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REMOVAL OF Pb AND As BY BACTERIA ISOLATED FROM SEDIMENTS OF LAS VÍRGENES DAM AND RÍO CONCHOS IN THE STATE OF CHIHUAHUA, MEXICO

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Abstract. Lead (Pb) and Arsenic (As) are elements that negatively affect all living organisms if present in the environment. These elements have been reported in the sediments of water supply basins in the State of Chihuahua, Mexico. The current study consisted of the isolation of bacteria and investigating their growth capacity and the potential removal of Pb and As. Thus, two Gram-positive bacteria resistant to Pb and two Gram-positive bacteria resistant to As were isolated. The phylogenetic analysis -based on the 16S rRNA gene- identified them as *Paenibacillus illinoisensis* (Pb114002), *Bacillus luti* (Pb214001), *Paenibacillus ginsengagri* (As115004), and *Bacillus freudenreichii* (As215002). *Paenibacillus illinoisensis* (Pb114002) showed a Pb removal percentage of 65.67% in the supernatant and *Bacillus luti* (Pb214001) displayed 6.57%. Regarding As, removal percentages in the supernatant shown were 11.96% by *Paenibacillus ginsengagri* (As115004) and 42.72% by *Bacillus freudenreichii* (As215002).
Keywords: *morphological characterization, 16S rRNA, heavy metal, metalloid, bioremediation*

Introduction

The Las Vírgenes dam belongs to the municipality of Rosales in the State of Chihuahua, located to the North-West of the municipalities of Meoqui and Delicias. This dam belongs to the sub-basin of the San Pedro River, which is located in the Municipality of Satevó, while the Río Conchos is located near the city of Ojinaga. Both are considered vital basins for the supply of hydric resources for the habitants who live near, as well as their use for economic activities in the region. However, some studies report the presence of heavy metal contamination at these sites (Holguín et al., 2006; Rubio et al., 2013), which affects water quality and consequently cause health and environmental problems. Among the contaminants present in the water, particularly those used for human consumption and food production, arsenic (As) occupies an important place due to the impact on health due to its intake, which has caused the spread of chronic regional endemic hydroarsenicism (HACRE), a chronic disease that can evolve into more serious pathologies such as different types of cancer, the maximum permissible limit of As recommended by World Health Organization (OMS) in drinking water is 10 µg/L (Medina-Pizzali et al., 2018; Olmos and Ridolfi, 2018). Likewise, lead is highly toxic and can accumulate in living organisms because it does not have a defined biological function

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and is regarded as biologically nonessential (Ali et al., 2019), but it has adverse effects on different essential biochemical processes, and it is toxic at even low levels of exposure (Rodríguez-Rey et al., 2016). Environments contaminated by heavy metals and metalloids tend to be ideal niches for other organisms which can develop in extreme conditions, these organisms are called extremophiles (Jorquera et al., 2019), as well as microorganisms that grow in high concentrations of metals, called metal resistant. Bacteria that are tolerant to heavy metals are capable of accumulating them intracellularly and the presence of metal-binding proteins internally in the cell is associated with the reduction of their toxicity (Suárez and Reyes, 2002). Thus, the use of metalophilic microorganisms is of great importance and scientific interest, in addition to the fact that they can be applied in environmental biotechnology processes for the removal of metals (Orji et al., 2021). The interaction between microorganisms and metals or metalloids has environmental importance, metalophilic bacteria can play a very important role in the biogeochemical cycle of heavy metals, as well as the remediation of contaminated environments by heavy metals or metalloids (Hu et al., 2021). Currently, environmental biotechnology is used to decontaminate or alleviate contamination, therefore, the objective of this study was to evaluate the potential of metalophilic bacteria in the removal of lead (Pb) and arsenic (As), isolated from sediments of bodies of water in the State of Chihuahua, Mexico.

Materials and methods

Microbial isolation and morphological characterization

The sediment sampling of the Las Vírgenes dam and the Conchos river was carried out at the coordinates 28°09'58.82" N 105°37'43.95" W and 29°32′41.59" N 104°28′35.66" W, respectively. The sediment samples were taken in the summer of 2018. Fifteen samples were collected in sterile 50 mL Falcon tubes, which were stored in a cooler at 4˚C and transported to the laboratory for analysis (Hernández-Peña et al., 2021). Subsequently, 10 g of sediment was weighed and incubated at 37°C for 21 days at 200 rpm in nutrient broth (DIFCO) supplemented with lead nitrate [$Pb(NO_3)_2$] or arsenic trioxide (As_2O_3) for a concentration of 10 mg/L of Pb or As. Samples of the culture medium were taken after 0, 3, 7, 14, and 21 days of incubation. The collected samples were cultured in Petri dishes with nutrient agar enriched with 10 mg/L of Pb or As, for which serial dilutions (10^6) were carried out. Morphologically different colonies were selected for isolation on Petri dishes containing the culture medium indicated above, by using Scottish stria. The scanning electron microscope (SEM) (Hitachi SU5000) was used to determine cell morphology; for this, bacteria cells with a growth time of 48 hours were collected, washed, fixed with 2% formaldehyde, and dried by dehydration by applying acetone and ethyl alcohol (Soto-Padilla et al., 2018).

Molecular identification of isolated bacteria

The DNA extraction of the isolated strains was carried out with the phenol-chloroform-isoamyl alcohol method (Guo et al., 1997). The 16S rRNA amplification, sequencing, and bioinformatic analysis were carried out as reported by Soto-Padilla et al. (2018). For the amplification protocol, the universal primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'TACGGYTACCTTGTTACGACTT-3') were used for fragments of 1500 bp, Master mix (PROMEGA) was used with a final volume of 50 µL, the purification was

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carried out using UltraClean® 15DNA Purification Kit. The sequencing was carried out by the National Laboratory of Genomics for Biodiversity (LANGEBIO) using the Sanger platform. The bioinformatic analysis of the sequences obtained from the bacterial strains was carried out by Finch TV, CLUSTALX 2.1, SEAVIEW, and MEGA 7.0 (Hernández-Peña et al., 2021). Phylogenetic trees were constructed using the Neighbor-joining method; and the Tamura-Nei model of distance analysis and 500 Bootstrap replications were assessed to support internal branches (Soto-Padilla et al., 2018).

