and fungi is the production of siderophores, which are low molecular weight compounds, chelator of Fe3+, which are synthesized under conditions of deficiency of this micronutrient. There are different types of siderophores and they are classified into three categories according to the functional groups they use as ligands for iron ions, including catecholates such as enterobactins and vibriobactins, hydroxamates such as staphyloferrin and mixed-type siderophores such as mycobactin [2].

Recent studies indicate that microbial siderophores can form stable complexes with other metals present in the environment (Aluminium, Cadmium, Copper, Gallium, Lead, Nickel, among others) as well as with Uranium [3]. Siderophores are produced by several groups of microorganisms including: plant and animal pathogens, free-living microorganisms and nitrogen-fixing symbiotic. Siderophore production is most common in plant growth-promoting rhizospheric bacterial species, which possess the ability to produce siderophores under extreme environmental conditions: Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum, Rhizobiun and steptomyces [1,4]. Many other plant-associated bacteria such as endophytes can synthesize siderophores, which gives them a competitive advantage in the colonization of plant tissues by helping them to exclude other microorganisms from their ecological niche [5,6].

There is evidence for the synthesis of siderophores by bacteria when they grow in environments with toxic heavy metals, linking these chelating agents as responsible for the homeostasis of these metals [7]. Several studies suggest that siderophores form stable complexes with other metals, such as Al, Cd, Cu, Ga, In, Pb and Zn3, [7]. According to work carried out by [8], he observed that the addition of heavy metals such as Al, Cu, Ga, Mn and Ni induced the production of pioverdin in Pseudomonas aeruginosa.

The increasing rate of heavy metal contamination (Hg, Cr, Pb, Cu, Ni, Cd, As, Sn) in different environments has gained much attention worldwide due to the toxic effects and long persistence in the biosphere. In agricultural soils, three heavy metals, cadmium, arsenic and lead, are reported to be the main contaminants (Zhao et al., 20119). Lead (Pb) is a naturally occurring metal in the environment, however, anthropogenic activities have significantly contributed to increased levels in some environments. Activities such as Pb mining, industrial processes that use Pb as a raw material, coal and oil combustion among other processes, contribute to the release of this metal into the environment [9]. This metal is persistent in the environment where it is deposited and retained [10,11]; when it accumulates in soils, it creates public health and environmental problems [12,13,14].

Currently, to mitigate the effects of heavy metal contamination, techniques such as phytoremediation are being developed, which is an effective, economical and environmentally friendly technology that is receiving much attention worldwide. The success of phytoremediation depends on the plant's ability to tolerate high concentrations of metals and produce large amounts of biomass [18]. Endophytic bacteria living in the internal tissues of plants enhance the efficiency of the phytoremediation process and increase plant biomass production through three mechanisms: increased root surface area and root hair production, increased metal availability and increased transfer of soluble metals from the rhizosphere to the plant [19,20]; some of the endophytic bacteria studied.

Endophytic bacteria are found in the internal tissues of the plant and play an extremely important role, which consists of contributing to the adaptation of plant species to contaminated sites, and in this way boosting their phytoremediation capacity and tolerance to the contaminants present in soils, such as heavy metals [21]. Similarly, these bacteria also have effects on plant development by promoting plant growth and increasing biomass through the production of phytohormones such as indo acetic acid, while improving the nutritional status of plants through nitrogen fixation, phosphate solubilization and production of siderophores for the uptake of essential nutrients in their development [22]; Therefore, the purpose of this research was to evaluate in-vitro the ability of endophytic bacteria isolated from mercury-contaminated environments to produce siderophores at different lead concentrations.

MATERIALS AND METHODS

Endophytic bacteria.

The bacteria used were taken from the collection of the microorganism bank of the Microbiological Research Laboratory of the University of Sucre; isolated from plant species of the genera Cyperus and Paspalum adapted to the mercury-contaminated environment of the Santa Cruz mine, department of Bolivar, Colombia, molecularly identified as 1BR1 (Burkholderia sp), 1CR3 (Burkholderia cepacea) and TLOM21 (Bacillus cereus).

Tolerance assays to different concentrations of lead.

