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In vitro efficiency of mercury biosorption capacity by rhizospheric bacteria

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ABSTRACT
The aim of this study was to isolate rhizospheric bacteria from mercury-contaminated soils and to evaluate them in vitro tolerance to different mercury concentrations. Rhizosphere soil was collected from each site. The determination of mercury concentration in the rhizosphere was carried out by instrumental cold vapors atomic absorption spectrophotometry. Bacteria were isolated from the rhizosphere; sensitivity and tolerance to different mercury concentrations were evaluated in vitro. The average concentration of mercury in rhizosphere was 2,56 ± 5,6 mg/kg. The amount of bacteria in the rhizosphere was 1,2± 9,7 $10x10^{10}$ CFU/g soil. The bacterial species with the ability to tolerate mercury were *Bacillus cereus* strain ML259; Bacillus mycoides O-1 and *Bacillus cereus* strain LB1016. The phylotype identified as *Bacillus cereus* showed the ability to tolerate up to 500 mg/L of $HgCl_2$, showing an alternative for the bioadsorption of this metal in the field. Key words: Bacteria, rhizosphere mercury, tolerance.

1. INTRODUCTION

In the environment, heavy metals, such as cadmium (Cd), lead (Pb), chromium (Cr) and mercury (Hg), are released by industrial and technological activities, generating a high impact on the environment. In particular, Hg is a highly toxic pollutant and is dispersion in soil and wáter threatens human and environmental health (Baldi et al., 2012; Parkash Dhankher et al., 2012).

As stated by (Gaiolí, et al., 2012) since the beginning of the industrial era, mercury (Hg) levels in the environment have increased considerably, reaching concentrations that affect ecosystems and human health. In its pure form, known as elemental mercury (Hg0), it volatilizes easily to form colorless, odorlessvapors. This metal, when it reaches nature and some time has elapsed, is transformed to methylmercury (CH3Hg+). Methylmercury, like other organometallic compounds, is fat-soluble, and therefore highly toxic, as it can easily pass through biological membranes, particularly the skin, and from here the metal is incorporated into the trophic chain (Posada and Arroyave 2006).

Soil contamination by mercury arises as a consequence of human activities, both industrial and agricultural, and the improper processing and disposal of mercury-containing waste. Worldwide, it has been confirmed that exposure to high concentrations of mercury causes adverse health effects, such as central nervous system, cardiovascular, immune and renal disorders (Holmes et al., 2009). There are different techniques used in the removal of metals when high concentrations are present, but when very low concentrations are present these techniques are not affordable and it is in these situations that the application of biological techniques is proposed as a removal alternative, which allows for efficient removal, in addition to the fact that it can be selective in terms of contaminating metals, with minimal impact on soil characteristics.

A large number of metal-tolerant or metal-accumulating micro-organisms can be found in nature and the study of these will allow the development of more effective biological remediation technologies. The organisms used in this technology can be bacteria and fungi (bioremediation), algae (phyco-remediation) or plants (phytoremediation). More recently, rhizoremediation has emerged as analternative technology involving the joint action of rhizospheric macroorganism and plant (Paisio et al., 2012).

Soil bacteria can influence plant growth and the mobility of nutrients, metals and contaminants in soil, and in recent years attention has focused on the interaction between the plant and the soil microbial community (Dixit et al., 2015). Understanding the complex interaction between metal-accumulating plants and rhizosphere-associated microorganisms has led to the use of new technologies such as microbial-assisted phytoremediation (Muehe et al., 2015). Plant growthpromoting rhizobacteria (PGPR) are a group of rhizosphere bacteria that can increase plant toleranceagainst heavy metal toxicity and promote plant growth.

Phytoremediation is now considered an effective, economical and environmentally friendly technology that has received worldwide acceptance due to its benefits as opposed to the traditional technology of accumulating heavy metals from soil. Such advantages are its low cost and negligible impact on humans and ecosystems (Glick, 2010; Sheng et al., 2008). The success of phytoremediation depends on the plant's ability to tolerate high concentrations of metals and produce large amounts of biomass (Ma et al., 2011). The efficiency of heavy metal phytoremediation is dependent on the performance of the plant and its ability to accumulate metal ions, the microorganisms associated with these plant species provide benefits to the plant as they can provide nutrients and reduce the harmful effects caused by heavy metals (Belimov et al., 2002; Ma et al., 2011).

The availability of heavy metals is a crucial factor for successful remediation. Microorganisms associated with plants bind metals in bioavailable form through methylation, altering soil pH, redox reactions, production and secretion of siderophores, organic acids and biosurfactants to make the phytoremediation process more efficient. As a strategy to respond to the problem of mercury contamination, it was proposed to isolate rhizosphere bacteria in areas close to the Santa Cruz Mine, Bolivar, and to evaluate the in vitro tolerance capacity to different concentrations of this metal.

2. MATERIALS AND METHODS

Study site. Soil samples of rhizosphere and plant species from Hg-contaminated soils were collected in areas near the Santa Cruz Mine, Bolivar, Colombia (8º 42‘and 8º 45’ N, 74º 10‘and 74º 14’ W). Sampling was carried out in the south of the department of Bolívar. At each selected site, a random zig-zag sampling was carried out to take soil and soil samples. Soil and rhizospheric soil and plant tissue samples were labelled with georeferencing of the sampling site. Part of these samples were sent to specialized laboratories for mercury concentration determination and the other part of the samples were taken to the microbiological research laboratory of the University of Sucre for the respective analyses (Perez et al., 2016 and 2018).