Bacterial resistance to Pb or As

This test was carried out to evaluate the minimum inhibitory concentration (MIC), which the strains tolerate lead and arsenic, the test was carried out in Petri dishes with nutritive agar (DIFCO) enriched with 100, 200, 400, 600, 800, 1000, and 1200 mg of Pb/L or As/L. Petri dishes were prepared in triplicate for each concentration and bacterial strain; in addition, uninoculated control was used for all concentrations (Pellizzari et al., 2015). The Petri dishes were incubated at 37°C and growth was observed at 72 h.

Bacterial growth kinetics in presence of Pb or As

The culture medium (nutrient broth added with Pb or As at a concentration of 100 mg/L) was inoculated with 10% (v/v) of each isolated bacteria and incubated in Erlenmeyer flasks. The inoculated flasks were incubated on an orbital shaker (222DS-LABNET) at 37°C at 200 rpm. The experiments were carried out in triplicate. For the evaluation of bacterial growth, 3 mL aliquots of the culture medium were taken at intervals of 2 h. Bacterial growth was estimated by turbidimetry at a wavelength of 600 nm in the UV-Vis spectrophotometer (Lambda 2) (Thacker et al., 2007).

Pb or As removal kinetics

The removal of Pb or As was evaluated by taking 3 mL aliquots of the culture medium at 2 h intervals, the samples were centrifuged at 3000 rpm for 15 minutes. The concentration of Pb or As was determined using the atomic absorption (Perkin-Elmer, Canada) spectrometry method (Hernández-Garcia et al., 2008). All experiments were conducted in triplicate.

Results and discussion

Microbial isolation and morphological characterization

Four bacterial strains were isolated under the presence of Pb or As from the sampling sites located in the State of Chihuahua. Both Pb and As are elements that affect human health and also cause an imbalance in the trophic chain of ecosystems (Nava-Ruíz and Méndez-Armenta, 2011). Strains Pb114002 and Pb214001 were isolated from both sampling sites in the presence of Pb (10 mg/L), as well as strains As115004 and As215002 in the presence of As (10 mg/L). Strains Pb114002 and Pb214001 showed a difference in growth in circular and massive forms, respectively; in the same way, they differ in pigmentation, strain Pb114002 showed yellow colonies, and strain Pb214004 showed white colonies. Regarding the colonies of strains with the ability to grow under As, differences between strain As115004 (beige) and As215002 (white). Based on the microscopic morphology (*Figure 1*) using the scanning electron microscope (SEM), cells with the shape of a bacillus (Pb114002) and coccobacillus (Pb214001) were observed,

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which have an average size of 1.5 µm and 1.0 µm, respectively. The strains that grew in As showed a bacillary form, with an average size of 1.4 µm. For the Gram stain, the four strains are identified as Gram-positive bacteria. The presence of heavy metals and metalloids in sediments of reservoirs in the state of Chihuahua, Mexico has been reported (Hernández-Garcia et al., 2008). Pb concentrations ranged from 58 to 94 mg/Kg in sediments, exceeding the recommended limit of 50 mg/Kg; the metalloid As was presented in concentrations around 17 mg/Kg of sediment, coinciding with different studies that show the presence of these elements in the riparian zones of the Conchos River (Holguín et al., 2006; Hernández-Garcia et al., 2008). Various studies have been carried out regarding the isolation of bacteria resistant to Pb or As in different environmental sites, and detected a low number of bacterial isolates (Chatterjee et al., 2012; Pandey and Bhatt, 2015; Dey et al., 2016; Kalita and Joshi, 2017; Uqab et al., 2020), coinciding with our research which shows that there is not a high presence of microorganisms living to on the presence of Pb and As. The morphological characterization observed by the isolated bacterial strains is shown in *Figure 1*, it is observed that Gram-positive bacteria predominate in the form of short bacilli that range from 1 to 1.5 µm.