The in-vitro tolerance assays of 1BR1, 1CR3, TLOM21, were carried out in tris-MMT minimal medium proposed by [23], with 5 treatments (concentrations) of lead in the form of PbCl2. The initial concentration of lead used was 0.01 mg/mL and from these the different treatments were prepared: T1: 100 (0.1); T2: 200 (0.2); T4: 300 (0.3); T5: 400 (0.35); T4 400 (0.4); and T5:500 (0.5 mg/mL). Aliquots of each strain in log phase were inoculated onto MMT medium. MMT medium without PbCl2 was used as a control. The experiment was carried out for each strain, which was incubated under agitation at 150 rpm at 32 °C for 24 hours [24]. The growth of each bacterium was determined by turbidimetry at 600 nm every hour for one day.

Siderophore production.

At the end of the experiment for each treatment, a sample was taken to determine the qualitative production of siderophores. The qualitative analysis was carried out in chromium azurol-S (CAS) medium used by [24]. For this purpose, 60.5 mg CAS was dissolved in 50 ml of distilled water and combined with 10 ml of an iron(III) solution (1 mM FeCl3.6 H2O and 10 mM HCl). Under stirring, this solution was mixed with 72.9 mg of HDTMA dissolved in 40 ml of water. The resulting blue liquid was sterilized at 121°C for 15 minutes. In another vessel, a mixture of 750 ml of water, 15 g of agar, 30.24 g of pipes, and 12 g of a 50% (w/w) solution of NaOH was sterilized to reach a pH of 6.8. To the medium, 4 g of glucose was added as a carbon source. The bacterial strains were incubated for 7 days at 30°C. The ability of the bacteria to produce siderophores is evidenced by the formation of a ring.

RESULTS AND DISCUSSION

The results obtained in the present study show that as the concentration of lead in the medium increases, the percentage of tolerance of the strains evaluated (1BR1, 1CR3, TLOM21) decreases, highlighting a growth up to 8 hours of the experiment for Bhurkoldelia sp as shown in figure 1. The species Bhurkoldelia cepacia showed a slight growth up to 10 hours of the experiment as shown in figure 2; and in the bacterium Bacilus cerius a growth up to 7 hours of the experiment was observed as shown in figure 3. Showing a longer adaptation time of the bacterial strains when entering in contact with the metal and developing physiological mechanisms to survive to the different concentrations of lead evaluated.

Taking into account the adaptation and growth behaviour of the bacteria evaluated and reinforcing this hypothesis by the results of other studies. The pb^{2+} generates toxicity to bacterial cells, producing oxidative damage, interacting with nucleic acids, binding to proteins essential for respiration and producing reactive oxygen forms. To avoid toxicity with pb^{2+} it must be converted into a biologically inactive form and/or eliminated rapidly and efficiently from the bacterial cell. Two basic possible mechanisms of pb^{2+} resistance are known, intracellular or extracellular complexing of toxic metal ions and reduced accumulation based on active cation flux. The latter is the main mechanism developed in prokaryotes. However, enzymatic. However, enzymatic transformations of metal ions (oxidation, reduction, methylation and demethylation) are also defense mechanisms in bacteria [25,26]. These microorganisms achieve resistance to heavy metals by processes known as biosorption, bioaccumulation, precipitation, complexing and metal outflow. Another tool used by bacteria to counteract lead is through the presence of specific genes, as highlighted in the study by [27]. In which they conclude that the genera Pantoea, Pseudomonas, Enterobacter and Bacillus are resistant to cadmium and lead, as they possess the cadA and pbrA genes that encode for proteins of a metal expulsion pump, which prevents the metal from damaging the bacterial cell.