Determination of mercury levels in the rhizosphere. The determination of total mercury concentration in the rhizosphere was determined by cold vapors atomic absorption spectrophotometry technique, according to procedures described in Marrugo-Negrete et al. (2015).

Isolation of rhizosphere bacteria. For the isolation of rhizosphere bacteria, root soils of plants present in mercury-contaminated environments were taken, removing the soil adhering to the roots and washed with 100 mL of sterile distilled water, then shaken for 30 min. Once sedimented, an aliquot of the suspension was taken and serial dilutions were prepared from 104 to 108, and then inoculated in nutrient agar and incubated at 30±1°C for 72 h. All dilutions were performed in triplicate. Isolates were identified according to their characteristics and stored on nutrient agar slants at 4°C (Salgado et al., 2012).

Sensitivity tests to mercury concentrations. For isolation of rhizospheric bacteria, 10 g of root-attached soil was suspended in 100 mL of 0.85% normal saline, then the suspension was shaken for 30 min. The extraction solution was serially diluted and spread on Luria-Bertani (LB) plates. For endophytic bacteria, plant tissues were subjected to surface disinfection process and isolation of endophytic bacteria was performed on the surface of R2A agar medium (Pérez et al. 2016). Increasing concentrations of HgCl2 were added to each medium up to 250 mg/L and incubated at 37 °C for 7 d. Colonies that grew and morphologically different were re-isolated in pure cultures.

Evaluation of the growth of rhizospheric bacterial strains in the presence of mercury. In order to determine the capacity of the strain to tolérate higher concentrations of mercury, the growth of each strain was evaluated for three consecutive days at 100, 200, 300, 400 and 500 mg/L $HgCl_2$.

Bacterial growth was assessed by measuring the optical density at 595.0 nm in 96-well cell culture dishes for each of the selected strains, for a total of five dishes. For the experiment, the selected strain was reactivated by inoculating 500 µL from a cryopreservation tube into 10 mL of LB medium and incubated at 30°C for 24 hours at 150 rpm. Subsequently, 2.5 mL of the previously reactivated selected strain was taken and inoculated into 50 mL of LB medium. Immediately after inoculation, 1 mL was taken, placed in a cuvette and the optical density was measured at 600 nm in a spectrophotometer (Thermo Genesys 180). Measurements were continued every half hour until an OD of 0.8 was reached for the strain under study. Once the optical density was reached, these bacterial inoculate were used to proceed with the mounting and inoculation in the 96-well plates.

The first six columns of the plate were control (no bacterial inoculum) and were arranged as follows: The first column was filled with 200 µL of LB medium without Hg supplementation, the following columns were filled with 200 µL of LB medium supplemented with the different concentrations of Hg proposed and functioned as reading blanks. The remaining six columns were filled in the same order as mentioned for the media without inoculum, with a volume of 150 µL, which was completed with the addition of 50 µL of bacterial inoculum, with the aim that all morphotype would start growth with an absorbance of 0.2 (Bravo et al., 2018). The final volume of all plate wells was 200 µL and each treatment had a total of eight replicates. The five dishes were incubated at 30°C and 70 rpm. Optical density was measured on the iMarkTM Microplate Absorbance Reader spectrophotometer from time zero to verify the initial absorbance and measurements were continued every 90 minutes for three consecutive days.

Identification of rhizospheric bacteria tolerant to different mercury concentrations. Isolates of rhizospheric bacteria showing the highest tolerance to different mercury concentrations were used for molecular identification. Before starting the DNA extraction process, morphotype were differentiated by Gram staining. Genomic DNA extraction was performed according to the protocol described by (Oliveira et al., 2013). Amplification of 16S rDNA from endophytic bacterial communities was performed by PCR technique. Amplification of rDNA fragments was carried out with the use of specific oligonucleotides for eubacterial groups (Oliveira et al., 2013). PCR products were sent for sequencing to Macrogen (Seoul, South Korea) on an automated 3730XL capillary sequencer. The nucleotide sequence entities obtained were compared with those stored in databases of the National Center For Biotechnology Information (NCBI). Base alignment was performed by means of the Clustal W program and analysis and correlation with the MEGA 6® program. Phylogenetic inferences were obtained by distance and maximum parsimony Neighbor-joining with bootstrap test (1,000 replicates). Trees for phylogenetic analysis of the sequences were reconstructed with MEGA 6.0®.

3. RESULTS AND DISCUSSION

Determination of mercury in the rhizosphere. The mercury content in the root was 5.6 mg/L Hg. The normality criterion was corroborated using the Shapiro-Wilks test (p-value: 0.8480). ANOVA shows significant statistical differences (p-value<0.05) between mercury levels (mg/kg) per site. Tukey's test shows significant statistical differences (p-value<0.05) between mercury levels (mg/kg) at the sampled sites (figure 1). The lowest mercury concentration was found at site 1 with values of 2.57 mg/kg, while the highest values were reported for site 9 with 5.6 mg/kg.