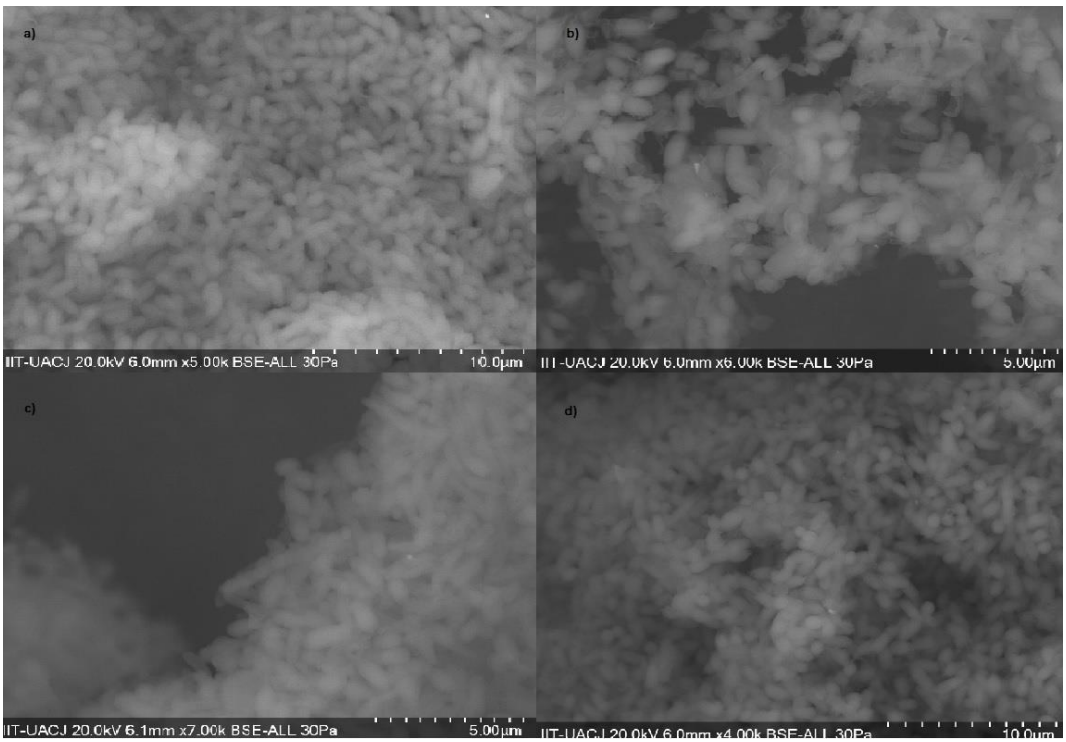

Figure 1. *A high-resolution image of bacterial colonies of the strain taken under a scanning electron microscope (SEM), a) Pb114002, b) Pb214001, c) As115004, d) As215002*

Molecular identification of isolated bacteria

Based on sequencing the 16S rRNA gene, the isolated bacteria strains tolerant to Pb (Pb114002 and Pb214001) were taxonomically affiliated to the species *Paenibacillus illinoisensis* (MW363241.1) and *Bacillus luti* (NR_157730.1), respectively (*Figures 2 and 3*). Bacterial strains tolerant to As (As115004 and As215002) were identified as belonging to the species *Paenibacillus ginsengagri* (MH491058.1) and *Bacillus*

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freudenreichii (KT291163.1), respectively. The analysis of the percentage of similarity showed values between 97.5% and 98.4% with the reference strains. Most of the reports of isolation and specie identification in soils, sediments, and waters with high concentrations of heavy metals and metalloids belong to the genera *Staphylococcus, Bacillus, Micrococcus, Achromobacter, Pseudomonas*, and *Klebsiella* (Carrillo-Pérez et al., 2004; Cervantes et al., 2006; Murthy et al., 2012; Oves et al., 2013; Dey et al., 2016; Kalita and Joshi, 2017; Uqab et al., 2020). Based on the phylogenetic analysis (16S rRNA) of these bacterial strains showed the presence of two genera, *Bacillus* and *Paenibacillus*, which correspond to two families Bacillaceae and Paenibacillaceae, these families belong to a Bacillales order and a Bacilli class. Research works related to the genus *Paenibacillus* have been reported from soil and water samples, but none of them in the presence of Pb or As. Mead et al. (2012) report that *Paenibacillus lautus* was isolated from Yellowstone National Park, and report that its presence in environments similar to our sampling site. Doukyu et al. (2003) report the isolation of *Paenibacillus illinoisensis* from soil samples in the Kanton area of Japan. Regarding the presence of the genus *Bacillus*, they have been identified in sites with both Pb and As (Murthy et al., 2012; Oves et al., 2013; Dey et al., 2016; Satyapal et al., 2016; Uqab et al., 2020), most of them from soils and sediments with contamination by heavy metals and metalloids, as the sampling sites of the present study.

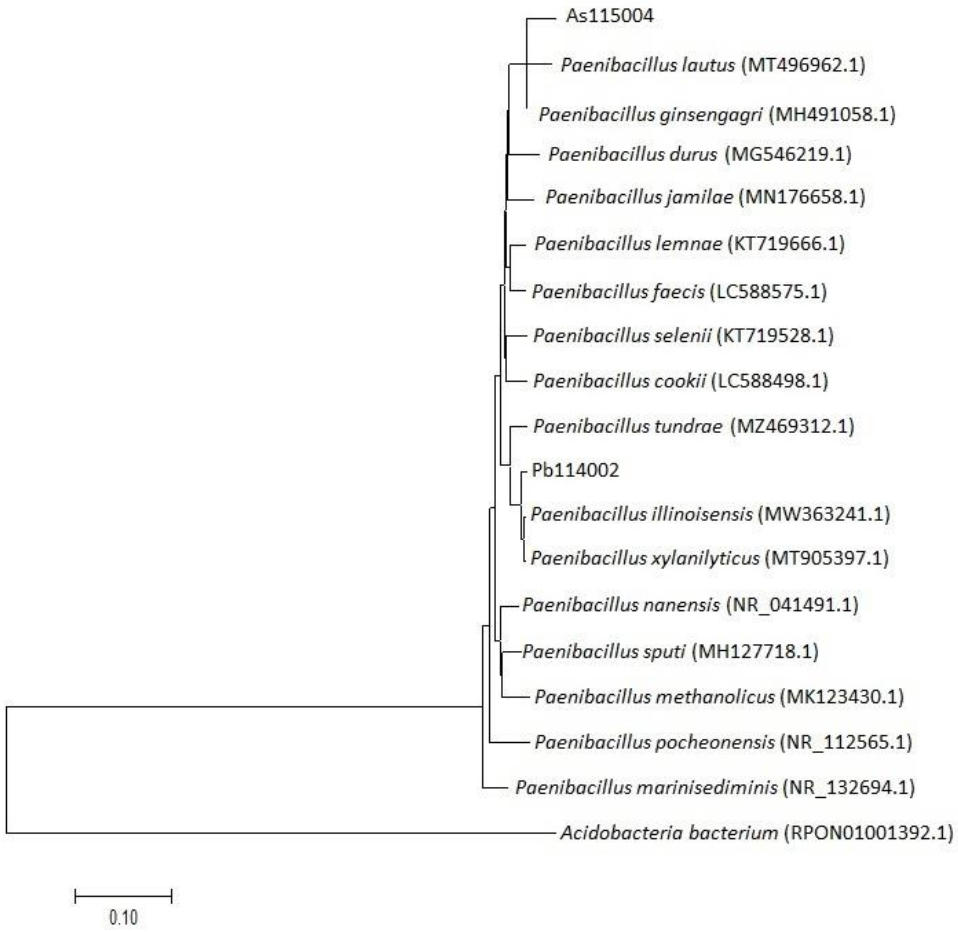

Figure 2. *Phylogenetic tree of the strains Pb114002 and As115004 with respect to the species of Paenibacillus conducted with the Neighbor Joining method, the accession number registered on the NCBI for the species utilized in the analysis is shown in parenthesis*