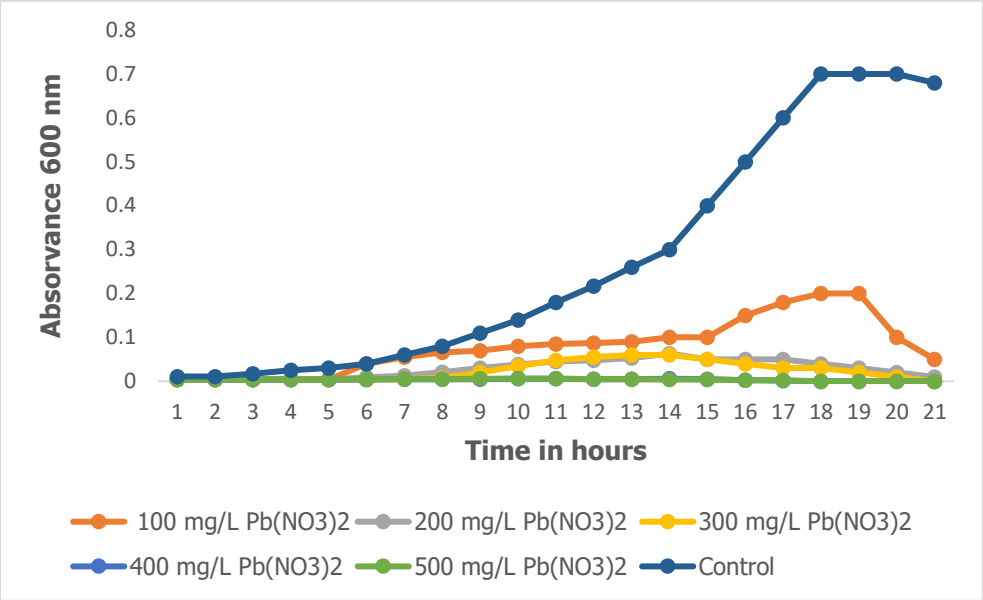

Figure 1. Tolerance of 1BR1(Burkholderia sp) at different lead concentrations.

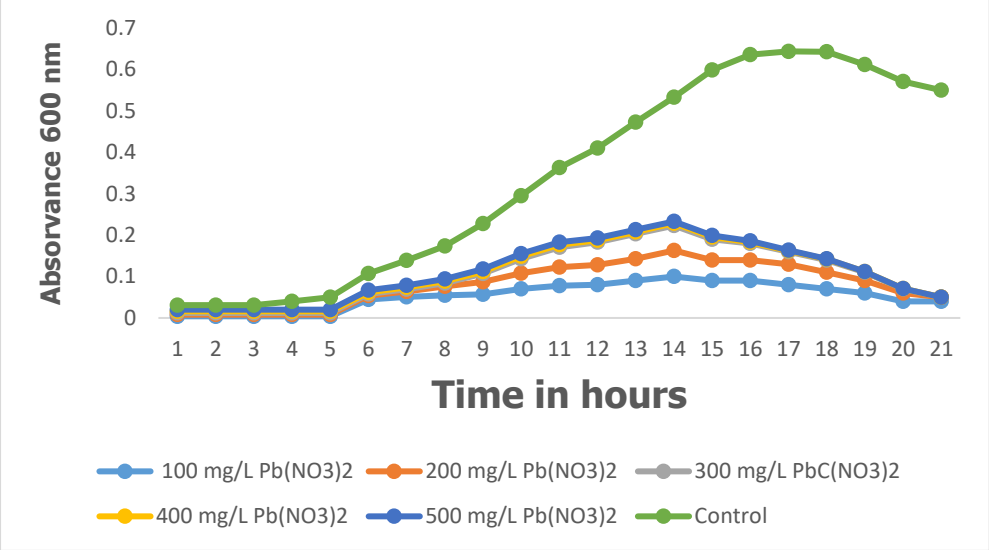

Figure 2. Tolerance of 1CR3 (Burkholderia cepacia) at different lead concentrations.