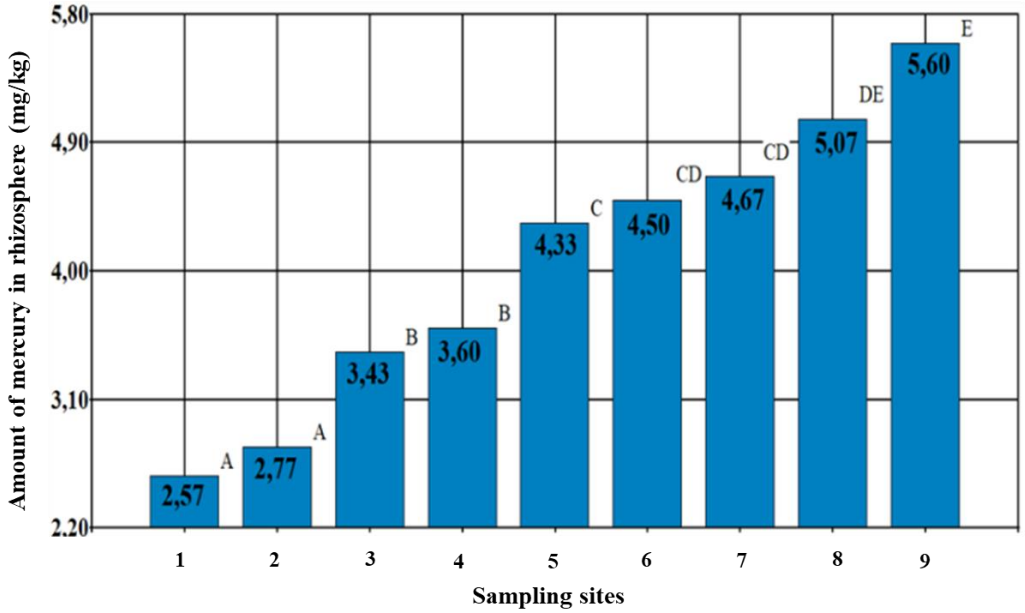

Figure 1. Tukey's test results for mercury levels in the rhizosphere.

The average mercury levels found in soil taken from the mercury-contaminated area near the Santa Cruz Mine, Sur de Bolivar, Colombia are 5.6 mg/kg rhizosphere (Figure 1), which is above the permissible values at which it can cause adverse effects on human health. Investigations on heavy metals in this critical area of the Colombian Caribbean have also shown the presence of heavy metals in sediments with mercury levels of 7.67 µg/g, which is above the permissible standard (0.5 µg/g). The maximum contamination levels permitted by the Ministry of Environment and Sustainable Development for the particular case of mercury (0.02 mg/L) are much higher (MADS, 2015) than the values permitted for Hg 0.00003 mg/L by the United States Environmental Protection Agency (USEPA) (Nguyen et al., 2013).

ANOVA shows significant statistical differences (p-value<0.05) between rhizospheric bacteria density per site. In the present study, the average range of rhizospheric bacteria was found to be 9.7x1010 ± 3.1 x1010 CFU/g soil (figure 2).

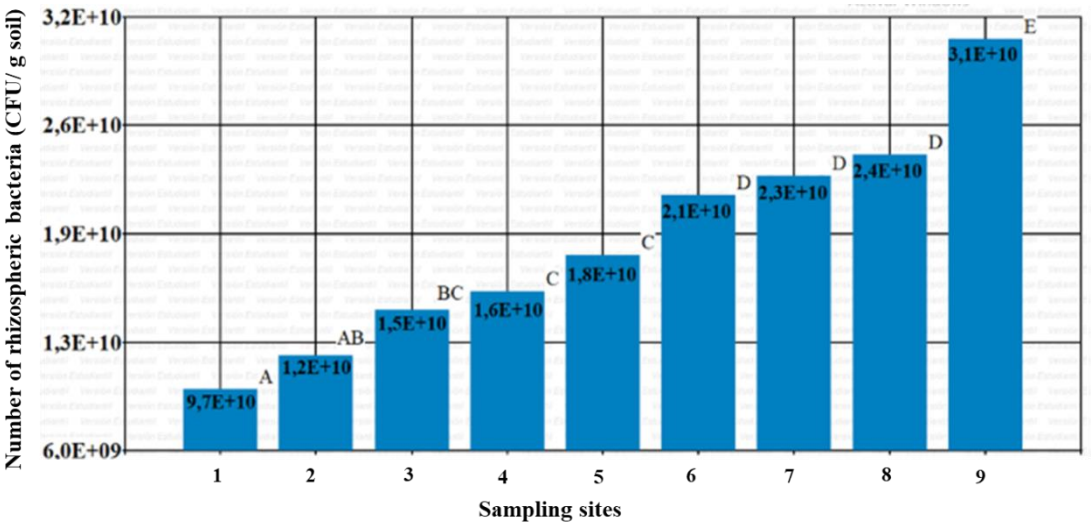

Figure 2. Tukey's test results for rhizospheric bacterial count.

Regarding mercury levels and bacterial population density per tissue (figure 2), higher densities of rhizospheric bacteria were observed at sites where mercury concentrations were lower. Although lower mercury concentrations were found in the roots compared to soil levels.

Evaluation of the sensitivity of rhizospheric bacteria to mercury. A DCA was carried out to evaluate the percentage of sensitivity to mercury of the bacterial morphotype isolated from plant tissues, indicating that there are significant statistical differences (p-value<0.05) between the susceptibility of the bacterial morphotype to the different concentrations of mercury, with the species Bacillus cereus presenting the highest percentage of tolerance to mercury with 85.4% and Bacillus mycoides with 79.1% respectively at concentrations of 500 mg/L. This was followed by Bacillus thuringiensis with 77.1% at 400 mg/L, Bacillus spp with 72.8% at 350 mg/L and finally Pseudomonas spp with 68.7% at 300 mg/L (figure 3).