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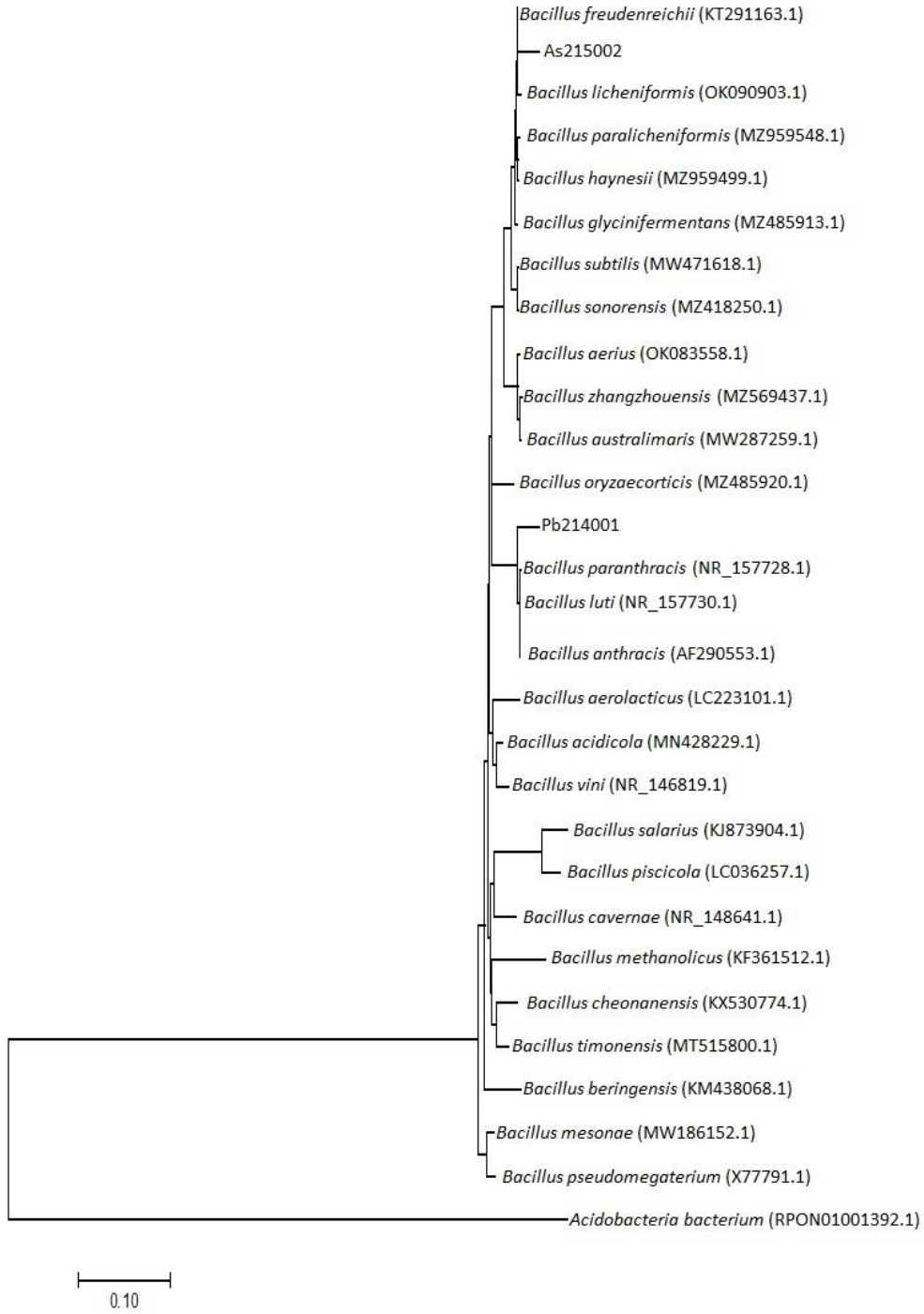

Figure 3. *Phylogenetic tree of the strains Pb214001 and As215002 concerning the species of Bacillus conducted with the Neighbor-Joining method, the accession number registered on the NCBI for the species utilized in the analysis is shown in parenthesis*

Bacterial resistance to Pb or As

The evaluation of the minimum inhibitory concentration (MIC) Pb or As of the identified bacterial strains is observed in *Table 1*. The strain *Paenibacillus illinoisensis* (Pb114002) showed resistance to 400 mg/L Pb and *Bacillus luti* (Pb214001) showed a

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MIC of 800 mg/L Pb. For the bacterial strains resistant to As, *Paenibacillus ginsengagri* (As115004) showed a MIC of 1000 mg/L As, and the strain *Bacillus freudenreichii* (As215002) showed a MIC of 400 mg/L As. Regarding the MIC for the studied strains, it can be observed that strains isolated in the presence of Pb coincide with the values shown by the bacterium of the genus *Paenibacillus* reported by Govarthanan et al. (2016), where a MIC of 400 mg/L was observed; regarding the MIC values for *Bacillus* strains in the presence of Pb have ranged between 125 to 1700 mg/L of Pb (Murthy et al., 2012; Oves et al., 2013; Uqab et al., 2020), the obtained value for the bacterial strain of this study was of 800 mg/L. Govarthanan et al. (2016) report the effect on the growth kinetics of bacteria of the genus *Paenibacillus* in the presence of As, showing a MIC of 400 mg/L, a value below the MIC obtained in our study for the strain of the genus *Paenibacillus* in the presence for this metalloid. The reports of the species of the genus *Bacillus* show MIC values between 550 and 750 mg/L (Pandey and Bhatt, 2015; Dey et al., 2016), without coinciding with the MIC values presented by the bacterial strain identified in this research, which presented a value of 400 mg/L.