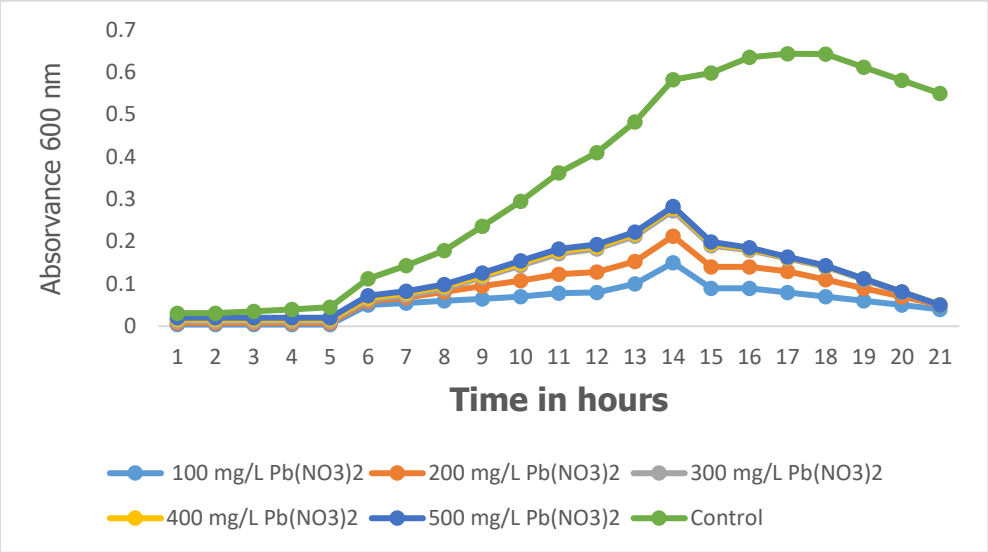

Figure 3. Tolerance of TLOM21(B. Cereus) at different lead concentrations

According to the growth curve, strain 1BR1 produced siderophores in the first 8 hours evaluated, at concentrations of 100, 200 and 300 mg/L; strain 1CR3 produced siderophores in the first 9 hours evaluated, at a concentration of 100, 200 and 300 mg/L; and strain TLOM21 produced siderophores at a concentration of 100, 200 and 300 mg/L, in the first 7 hours evaluated, as shown in figure 4.

In general, the production of siderophores by endophytic bacteria can possibly help plants to reduce the toxicity caused by the presence of heavy metals and supply the need for iron as an essential micronutrient, as it helps to reduce nitrates and sulphates and energy production within the plant; it is also essential for chlorophyll formation and promotes plant growth and development in polluted environments [28]. In addition to iron chelation, siderophores serve as a mechanism for bioremediation, where the role of siderophores in reducing cadmium and lead toxicity has been observed.

