



Phytoremediation of mine tailings by *Brassica juncea* inoculated with plant growth-promoting bacteria

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ABSTRACT

Mine tailings represent a serious environmental pollution problem and techniques such as phytoremediation using plant growth-promoting bacteria become an important solution due to their environmentally friendly nature. The study performed using *Brassica juncea* L. (Indian mustard) and plant growth-promoting bacteria such as *Serratia K120*, *Enterobacter K125*, *Serratia MC107*, *Serratia MC119* and *Enterobacter MC156* showed that plant roots colonization favored the transfer of metals to the plant, mainly Al and Pb from the 8 analyzed metals with bioaccumulation factors > 1 for Al, Pb, Cd and Fe obtained with *Serratia K120*, *Enterobacter K125*, *Serratia MC107*, *Serratia MC119* and *Enterobacter MC156*. Based on these results, this system could be used in phytoextraction processes whereas *Enterobacter MC156* reduced the bioaccumulation of metals, indicating the possible phytostabilization of metals present in mine tailings.

1. Introduction

Mining is one of the main industrial activities in the world and Mexico (Espinosa-Reyes et al., 2014). This activity generates a big amount of solid mine waste, known as mining tailings, which is considered as one of the main causes of soil degradation. Such mining tailings, which consist mainly of metal and metalloids such as Pb, Hg, As, Cu, Fe, Mn, Ni and Cr, among others, are released into the environment and dispersed through the air and water, damaging organisms and interfering with the dynamics of ecosystems (Moreira et al., 2016). In general, these residues are deposited in the open air and, due to the environmental conditions, suffer a series of chemical transformations through redox reactions. In this sense and according to the report by the Mexican Economy Office in 2016 (S.E., 2016), Mexico is the first producer of silver, eleventh of gold, twelfth of copper, second of fluorite, fifth of lead and third of bismuth, and the generated waste, which contains high amounts of metals, is deposited in the open air without any treatment (Babel et al., 2016), provoking serious pollution problems at local, regional and global levels. In order to face this problem, the diverse bioremediation techniques are viable options due to their economical, efficient, ecological and technical features. Among these techniques, phytoremediation, by means of plants, can remove

soil pollutants through processes that allow the stabilization, extraction or volatilization of heavy metals and xenobiotic compounds, which depends on the selected plant to carry out a specific function. This technique helps treat the pollutants without damaging the soil upper layers, which is directly reflected in the use of soils and fertility (Wani et al., 2017). Phytoremediation is beneficial to processes such as phytoextraction and phytostabilization of soil metals, biomass and biofuel production, and carbon sequestration due to the fact that many plants have the capacity of producing biomass and accumulating various environmental pollutants (Wang et al., 2017). Bacteria can remove xenobiotics compounds (Singh et al., 2017) or when combined with plants, they can enhance the phytoextraction through the modification of soil factors such as solubility, viability, heavy metal and nutrient transport by soil pH reduction, chelate release, P solubilization or redox changes (Ma et al., 2011); more specifically, rhizobacteria, which promote the plant growth and are involved in the processes mentioned above, activate the plant growth through direct and indirect methods (Kumar et al., 2015; Ahemad and Kibret, 2014) and diminish the plant stress caused by the presence of metals. One of the advantages of using endemic microorganisms is the adaptation capacity to the changing environment. The microorganism communities, adapted to the presence of metals, can promote the growth of plants through the production of