Table 1. *The minimum inhibitory concentration of the bacterial isolated after 72 h of incubation*

Bacterial strain	Metal concentration (mg/L)						
	100	200	400	600	800	1000	1200
Paenibacillus illinoisensis	+	+	+	-	-	-	-
Bacillus luti	+	+	+	+	+	-	-
Paenibacillus ginsengagri	+	+	+	+	+	+	-
Bacillus freudenreichii	+	+	+	-	-	-	-

+ Microorganism growth, - Absence of microorganisms

Bacterial growth kinetics in presence of Pb or As

The average values of the triplicates of the microbial growth curve for the strains ANPb114002 and ANPb214001 at a concentration of 100 mg/L Pb is shown in *Figure 4*, and the average values of the microbial growth curve corresponding to the strains As115004 and As215002 is shown in *Figure 5*. The growth kinetics of the four strains analyzed showed an average adaptation time from 3 to 4 hours, the maximum absorbance value is presented at 12 hours of incubation. Jarosławiecka and Piotrowska-Seget (2014), attribute various mechanisms for the resistance of microorganisms to Pb that involve adsorption by extracellular polysaccharides, and regulation through protein expression. For their part, Pandey and Bhatt (2015) mention that the microbial capacity to grow in high concentrations of arsenic is favored by a variety of specific mechanisms that include cell accumulation, surface sorption, biotransformation, and precipitation by oxidation/reduction reaction. When performing the growth kinetic analysis in the presence of Pb and As we can see that the maximum absorbance value is shown at 12 hours of incubation. Govarthanan et al. (2016) report the growth kinetics for the bacterium *Paenibacillus* sp. as maximum absorbance values for both Pb and As in a time of 24 hours.

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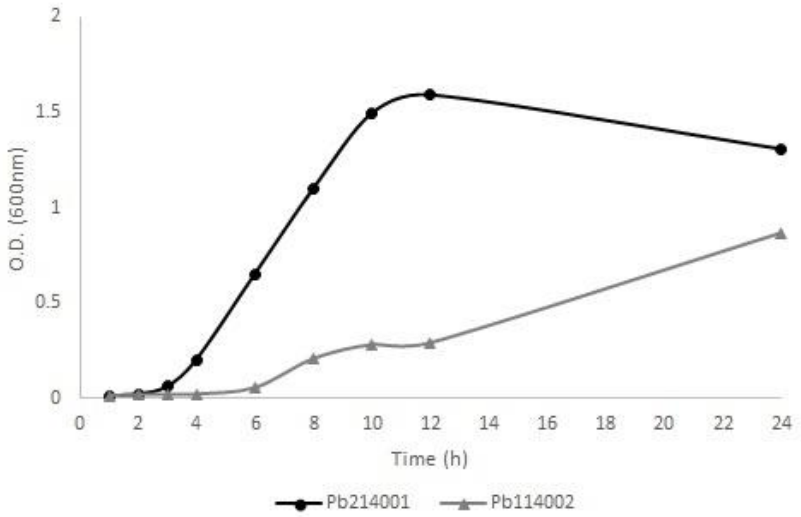

Figure 4. *Kinetic cellular growth of the strains Pb114002 and Pb214001*

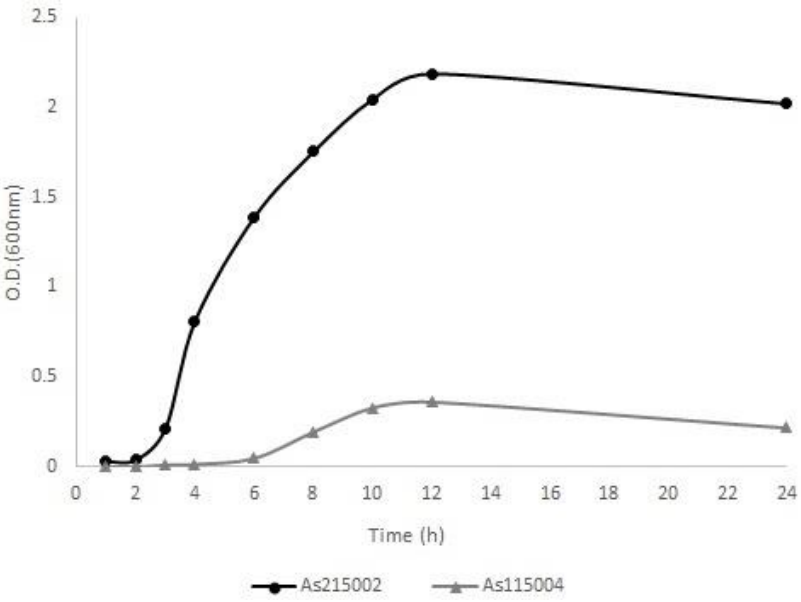

Figure 5. *Kinetic cellular growth of the strains As115004 and As215002*

Pb or As removal kinetics

The kinetics of removal of Pb (Pb114002 and Pb214001) showed that strains exposed to an initial concentration of this metal (100 mg/L) were able to remove 65.67% of Pb in a time of 24 hours (*Figure 6*). On the other hand, strains (As115004 and As215002) exposed to an initial concentration of 100 mg/L As showed removal of 42.72% in 24 hours (*Figure 7*). *Figures 6 and 7* show the average values of the triplicates. Thus, according to data obtained from the metal and metalloid removal kinetics, *Table 2* shows the estimated removal percentages for the *Paenibacillus* and *Bacillus* strains of the Las Vírgenes Dam and the Conchos River of the State of Chihuahua. Mexico.