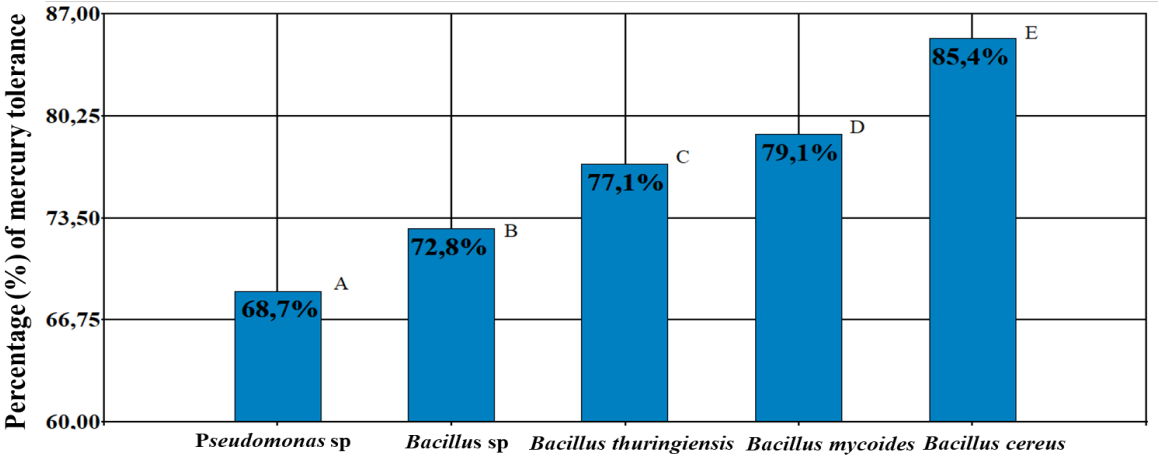

Figure 3. Percentage mercury tolerance test for rhizospheric bacteria.

Evaluation of the growth of rhizosphere bacterial strains in the presence of mercury. The strains with the highest percentage of tolerance to was inoculated in TLP at concentrations of 100; 200; 300; 350, 400, and 500 mg/L mg/L HgCl2. The evaluation of bacterial growth was carried out by measuring the optical density at 595.0 nm in 96-well cell culture dishes for the selected strain, a total of five dishes, with a total of five in Figure 4 shows the growth behaviours of the Bacillus cereus strain tolerance and tolerance by ELISA assay in the 96-well plates. The observed results show that Bacillus cereus grew up to 500 mg/L mercury solution in the form of $HgCl_2$.

Mercury is a highly reactive metal when it is in cationic form or bound to other compounds; biochemically it has an affinity for functional groups (e.g. sulphhydryl groups) present in enzymes that catalyze critical reactions in an organism, metal ions have been found to interact with cellular components such as DNA and proteins, causing damage and conformational changes that can alter the cell cycle (Tchounwou, et al, 2012).

Molecular identification of mercury-tolerant endophytic bacteria. The sequences of the isolates obtained by sequencing technique were compared with sequences present in the NBCI library. Phylogenetic analysis of 16S rDNA gene of endophytic bacteria with the ability to tolerate different concentrations of mercury showed similarity with Bacillus thuringiensis, Bacillus cereus, Bacillus sp and Pseudomona sp species (figure 5).

	(LBsin mercurio)	(LB + 100 mg/mLHgCl_2)	(LB + 200 mg/mLHgCl_2)	(LB + 300 mg/mLHgCl_2)	(LB+400 mg/mLHgCl_2)	(LB+500mg/mLHgCl_2)	(LB + Bacteria)	H (LB + Bacteria + 100 mg/mLHgCl_2)	I(LB + Bacteria + 200 mg/mLHgCl_2)	J (LB + Bacteria + 300 mg/mLHgCl_2)	K (LB + Bacteria + 400 mg/mLHgCl_2)	L(LB + Bacteria + 500 mg/mL$HgCl_2$)
1												
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Figure 4. Schematic of the treatments and their distribution in the 96-well ELISA plates. The name of each column indicates the treatment that was arranged in the eight wells that make up the column.

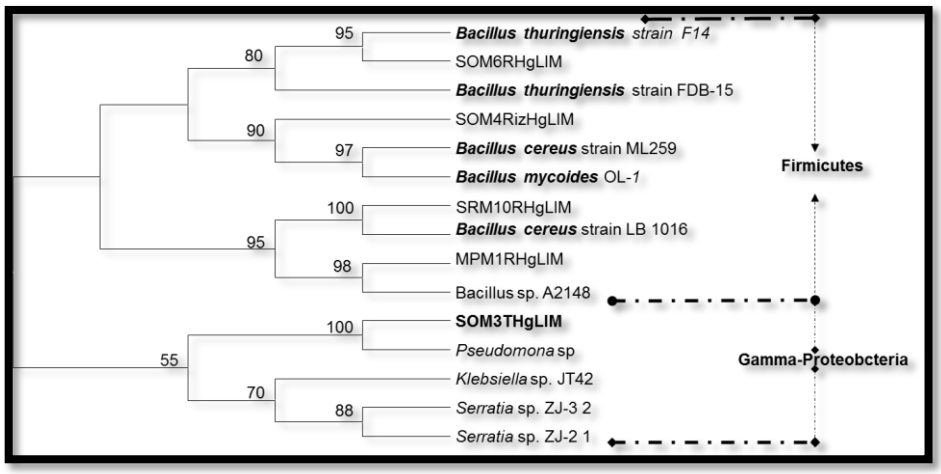

Figure 5. Maximum similarity phylogenetic tree derived from the analysis of 16S rDNA gene sequences of rhizosphere bacteria isolated from mercury-contaminated environments. At the base of each clade, we observe the branch support expressed as the percentage of times the analysis produced the same association between sequences.