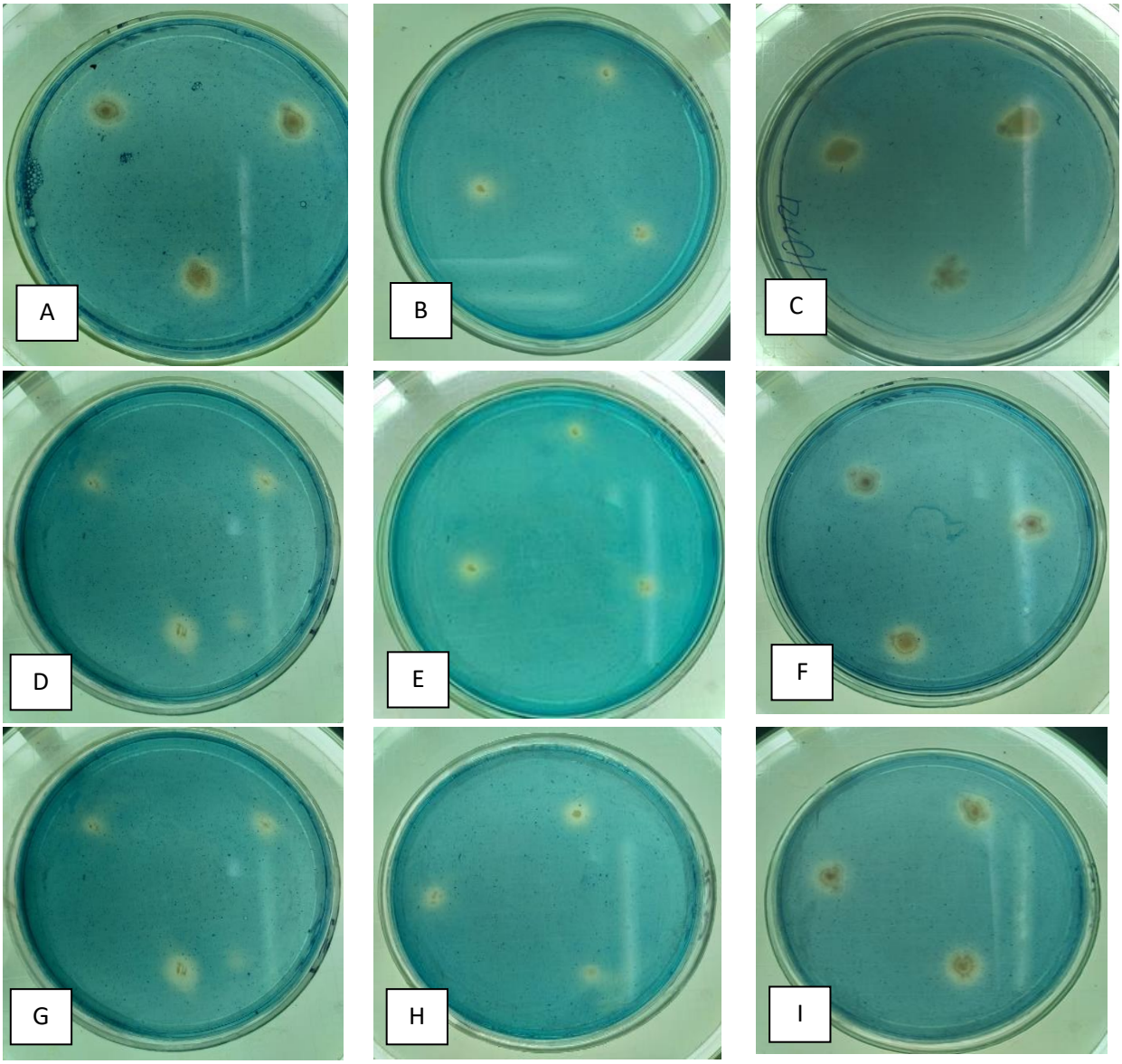

Figure 4. Siderophore production by the strains 1BR11CR3, TLOM21 in different lead concentrations mg/L 100, 200 y 300. A: 100 mg/L $Pb(NO_3)_2$ (1BR1), B: 100 mg/L $Pb(NO_3)_2$ (1CR3), C: 100 mg/L $Pb(NO_3)_2$ (TLOM21) D: 200 mg/L Pb(NO3)2 (1BR1), E: 200 mg/L $Pb(NO_3)_2$ (1CR3), F: 200 mg/L $Pb(NO_3)_2$ (TLOM21), G: 300 mg/L Pb(NO3)2 (1BR1), H: 200 mg/L $Pb(NO_3)_2$ (1CR3) y I: 300 mg/L $Pb(NO_3)_2$ (TLOM21).

The results obtained in this research represent a novelty with respect to the production of siderophores by endophytic bacteria at different concentrations of heavy metals, in this case lead, as there are not many specific studies known that evaluate the production of these iron chelating compounds by endophytic bacteria when stressed with this metal.

However, studies carried out by [29] conclude that the endophytic bacterium Bacillus cereus 1DH1LIM has the capacity to tolerate up to 400 ppm of Pb in the form of $Pb(NO_3)_2$ and to produce siderophores. Other results show that the quantity and quality of siderophore production by the plant growth-promoting Pseudomonas fulva bacterium species was due to increasing $Cd2^+$ exposure (0, 0.5, 1.0, 2.0 mM). The study aimed to determine the changes in siderophore production. The results showed that in the presence of 2.0 mM Cd^{2+}, the synthesis of siderophores of the hydroxymates, catecholates and phenolates group is produced with respect to the lower concentrations of Cd^{2+} (0.5 and 1.0). What we can infer is that possibly the mechanism of siderophore production around the environment where the bacteria grow facilitates them to sequester heavy metals and decrease their accumulation inside the cell. Therefore, the bacteria reduce the transport of heavy metals in high concentrations to the cytoplasm, preventing the intoxication of the cell [30].

CONCLUSION

Burkholderia sp (1BR1), Burkholderia cepacia (1CR3) and Bacillus cereus (TLOM21) are Gram-negative and Gram-positive endophytic bacteria, respectively, tolerant to lead. These strains were isolated from the root of plants belonging to the genera Cyperus and Paspalum growing in soils with high heavy metal contents. In-vitro tests indicate that it is able to tolerate up to 500 mg/L $Pb(NO_3)_2$, and produces siderophores at different concentrations of this metal (100, 200 and 300 mg/L). The results obtained in this study emphasize the possibility of exploring the possibility of achieving lead-tolerant endophytic bacteria for remediation and safe production of plant species in lead-contaminated soils.

ACKNOWLEDGEMENTS

The authors are grateful for the support of the microbiological research laboratory of the University of Sucre for allowing the respective tests to be carried out.

REFERENCES

1. Ali SS, Vidhale NN. Bacterial siderophore and their application: a review. Int J Curr Microbiol App Sci. 2013, 2(12):303-312.

2. Saha R, Saha N, Donofrio RS, Bestervelt LL. Microbial siderophores: a mini review. Journal of basic microbiology. 2013, 53(4):303-317.

3. Rajkumar M, Ae N, Narasimha M, Prasad V. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol. 2010, 28:142–9.

4. Złoch M, Thiem D, Gadzała-Kopciuch R, Hrynkiewicz K. Synthesis of siderophores by plant-associated metallotolerant bacteria under exposure to Cd2+. Chemosphere. 2016, 156:312-25.

5. Hrynkiewicz K, Baum, C, Leinweber P. Density, metabolic activity, and identity of cultivable rhizosphere bacteria on Salix viminalis in disturbed arableand landfill soils. Journal of Plant Nutrition and Soil Science. 2010, 173:747-756.