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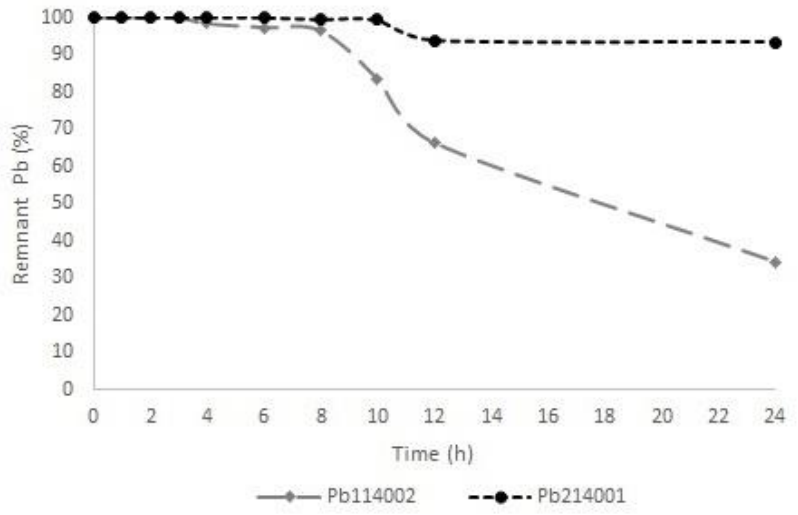

Figure 6. *Kinetics of the removal of Pb to 100 mg/L by the strains Pb114002 and Pb214001*

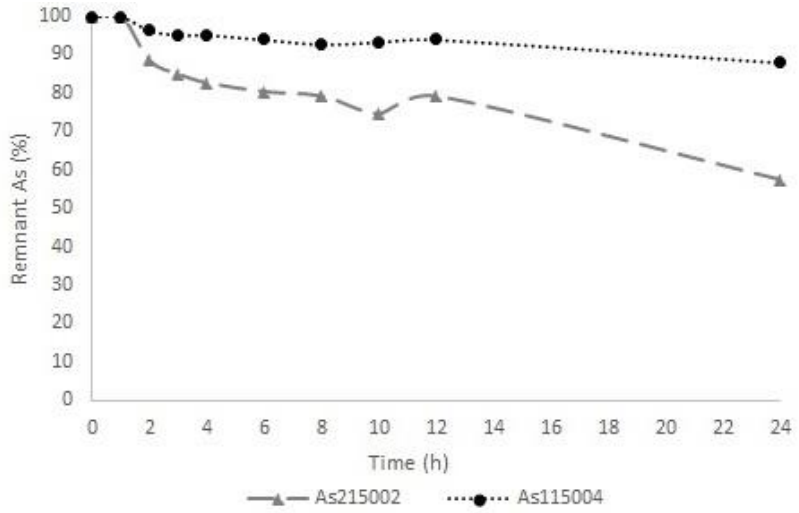

Figure 7. *Kinetics of the removal of As to 100 mg/L by the strains As115004 and As215002*

Table 2. *Removal of Pb and As from isolated bacteria*

Bacterial strain	Remotion (%)
Paenibacillus illinoisensis	65.67
Bacillus luti	6.57
Paenibacillus ginsengagri	11.96
Bacillus freudenreichii	42.72

Various microorganisms have been applied for the bioremediation of different heavy metals and metalloids, these microorganisms are identified within the group of otorhinophilic microorganisms classified as metalophiles due to their tolerance to heavy metal concentrations. A variety of mechanisms allow them to tolerate high concentrations of these compounds (Chien and Han, 2010; Oliart-Ros et al., 2016). In the bioremediation

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processes of Pb and As, various mechanisms such as biosorption, bioaccumulation, and biotransformation have been studied both by bacteria of the genus *Bacillus* and *Paenibacillus*. Chatterjee et al. (2012) mention that in bioremediation processes, microorganisms decrease the bioavailability of heavy metals in such a way that they provide an alternative to detoxify the pollutant in the environment, in the evaluation carried out shows a Pb removal of 81% at 72 h. Thus, Govarthanan et al. (2016) report removal of 40% at 48 h using a bacterium of the genus *Paenibacillus*. The bioadsorption process is characterized by non-specific binding from metal ions to polysaccharides and extracellular proteins (Satyapal et al., 2016), the Pb biosorption process was evaluated by Oves et al. (2013) demonstrating with their results the application of *Bacillus thuringiensis* in the biosorption process of different heavy metals. Similarly, Murthy et al. (2012) reported the biosorption of Pb using the bacterium *Bacillus cereus* with a reduction of the concentration in the medium of 90% in a time of 48 h. The bioaccumulation mechanism is the entry of heavy metals through bacterial membranes, including ion pumps, ion channels, carrier-mediated transport, endocytosis, and lipid permeation (Satyapal et al., 2016). Uqab et al. (2020) report the sequestration of 65% of Pb by the bacterium *Bacillus thuringiensis*, a study similar to the accumulation reported by Pandey and Bhatt (2015), but in their case, it is the accumulation of As by *Bacillus* sp. reporting values of 60%. Bacteria have been reported to have arsenic redox potential, as well as the production of genes and enzymes involved in the transformation of arsenic (Satyapal et al., 2016; Suhadolnik et al., 2017). Dey et al. (2016) report a 51.45% removal of As applied to a bacterium isolated from groundwater in India and belonging to the genus *Bacillus*. On the other hand, Govarthanan et al. (2016) report removal of around 30% at 24 h using the bacterium *Paenibacillus* sp. isolated from the roots of *Tridax procumbens*. The results obtained in our investigation regarding the removal of Pb and As, shown in table 2, exceed the values shown by *Paenibacillus* reported by Govarthanan et al. (2016) for removal of Pb by *Paenibacillus illinoisensis*, but not for removal As by *Paenibacillus ginsengagri*.

Conclusion

Based on the results of this research the presence of microorganisms with resistance to contaminating metals in the sediments sampled from water bodies was demonstrated. Similarly, the genus *Paenibacillus* and *Bacillus* were identified within these microorganisms. The research also showed that a noticeable remotion of metal occurred after 24 hours of bioremediation up to 50% in the case of Pb. Based on the promising results obtained in our work, further research needs to be developed to understand the biochemical and/or molecular mechanisms used by the strains to design bioproducts for Pb and As remediation *in situ*.

REFERENCES

[1] Ali, H., Khan, E., Ilahi, I. (2019): Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. – Journal of chemistry 4: 1-14. https://doi.org/10.1155/2019/6730305.