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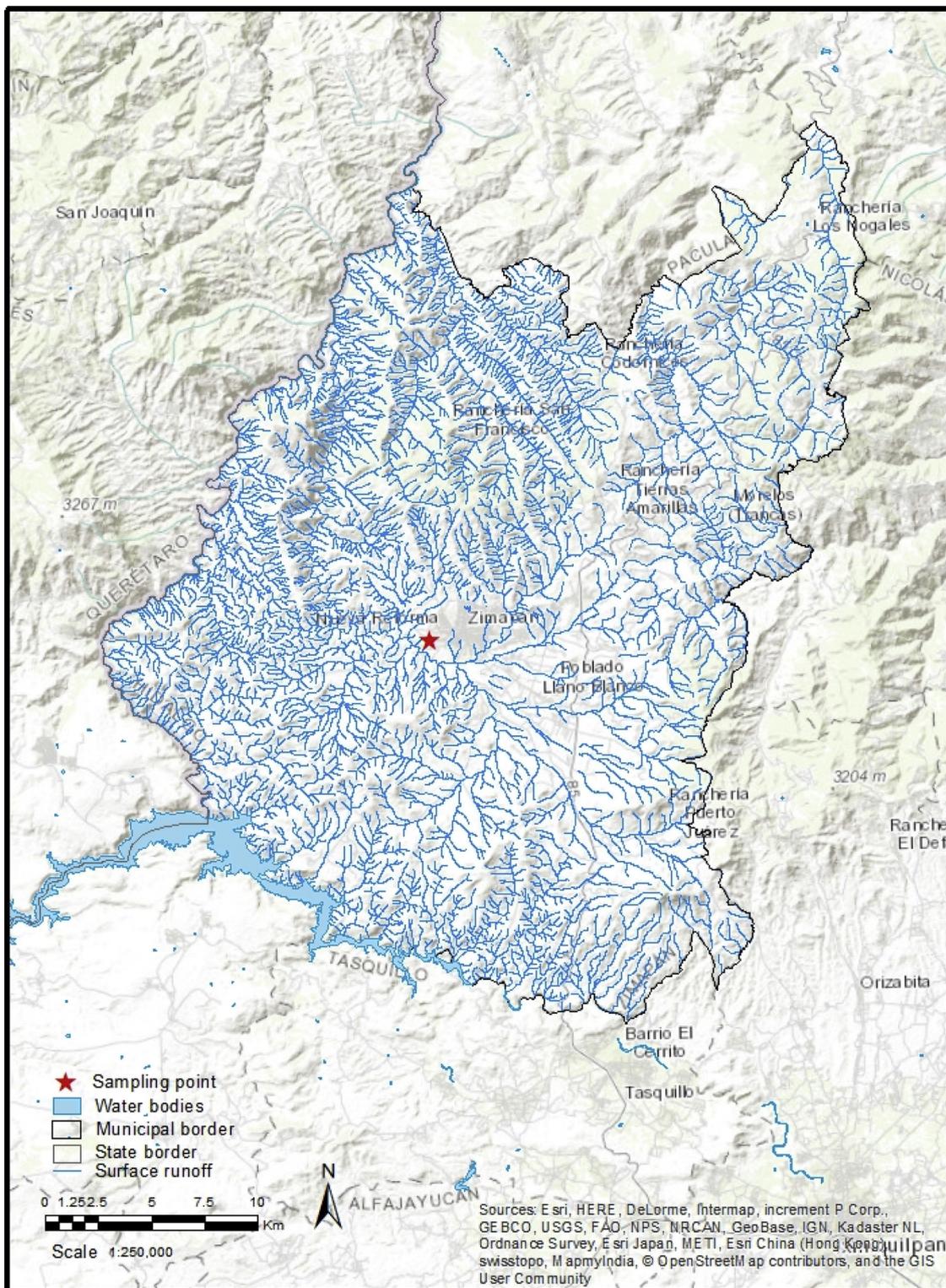


Fig. 1. Location of sampling site.

siderophores, solubilization of phosphates, production of phytohormones such as the indole acetic acid, activity of the enzyme ACC deaminase and nitrogen fixation, thus diminishing the plant stress caused by the hostile surrounding conditions (Das et al., 2014).

The use of plant growth-promoting bacteria (PGPB) to support phytoremediation processes is considered as a feasible technique due to the modification of the medium conditions, which can increase the production of biomass and bioavailability of metals, thus being highly

useful for the bioremediation of these sites (Aggangan et al., 2017). The aim of the present work is to propose a phytoremediation system using *Brassica juncea* L. and plant growth-promoting bacteria.

2. Materials and methods

2.1. Sampling

Random sampling was carried out in Zimapán Hidalgo, Mexico (458459.6, 2292938.7) according to NOM-021-SEMARNAT-2000 (Fig. 1), keeping the composite samples at 4 °C for transportation. The preparation of the samples consisted on drying, grinding and sieving to homogenize them to a particle size < 2 mm for later use in the physical and chemical characterization and pot trial.

2.2. Physical and chemical characterization of mine tailings

Mine tailing samples from Zimapán Hidalgo, Mexico, were physicochemically characterized by measuring the organic matter percentage, pH, electrical conductivity and the concentration of soluble, extractable and total metals. The pH was measured in a soil/water (1:2) suspension (Jackson, 1976) and the electrical conductivity was measured after 24 h (Richards, 1973). The organic matter percentage was analyzed by using the Walkley and Black method (Walkley and Black, 1934). The total metal concentration was analyzed by using the EPA Method 3052 (US EPA, 1996) and the soluble metals and extractable metal concentration was determined by using the DTPA-TEA-CaCl₂ method (Lindsay and Norvell, 1978). Determination of heavy metals was by atomic absorption spectrophotometry (Analyst 400 Perkin Elmer).

2.3. Bacterial strains

The used bacterial strains were *Serratia K120*, *Enterobacter K125*, *Serratia MC107*, *Serratia MC119* and *Enterobacter MC156*, isolated from the rhizosphere of the Zimapán Hidalgo mining residues and characterized as PGPB (Mendoza-Hernández et al., 2016).

2.4. Pot experiment

2.4.1. Inoculant preparation

The chosen strains were cultivated in 250 mL flasks that contained LB Broth. The flasks were incubated for 48 h at 30 °C with orbital stirring at 80 rpm. Later on, bacteria were separated from the liquid medium by centrifugation at 8000 rpm for 15 min. Afterwards, the culture was centrifuged at 9000 G for 10 min, and the pellet was resuspended in 0.1 M (pH 7.4) of phosphate buffer solution, and the bacterial suspension was adjusted to 0.5 Å at 600 nm to obtain an approximate concentration of 1×10^9 CFU mL⁻¹.