Isolate SOM6RHgLIM has high homology with sequences from Bacillus thuringiensis strain F14; SOM4RizHgLIM with Bacillus cereus strain ML259 and Bacillus mycoides O-1; SRM10RHgLIM with Bacillus cereus strain LB1016; MPM1HgLIM with species belonging to the genus Bacillus sp and SOM3HgLIM with species belonging to the genus Pseudomonas sp.

Tolerance to heavy metals in bacteria has been extensively studied worldwide. Several bacterial species have been reported for metal resistance. Most of the bacterial species that claim to be possible candidates for heavy metal bioremediation belong to the genera Bacillus, Pseudomonas and Streptomyces (Uslu and Tanyol, 2006).

The genus Bacillus belongs to the family Bacilliaceae, a genus that today includes more than 60 species of bacilli. This genus consists of Gram-positive, endospore-forming, chemo-heterotrophic bacillary microorganisms that are normally motile and surrounded by periotic flagella. They are anaerobic or facultative aerobic, catalase positive. This genus is commonly found in soils and plants where they play an important role in the carbon and nitrogen cycle. They are common inhabitants of fresh and stagnant waters, and are particularly active in sediments (Koneman, 2001).

Molecular analysis by Denaturing Gradient Gel Electrophoresis (DGGE) identified bacteria belonging to the genera: Arthrobacter, Bacillus, Brevibacterium, Brochothrix, Comamonas, Cytophaga, Deinococcus, Enterobacter, Hafnia, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Rathayibacter, Rhodococcus, Salmonella, Serratia, Staphylococcus, Variovorax and Xanthomonas with the ability to tolerate heavy metals in vitro (Ellis et al, 2003).

It has been found that some metal resistance systems are encoded by chromosomal genes in some microorganisms, as is the case of Bacillus sp. that showed resistance against mercury. Kavaruma and Esposito (2010) conducted studies with Bacillus subtilis in order to study the genes that are expressed by exposure to metals, identifying that different types of affected genes were regulated by metallo-regulatory proteins known as Fur, MntR, Per R, ArsR and CueR.

Lin and Harichund (2011), studied the removal of heavy metals from chemical industry effluent and found that Bacillus sp. was able to remove As by 20.3% and Hg by 16.7% effectively from the effluent. Nanda et al. (2011a, b) reported different bacteria for the efficient removal of heavy metals in effluent from the pharmaceutical industry and found bacteria of the genus Bacillus sp. in the removal of Hg (45%) and Cu (62%). Pseudomonas sp. was able to remove 56% of Cd, 34% of As and 53% of Co.

Sinha et al. (2012), immobilized a Bacillus cereus strain on calcium alginate, with which they obtained high Hg(II) removal efficiencies through bioadsorption to the bacterial biomass (80% removal of initial 20 mg/L Hg(II), in 120 h), in both batch and continuous systems.

Highly mercury-resistant bacteria (*Bacillus thuringiensis*) have been isolated from coastal marine sediments in India and are able to tolerate up to 50 ppm mercury chloride (Dash et al., 2013). Furthermore, mercury-resistant aerobic bacteria were isolated from sediments of the mercury-contaminated area of the Tagus estuary in Portugal that were mostly Bacillus spp. and were able to tolerate up to 10 ppm Hg2+ and can reduce Hg^{2+} to Hg0 (Figueiredo et al., 2016). In addition, a strain of Bacillus thuringiensis PW-05 was isolated from the Odisha coast in India and was found to resist 50 ppm Hg (Dash et al., 2013).

Bacillus cereus, has been reported as an endophytic bacterium of plants of the genera Cyperus and Paspalum with the in vitro capacity to tolerate up to 400 ppm (400 mg/L) of mercury in the form of HgCl2 and also of rice plants with the capacity to tolerate up to 400 ppm of Pb in the form of Pb(NO3)2 and to produce siderophore (Perez et al., 2015; Perez et al., 2018).

On the other hand, Pseudomonas is the most heterogeneous and ecologically important group of known bacteria. Because the nutritional requirements of the species of this genus are very simple, representatives have been detected in virtually all natural habitats and tend to be predominant among bacteria associated with the rhizosphere of plants (Arora, 2015; De Oliveira et al., 2015). The role of Pseudomonas in bioremediation is a consequence of their environmental importance and their metabolic diversity thanks to their ability to degrade a wide range of organic compounds as demonstrated by many authors in their research where they have shown to be efficient in the bioaccumulation of heavy metals (Ramteke, 2000), this process has gained importance in recent years due to its good performance, low cost, specificity and easy reuse (Ahu¬ja et al., 2001).