[2] Carrillo-Pérez, E., Ruiz-Manríquez, A., Yeomans-Reina, H. (2004): Aislamiento, identificación y evaluación de un cultivo mixto de microorganismos con capacidad para

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DOI: http://dx.doi.org/10.15666/aeer/2003_28172829

degradar DDT. – Revista Internacional de Contaminación Ambiental 20: 69-75. https://www.redalyc.org/articulo.oa?id=37000203.

[3] Cervantes, C., Espino-Saldaña, A. E., Acevedo-Aguilar, F., León-Rodríguez, I. L., Rivera-Cano, M. E., Avila-Rodríguez, M., Wróbel-Kaczmarczyk, K., Wróbel-Zasada, K., Gutiérrez-Corona, J. F., Rodríguez-Zavala, J. S., Moreno-Sánchez, R. (2006): Interacciones microbianas con metales pesados. – Revista Latinoamericana de Microbiología 48: 203-210. https://www.medigraphic.com/pdfs/lamicro/mi-2006/mi062v.pdf?q=metales.

[4] Chatterjee, S., Mukherjee, A., Sarkar, A., Roy, P. (2012): Bioremediation of lead by lead-resistant microorganisms, isolated from industrial simple. – Advances in Bioscience and Biotechnology 3: 290-295. http://dx.doi.org/10.4236/abb.2012.33041.

[5] Chien, C. C., Han, C. T. (2010): Tellurite Resistance and Reduction by a *Paenibacillus* sp. Isolated from Heavy Metal-Contaminated Sediment. – Environmental Toxicology and Chemistry 28: 1627-1632. https://doi.org/10.1897/08-521.1.

[6] Dey, U., Chatterjee, S., Mondal, N. K. (2016): Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation. – Biotechnology Reports 10: 1-7. http://dx.doi.org/10.1016/j.btre.2016.02.002.

[7] Doukyu, N., Kuwahara, H., Aono, R. (2003): Isolation of *Paenibacillus illinoisensis* that produces cyclodextrin glucanotransferase resistant to organic solvents. – Bioscience, Biotechnology, and Biochemistry 67: 334-340. https://doi.org/10.1271/bbb.67.334.

[8] Govarthanan, M., Mythili, R., Selvankumar, T., Kamala-Kannan, S., Rajasekar, A., Chang, Y. C. (2016): Bioremediation of heavy metals using an endophytic bacterium *Paenibacillus* sp. RM isolated from the roots of Tridax procumbens. – Biotechnology 6: 1-7. https://doi.org/10.1007/s13205-016-0560-1.

[9] Guo, C., Sun, W., Harsh, J. B., Ogram, A. (1997): Hybridization Analysis of Microbial DNA from Fuel Oil–Contaminated and Noncontaminated Soil. – Microbial Ecology 34: 178-187. https://doi.org/10.1007/s002489900047.

[10] Hernández-Garcia, Y., Sosa-Cerecedo, M., Moreno, M., Alcalá, J., Puga, S. (2008): Evaluación de la contaminación por metales pesados y arsénico en sedimento en embalses del estado de Chihuahua, México. – Revista Latinoamericana de Recursos Naturales 4: 89-94. https://www.itson.mx/publicaciones/rlrn/Documents/v4-n2-7-evaluacion-de-la-contaminacion-por-metales-pesados.pdf.

[11] Hernández-Peña, C. C., Lares-Villa, F., Santos-Villalobos, S. D. L., Estrada-Alvarado, M. I., Cruz-Soto, A., Flores-Tavizón, E., Soto-Padilla, M. Y. (2021): Reduction in concentration of chromium (VI) by *Lysinibacillus macroides* isolated from sediments of the Chapala Lake, Mexico. – Anais da Academia Brasileira de Ciências 93: 1-11. https://doi.org/10.1590/0001-3765202120190144.

[12] Holguín, C., Rubio, A. H., Olave, M. E., Saucedo, R., Gutiérrez, M., Bautista, R. (2006): Calidad del agua del Río Conchos en la región de Ojinaga, Chihuahua: parámetros fisicoquímicos, metales y metaloides. – Universidad y Ciencia 22: 51-63. https://www.redalyc.org/articulo.oa?id=15402204.

[13] Hu, X., Wang, J., Lv, Y., Liu, X., Zhong, J., Cui, X., Zhang, M., Ma, D., Yan, X., Zhu, X. (2021): Effects of heavy metals/metalloids and soil properties on microbial communities in farmland in the vicinity of a metals smelter. – Frontiers in Microbiology 12: 1-13. https://doi.org/10.3389/fmicb.2021.707786.

[14] Jarosławiecka, A., Piotrowska-Seget, Z. (2014): Lead resistance in microorganisms. – Microbiology 60: 12-25. https://doi.org/10.1099/mic.0.070284-0.

[15] Jorquera, M. A., Graether, S. P., Maruyama, F. (2019): Editorial: Bioprospecting and Biotechnology of Extremophiles. – Frontiers in Bioengineering and Biotechnology 7: 204. https://doi.org/10.3389/fbioe.2019.00204.

[16] Kalita, D., Joshi, S. R. (2017): Study on bioremediation of Lead by exopolysaccharide producing metallophilic bacterium isolated from extreme hábitat. – Biotechnology Reports 16: 48-57. https://doi.org/10.1016/j.btre.2017.11.003.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28172829

[17] Mead, D. A., Lucas, S., Copeland, A., Lapidus, A., Cheng, J. F., Bruce, D. C., Goodwin, L. A., Pitluck, S., Chertkov, O., Zhang, X., Detter, J. C., Han, C. S., Tapia, R., Land, M., Hauser, L. J., Chang, Y. J., Kyrpides, N. C., Ivanova, N. N., Ovchinnikova, G., Woyke, T. (2012): Complete genome sequence of *Paenibacillus* strain Y4. 12MC10, a novel *Paenibacillus lautus* strain isolated from Obsidian Hot Spring in Yellowstone National Park. – Standards in Genomic Sciences 6: 366-385. https://doi.org/10.4056/sigs.2605792.