6. Loaces I, Ferrando L, Scavino AF. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microbial Ecology. 2010, 61, 606-618.

7. Schalk IJ, Hannauer M, Braud A. New roles for bacterial siderophores in metal transport and tolerance. Environ. Microbiol. 2011, 13:2844-2854.

8. Braud A, Jezequel, K, Bazot S, Lebeau T. Enhanced phytoextraction of an agricultural Cr-, Hg- and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere. 2009, 74:280-286.

9. Schwarz K, Pickett STA, Lathrop RG, Weathers KC, Pouyat RV, and Cadenasso ML. The effects of the urban built environment on the spatial distribution of lead in residential soils. Environ. Pollut. 2012, 163:32-39.

10. Dauvin JC. Effects of heavy metal contamination on the macrobenthic fauna in estuaries: the case of the Seine estuary. Mar. Pollut. Bull. 2008, 57:160-167.

11. Flora SJS, M Mittal and A Mehta. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J. Med. Res. 2008, 128:501-523.

12. Wong CSC, X Li, and I Thornton. 2006. Urban environmental geochemistry of trace metals. Environ. Pollut. 142(1):1-16.

13. Mielke HW, ET Powell, CR Gonzales and JPW Mielke. 2007. Potential lead on play surfaces: evaluation of the "PLOPS" sampler as a new tool for primary lead prevention. Environment Research. 103:154-159.

14. Mielke HW, MAS Laidlaw and CR Gonzales. 2011. Estimation of leaded (Pb) gasoline's continuing material and health impacts on 90 US urbanized areas. Environ. Int. 37:248-257.

15. Banat KM, F Howari and AA Al-Hamad. 2005. Heavy metals in urban soils of Central Jordan: should we worry about their environmental risks. Environ. Res. 97:258-273

16. Needleman H. 2004. Lead poisoning. Annu. Rev. Med. 55:209-22.

17. Guitart R, and T Vernon. 2005. Es el plomo empleado en deportes (caza, tiro y pesca deportiva) un problema de salud pública infravalorado?. Rev. Esp. Salud Pública. 79:621-632.

18. Ma Y; Prasad, M.; Rajkumar, M.; Freitas, H. 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol. Adv. 29:248-258.

19. Weyens N, Van Der Lelie D,Taghavi S, Vangronsveld J. 2009. Phytoremediation: plantendophyte partnerships take the challenge. Current Opinion Biotechn. 20:248-254.

20. Li H, Wei D, Shen M, Zhou Z. Endophytes and their role in phytoremediation. Fungal Diversity. 2012, 54(1): 11-18.

21. Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel W, Fallmann K, Puschenreiter, M. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biology and Biochemistry. 2013, 60(100):182 - 194.

22. Rathnayake IVN, Mallavarapu M, Krishnamurti GSR, Bolan NS, Naidu R. Heavy metal toxicity to bacteria -Are the existing growth media accurate enough to determine heavy metal toxicity. Chemosphere. 2013, 90(3):1195-1200.

23. Zhang YF, He LY, Chen ZJ, Zhang WH, Wang QY, Qian M, Sheng XF. Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape. Journal of Hazardous Materials. 2011, 186 (2-3):1720- 1725.

24. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry. 1987, 160(1), 47-56.

25. Leedjärv A, Ivask A, Virta M. Interplay of different transporters in the mediation of divalent heavy metal resistance in Pseudomonas putida KT 2440. J. Bacteriol. 2008, 190:2680–2689.

26. Silver S, Phung LT. A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. J. Ind. Microbiol. Biotechnol. 2005, 32, 587–605.

27. Guaman JFI, Moreno CGR, Guaman CFI. Determinantes genéticos y sus mecanismos de acción implicados en la resistencia bacteriana a metales pesados: una revisión. Perfiles. 2022, 1(27): 26-38.

28. Rajkumar M, Ae N, Prasad MNV, Freitas H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol. 2010, 28(3). 142-149.

29. Pérez A, Pérez-Espinosa A, Vitola D. Lead Resistance by Bacillus cereus 1DH1LIM Isolated from Contaminated Environments with Mercury. Indian Journal of Science and Technology. 2018, 11(38): 1-6.

30. Thiem D, Złoch M, Gadzała-Kopciuch Renata, Szymańska S. Baum C, Hrynkiewicz Katarzyna. Cadmium-induced changes in the production of siderophores by a plant growth promoting strain of Pseudomonas fulva. Journal of Basic Microbiology. 2018 :1-10.