Wagner-Döbler (2003), summarizes the results obtained after two years of work in a pilot plant built to treat effluent from a chlor-alkali electrolysis industry in the Czech Republic. In this work, a packed bed bioreactor with a capacity to treat 100 m3 of effluent per day (containing between 2 and 10 mg/L Hg) was used and operated continuously for 8 months with excellent results. This bioreactor was inoculated with a bacterial biofilm, consisting of seven strains of Pseudomonas immobilized on pumice, which has the capacity to reduce Hg(II). Optimization of this system involved testing various culture conditions, immobilization matrices, biofilm types and bioreactor operating conditions. Pepi et al. (2011) used biofilm-producing bacteria belonging to the genera Pseudomonas and Psychrobacter, both free and immobilized on a pumice matrix, to volatilize organic and inorganic Hg with high efficiency (up to 190 ng/mL in 5 min). Regarding the removal of MeHg.

Lee et al. (2012) described that Pseudomonas balearica reduced 97% of MeHg (20 μg/L) in 3 h while Cabral et al. (2012) indicated that Pseudomonas putida V1 volatilized 77% of MeHg (2.5 μM), in only 24 h. A recent contribution to the understanding of bacterial MeHg detoxification mechanisms was made by Adelaja and Keenan (2012), who reported for the first time that strains of *Pseudomonas fluorescens*, *Enterobacter cloacae*, Citrobacterbraakii and Alcaligenesfaecalis use MeHg as their sole carbon and energy source.

Deng and Wang (2012) reported that a bacterial strain of pseudomonas sp. isolated from marine sediments removed and accumulated more Hg2+ on the cell surface. The bio-capture of mercury was probably through functional groups attached to the bacterial cell wall, such as carboxyl, phosphate, hydroxyl, thio and pyridine groups that contributed to Hg2+ uptake, and carboxyl groups were the most important in this action.

4. CONCLUSIONS

The *Bacillus cereus* species presented the highest percentage of tolerance to mercury with 85.4% and *Bacillus mycoide*s with 79.1% respectively at concentrations of 500 mg/L. Followed by *Bacillus thuringiensis* with 77.1% at 400 mg/L, Bacillus spp with 72.8% at 350 mg/L and finally Pseudomonas spp with 68.7% at 300 mg/L. The biological systems evaluated to date have been efficient in remediating Hg, most have been studied in synthetic aqueous media. Therefore, it is necessary to expand studies concerning the application of these systems to remove Hg from industrial effluents, water sources, soils and sediments, which would contribute to reducing pollution of natural environments. There is a presence of bacteria in the rhizosphere and in the tissues of plant species in soils contaminated with a high concentration of mercury in the south of Bolívar that showed the ability to tolerate high concentrations of mercury in vitro and possibly accompanied by other studies could contribute to remediating environments contaminated with this metal.

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6. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis.Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. Allauthors have read and approved the manuscript.

7. CONFLICTOFINTEREST.Alltheauthorsofthemanuscriptdeclarethattheyhavenoconflictofinterest

8. REFERENCES

Ahemad, M. 2012. Implications of bacterial resistance against heavy metals in bioremediation: a review. *IIOABJ, 3*, 39-46.

Adelaja, O.A., Keenan, H.E. 2012. Tolerance of TBT-resistant bacteria isolates to methylmercury. *Res J Environ Sci*6(1):1-13.

Ahuja V, Vohra P, Kashyap D, Tewari R. 2001. Adsorption of heavy metals (Pb+2 and Cd+2). by free and immobilized biomass of *Acinetobacteranitratus*. 41:275- 279.

Arora, N.K. (2015). Plant Microbes Symbiosis: Plant Facets. India: Springer.

Baldi, F., Pepi, M., Filippelli, M. 1993. Methylmercury resistance in Desulfovibriodesulfuricans strains in relation to methylmercury degradation. ApplEnvironMicrobiol 59(8):2479-2485.

Benhamou, N., Gagné, S., Quéré, D.L., Dehbi, L. 2000. Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium *Serratiaplymuthica* on the protection against infection by *Pythium ultimum. Biochem. Cell Biol*., 90, 45-56.

Bravo,Daniel,Leon-Moreno,C.,Martínez,C.A., Varón-Ramírez,V.M., Araujo-Carrillo, G.A.,Vargas,R.,Quiroga-Mateus,R.,Zamora,A.,&Rodríguez,E.A.G.(2021). The first national survey of cadmium in cacao farm soil in Colombia. *Agronomy*, *11*(4), 1–18. https://doi.org/10.3390/agronomy11040761

Cabral, L., Giovanella, P., Gianello, C., Bento, F.M., Andreazza, R., Camargo, F.A. 2012. Isolation and characterization of bacteria from mercury contaminated sites in Rio Grande do Sul, Brazil, and assessment of methylmercury removal capability of a *Pseudomonas putida* V1 strain. *Biodegradation* doi 10.1007/s10532-012-9588-z, *In press*.

Cho, C., Yavuz-Corapcioglu, M., Park, S., Sung, K. 2008. Effects of Grasses on the Fate of VOCs in Contaminated Soil and Air. *Water, Air, & Soil Pollution, 187*, 243-250.

Compant, S., Clément, C., Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology Biochemical*, *42*, 669–78.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., AitBarka, E. 2005. Endophytic colonization of *Vitisvinifera* L. by a plant growth-promoting bacterium, *Burkholderia* sp. strain PsJN. *Applied Environmental Microbiology*, *71*, 1685–1693.

Cordy, P., Veiga, M., Salih, I., Al-Saadi, S., Console, S., García, O., Mesa, L., Velásquez-López, P. &Roeser, M. (2011). Mercury contamination from artisanal gold mining in Antioquia, Colombia: Thmoree world's highest per capita mercury pollution. *Science of the total environment, 410-411*, 154-160.