[18] Medina-Pizzali, M., Robles, P., Mendoza, M., Torres, C. (2018): Ingesta de arsénico: el impacto en la alimentación y la salud humana. – Revista Peruana de Medicina Experimental y Salud Pública 35: 93-102. https://doi.org/10.17843/rpmesp.2018.351.3604.

[19] Murthy, S., Bali, G., Sarangi, S. K. (2012): Biosorption of lead by *Bacillus cereus* isolated from industrial effluents. – Biotechnology Journal International 2: 73-84. https://core.ac.uk/download/pdf/72804029.pdf.

[20] Nava-Ruíz, C., Méndez-Armenta, M. (2011): Efectos neurotóxicos de metales pesados (cadmio, plomo, arsénico y talio). – Archivos de neurociencias 16: 140-147. https://www.medigraphic.com/pdfs/arcneu/ane-2011/ane113f.pdf.

[21] Oliart-Ros, R. M., Manresa-Presas, Á., Sánchez-Otero, M. G. (2016): Utilización de microorganismos de ambientes extremos y sus productos en el desarrollo biotecnológico. – CienciaUAT 11: 79-90. https://www.redalyc.org/articulo.oa?id=441946945006.

[22] Olmos, V., Ridolfi, A. S. (2018): Hidroarsenicismo: mecanismos de acción asociados a la toxicidad del arsénico. – Acta Toxicológica Argentina 26: 32-44. http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S1851-37432018000100004.

[23] Orji, O. U., Awoke, J. N., Aja, P. M., Aloke, C., Obasi, O. D., Alum, E. U., Udu-Ibiam, O. E., Oka, G. O. (2021): Halotolerant and metalotolerant bacteria strains with heavy metals biorestoration possibilities isolated from Uburu Salt Lake, Southeastern, Nigeria. – Heliyon 7: 1-8. https://doi.org/10.1016/j.heliyon.2021.e07512.

[24] Oves, M., Khan, M. S., Zaidi, A. (2013): Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil. – Saudi Journal of Biological Sciences 20: 121-129. https://doi.org/10.1016/j.sjbs.2012.11.006.

[25] Pandey, N., Bhatt, R. (2015): Arsenic resistance and accumulation by two bacteria isolated from a natural arsenic contaminated site. – Journal of Basic Microbiology 55: 1275-1286. http://dx.doi.org/10.1002/jobm.201400723.

[26] Pellizzari, E. E., Marinich, L. G., Flores, S. A., Giménez, C. M. (2015): Degradación de arsénico por *Pseudomonas aeruginosa* para bioremediaciónde agua. Estudio preliminar. – Avances en ciencias e ingeniería 6: 1-6. https://www.redalyc.org/articulo.oa?id=323635882001.

[27] Rodríguez-Rey, A., Cuéllar-Luna, L., Maldonado-Cantillo, G., Suardiaz-Espinosa, M. E. (2016): Efectos nocivos del plomo para la salud del hombre. – Revista Cubana de Investigaciones Biomédicas 35: 251-271. https://www.medigraphic.com/pdfs/revcubinvbio/cib-2016/cib163f.pdf.

[28] Rubio, A. H., Ochoa, R. J. M., Quintana, R. M., Saucedo, T. R., Ortiz, D., Rey, B. N. L., Espinoza, P. J. R. (2013): Development of a Water Quality Index (WQI) of an Artificial Aquatic Ecosystem in Mexico. – Journal of Environmental Protection 4: 1296-1306. https://doi.org/10.4236/jep.2013.411151.

[29] Satyapal, G. K., Rani, S., Kumar, M., Kumar, N. (2016): Potential role of arsenic resistant bacteria in bioremediation: current status and future prospects. – Journal of Microbial and Biochemical Technology 8: 256-258. http://dx.doi.org/10.4172/1948-5948.1000294.

[30] Soto-Padilla, M. Y., Gortares-Moroyoqui, P., Cira-Chavez, L. A., Estrada-Alvarado, M. I. (2018): Biochemical and molecular characterization of a native haloalkalophilic tolerant strain from the Texcoco Lake. – Polish Journal of Microbiology 67: 377-382. https://doi.org/10.21307/pjm-2018-047.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2003_28172829

[31] Suárez, P., Reyes, R. (2002): La incorporación de metales pesados en las bacterias y su importancia para el ambiente. – Interciencia 27: 160-164. https://www.redalyc.org/articulo.oa?id=33906702.
[32] Suhadolnik, M. L., Salgado, A. P., Scholte, L. L., Bleicher, L., Costa, P. S., Reis, M. P., Dias, M. F., Ávila, M. P., Barbosa, F. A. R., Chartone-Souza, E., Nascimento, A. M. (2017): Novel arsenic-transforming bacteria and the diversity of their arsenic-related genes and enzymes arising from arsenic-polluted freshwater sediment. – Scientific Reports 7: 1-17. https://doi.org/10.1038/s41598-017-11548-8.
[33] Thacker, U., Parikh, R., Shouche, Y., Madamwar, D. (2007): Reduction of chromate by cell-free extract of *Brucella* sp. Isolated from Cr(VI) contaminated sites. – Bioresource Technology 98: 1541-1547. https://doi.org/10.1016/j.biortech.2006.06.011.
[34] Uqab, B., Nazir, R., Ganai, B. A., Rahi, P., Rehman, S., Farooq, S., Dar, R., Parray, J. A., Al-Arjani, A. F., Tabassum, B., Abd_Allah, E. F. (2020): MALDI-TOF-MS and 16S rRNA characterization of lead tolerant metallophile bacteria isolated from saffron soils of Kashmir for their sequestration potential. – Saudi Journal of Biological Sciences 27: 2047-2053. https://doi.org/10.1016/j.sjbs.2020.04.021.

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DOI: http://dx.doi.org/10.15666/aeer/2003_28172829