Delgadillo-López, A., González-Ramírez, C., Prieto-García, F., Villagómez-Ibarra, J., Acevedo-Sandoval, O. 2011. Fitorremediación: Una alternativa para eliminar la contaminación. *Tropical and Subtropical Agroecosystems, 14*, 597- 612.

Deng, X., Wang, P., 2012. Isolation of marine bacteria highly resistant to mercury and their bioaccumulation process. Bioresour. Technol. 121, 342–347

Díaz-Arriaga, F. 2014. Mercurio en la minería del oro: impacto en las fuentes hídricas destinadas para consumo humano. *Rev. salud pública*, *16*(6), 947-957.

De Oliveira, G. B., Favarin, L., Luchese, R. H., &McIntosh, D. (2015). Psychrotrophic bacteria in milk: How much do we really know?. Brazilian Journal of Microbiology, 46(2), 313–321.

Ellis R, Morgan P, Weightman A, Fry J. 2003. Cultivation-dependent and independent approaches for determining bacterial diversity in heavy metal-contaminated soil. ApplEnvironMicrobiol, 69:3223-3230.

Gaioli, M., Amoedo, D., González, D. 2012. Impacto del mercurio sobre la salud humana y el ambiente. *Arch Argent Pediatr.*, *110*(3), 259-264.

Gaona, X. 2004. El mercurio como contamiante global: Desarrollo de metodologías para su determinación en suelos contaminados y estrategias para la reducción de su liberación al medio ambiente. Tesis doctoral en química. Universidad autónoma de Barcelona, Departamento de química, España.

Gelsomino, A., Cacco, G. 2005. Composition shifts of bacterial groups in a solarized and amended soil as determined by denaturing gradient gel electrophoresis. *Soil Biology e Biochemistry*, *38*(1), 91-102.

Ghosh, M., Singh, S. P. 2005. A review on phytoremediation of heavy metals and utilization of its by-products. *Applied Ecology and Environmental Research, 3*, 1-18.

Gordon, S., Weber, R. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol.*, 26, 192-195.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. CRC Press, Boca Ratón (Estados Unidos). p.304-312.

Koneman.E.W 2001.Diagnostico microbiológico: Texto y atlas decolor. Quinta Edicion.Editorial Médica Panamericana. Buenos Aires.

Hardoim, P., Van Overbeek, L., Van Elsas, J. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology*. *16*(10), 463-471.

Heuer, H., Krsek, M., Baker, P., Smalla, K., Wellington, E. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol*., *63*(8), 3233-3241.

Lara, M.C., Villalba, A.M., Oviedo, Z.L.E. 2007. Bacterias fijadoras asimbióticas de nitrógeno de la zona agrícola de San Carlos, Córdoba, Colombia. *Rev. Colombiana de biotecnología*, *9*(2), 6-14.

Lee, S.E., Chung, J.W., Won, H.S., Lee, D.S., Lee, Y.W. 2012. Removal of methylmercury and tributyltin (TBT) using marine microorganisms. *Bull Environ ContamToxicol*88:239-244.

Lin, J., Harichund, C., 2011. Industrial effluent treatments using heavy-metal removing
bacterial bioflocculants. Water SA 37 (2), 265–270.

Lindberg, S., Wallschlager, D., Prestbo, E., Bloom, N., Price, J., Reinhart, D. 2011. Methylated mercury species in municipal waste landfill gas sampled in Florida, USA. *Atmospheric Environ.*, 35, 4011-4015.

Magos, L., Clarkson T.W. 2006. Overview of the clinical toxicity of mercury. *Ann Clin Biochem.*, *43*, 257-268.

Marmur, J. 1961. A procedure for isolation of deoxyribonucleic acid from microorganism. *Journal of Molecular Biology*, *3*, 208-218.

Maron, P., Ranjard, L., Mougel, C., Lemanceau, P. 2006. Metaproteomics: A new approach for studying functional microbial ecology. *MicrobialEcology*, *53*, 486-493.

Ministerio de Ambiente y Desarrollo Sostenible República de Colombia (MADS). 2015. Resolución 0631 de 2015 "Por la cual se establecen los parámetros y los valores límites máximos permisibles en vertimientos puntuales a cuerpos de aguas superficiales y a sistemas de alcantarillado público, y se dictan otras disposiciones. pp. 62.

Mousa, W.K., Raizada, M.N. 2015. Biodiversity of genes encoding anti-microbial traits within plants associated microbes. *Front Plant Sci.,6*, 231.

Nguyen, T.A.H., Ngo, H.H., Guo, W.S., Zhang, J., Liang, S., Yue, Q.Y, Li, Q., Nguyen, T.V. 2013. Applicability of agricultural waste and by-products for adsorptive removal of heavy metals from wastewater. *Bioresour. Technol., 148*, 574-585.

Olivero, J., Johnson, B. 2002. El lado gris de la minería de oro: La contaminación con mercurio en el norte de Colombia. Cartagena, Colombia. Alpha impresores.

Oliveira, M.; Santos, T.; Vale, H.; Delvaux, J.; Cordero, P.; Ferreira, A.; Miguel, P.; Totola, M.; Costa, M.; Moraes, C.; Borges, A. 2013. Endophytic microbial diversity in coffee cherries of Coffeaarabica from southeastern Brazil. Can. J. Microbiol. 59:221-30.

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J.L., Thonart, P. 2007. Surfactin and fengycinlipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol., 9*, 1084-1090.

Pacyna, E.G., Pacyna, J.M., Steenhuisen, F., Wilson, S. 2006. Global anthropogenic mercury emission inventory for 2000. *Atmospheric Environment, 40*, 4048-4063.

Padmavathiamma, P.K., Li, L.Y. 2007. Phytoremediation Technology: Hyperaccumulation Metals in Plants. *Water, Air, & Soil Pollution, 184*, 105-126.

Parkash Dhankher, O., Lafferty Doty, S., Meagher, R.B., Pilon-Smits, E. 2012. Biotechnological approaches for phytoremediation. In: Plant Biotechnology and Agriculture. Altman, A., Hasegawa, M.P. (Eds). Oxford: AcademicPress. pp.309-328.

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S. Sa, T. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiol. Res., 160*(2), 127-133.

Pepi M., Gaggi, C., Bernardini, E., Focardi, G., Lobianco, A., Ruta, M., Nicolardi, V., Volterrani, M., Gasperini, S., Trinchera, G., Renzi, P., Gabellini, M., Focardi, S.E. 2011. Mercury-resistant bacterial strains *Pseudomonas* and *Psychrobacter*spp. isolated from sediments of Orbetello Lagoon (Italy) and their possible use in bioremediation processes. *IntBiodetBiodeg*65(1):85-91.

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, october; 11(38): 1-6.https://doi.org/10.17485/ijst/2018/v11i38/ 131974, October 2018..

Pérez A, Martínez D, Barraza Z, Marrugo J. Endophytic bacteria associated to genus Cyperus and Paspalum in soils with mercury contamination. Rev. U.D.C.A Act. & Div. Cient. 2016 enero; 19(1): 67-76. http://www.scielo.org.co/pdf/rudca/v19n1/v19n1a08.pdf.

Pérez -Cordero, Alexander; Barraza-Roman, Zafiro; Martínez-Pacheco, Dalila. 2015. Identificación de bacterias endófitas resistentes a plomo, aisladas de plantas de arroz Agronomía Mesoamericana, 26(2): 267-276.

Plan de Ordenamiento Territorial. 2002. Diagnóstico del Municipio de Corozal, Departamento de Sucre. p 1-5.

Posada, M.I., Arroyave, M. 2006. Efectos del mercurio sobre algunas plantas acuáticas tropicales. *Revista Esc. Ing. Antioq.*, (6), 57-67.

Reiter, B., Sessitsch, A. 2006. Bacterial endophytes of the wildflower Crocus albi-florus analyzed by characterization of isolates and by a cultivation-independent approach. *Can. J. Microbiol.*,*52*,140–149.

Ramteke P. 2000. Biosorption of Nickel (II) by *Pseudomonas stutzeri*. J EnvironBiol, 21:219-221.

Salgado-Bernal, I., Durán-Domínguez, C., Cruz-Arias, M., Carballo-Valdés, M.E., Martínez-Sardiñas, A. 2012. Bacterias rizosféricas con potencialidades fisiológicas para eliminar materia orgánica de aguas residuales. *Rev. Int. Contam. Ambie.*,*28*(1), 17-26.

Sheng, X., Xia, J., Jiang, C., He, L., Qian, M. 2008. Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environmental Pollution*, *156*, 1164–1170.

Sinha, A., Pant K.K., Khare, S.K. 2012. Studies on mercury bioremediation by alginate immobilized mercury tolerant *Bacillus cereus* cells. *IntBiodetBiodeg*71:1-8.

Stone, J.K., Bscon, C.W., White, J.R. 2000. An overview of endophytic microbes: endophytism defined [J]. In: Becon CW, White Jr JF, editors. Microbial Endophytes. New York: Marcel Dekker; p. 3-29

Sun, Y., Cheng, Z., Glick, B.R. 2009. The presence of a 1-aminocyclopropane-1- carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PSJN. FEMS. *Microbiol. Lett.*, 296, 131-136.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. 2012. Heavy Metals Toxicity and the Environment. In Molecular, Clinical and Environmental Toxicology. 101: 133–164). http://doi.org/10.1007/978-3-7643-8340-4_6

Unidad de planeación minero energética (UPME). 2007. Producción más limpia en la minería del oro en Colombia mercurio, cianuro y otras sustancias. Bogotá. Scripto Impresores S.A.

Uslu, G., Tanyol, M., 2006. Equilibrium and thermodynamic parameters of single and
binary mixture biosorption of lead (II) and copper (II) ions onto Pseudomonas putida,
effect of temperature. J. Hazard. Mater. 135, 87–93.

Vallejo, A., Zapata, F. 1998-2001. Especies forrajeras. Medellín: Agrosoft Ltda., p. 1.

Verma, S.C., Ladha, J.K., Tripathi, A.K. 2001. Evaluation of plant growth promot-ing and colonization ability of endophytic diazotrophs from deep-water rice. *J. Biotechnology*,*91*, 127-141.

Viloria, H.J. 2002. Documento de trabajo sobre economía regional: La ganadería bovina en las llanuras del Caribe Colombiano. Banco de la República, Cartagena de Indias.

Wagner-Döbler, I. 2003. Pilot plant for bioremediation of mercury-containing industrial wastewater. *ApplMicrobiolBiotechnol*62:124-133.