2.5. Pot trial

Mustard seeds (*Brassica juncea* L.) were superficially sterilized by immersing them in an ethanol solution at 95% for 5 min; then, they were immersed in sodium hypochlorite at 5% for 10 min, and finally washed with sterile distilled water. The seeds were planted in pots that contained 750 g of mining residues with 10% of Miracle Gro® substrate.

A totally randomized experimental design was used for assessing the PGPB effect, where the treatment factor was bacterial inoculation (5 treatments corresponding to the number of chosen strains). A witness treatment, which consisted of a mixture of mining residues and non-inoculated bacterial substrate, was added.

First, 3 seeds were planted and 25 mL of bacterial suspension were inoculated in each experimental unit. Then, 25 mL of 0.1 M (pH 7.4) phosphate buffer were added to the witness treatment case. Afterwards, the experimental units were kept in a greenhouse for 21 days. Next, irrigation with 30 mL of water was carried out every other day. During the experiment, the total height of the plant was monitored. At the end of the experiment (60 days), the plants were harvested and the total plant height and root length were measured by using the Imagin tool

3.0 software, and heavy metal (P, K, Na, Mn, Cu, Al, Ni, Pb, and As) levels were measured by using flame Atomic Absorption spectrophotometry (Analyst 400).

2.6. Scanning Electron Microscopy (SEM)

After 60 days growing, root samples were taken randomly to be analyzed. Prior to the microscopic analysis, the roots were washed thrice with sterile distilled water; afterwards, a longitudinal cut was done in order to observe the interior of the witness plant and the roots of the plants inoculated with the strains *Serratia K120*, *Serratia K131* and *Serratia MC156*. The samples were placed on conductive carbon tape and coated with gold in a sputtering unit (Denton Vacuum, DESK v SCD-030) before the SEM (Scanning Electron Microscope, JEOL JSM-6610LV) observation. SEM observations were carried out by using a secondary electron (SE) detector on a bulk sample system working in high vacuum mode at 20 kV of accelerating voltage. The SEM piece of equipment was coupled with an EDAX energy dispersive X-ray spectrometer (EDX) for chemical elemental microanalysis.

2.7. X-Ray diffraction (XRD)

The soil samples, after the treatment with plant growth-promoting bacteria, were characterized by means of the X-ray diffraction (XRD BrukerD8 Discover) technique. The data were collected on a Bruker diffractometer model D8 Discover in a Bragg–Brentano geometry using CuKα radiation ($\lambda = 0.15418$ nm) and step-scan mode (range: 3–90° of 2θ, step-time: 0.60 s, step-width: 0.04°).

2.8. Transfer, translocation and bioaccumulation factors

The transfer of metals from the mine tailing to the roots and aerial part of *Brassica juncea* L. was evaluated by studying the corresponding transfer factors, where (TF_H) is defined as the metal concentration in the aerial part (C_H) with respect to the concentration of the same metal present in the mine tailing (C_j) (Rehman et al., 2018) whereas the transfer factor in the root (TF_r) is defined as the concentration of the metal in the root (C_r) with respect to the concentration of the same metal in the mine tailing (C_j) (Ganeshkumar et al., 2018).

$$\text{Aerial part transfer factor } TF_H = \frac{C_H}{C_j}$$

$$\text{Root transfer factor } TF_r = \frac{C_r}{C_j}$$

The translocation of metals inside the plant was evaluated through the translocation factor TF , which is defined as the metal concentration in the aerial part (C_H) with respect to the concentration of the same metal in the roots (C_r) (Chandra et al., 2017; Forján et al., 2018).

$$\text{Translocation factor } TF = \frac{C_H}{C_r}$$

The bioaccumulation of metals was calculated through the bioaccumulation factor, which is defined as the metal concentration in the roots (C_r) plus the metal concentration in the aerial part (C_H) with respect to the concentration of the same metal in the mine tailing (C_j) (Chandra et al., 2017).

$$\text{Bioaccumulation factor } BF = \frac{C_r + C_H}{C_j}$$

2.9. Statistical analysis

The analysis of the growth of roots and stems and plant metal concentrations was done by a variance analysis for detecting differences between the treatments with respect to the witness, using Tukey's

Table 1
Concentrations of soluble, extractable and total metals.

Metal	Soluble fraction (mgKg ⁻¹)	Extractable fraction (mgKg ⁻¹)	Total fraction (mgKg ⁻¹)
Cu	10.23 ± 1.23	14.36 ± 2.32	492.69 ± 34.67
Pb	18.59 ± 2.35	39.38 ± 4.76	2211.60 ± 232.54
As	11.34 ± 1.89	16.75 ± 2.45	7889.83 ± 234.67
Ni	3.28 ± 1.14	5.57 ± 1.23	54.60 ± 9.87
Fe	8.47 ± 0.26	95.78 ± 0.58	4591.40 ± 9.98
Cr	5.39 ± 0.95	16.57 ± 2.4	456.90 ± 8.43
Cd	1.24 ± 0.15	4.97 ± 1.67	191.40 ± 7.89
Mn	3.21 ± 0.39	8.66 ± 2.34	917.93 ± 23.45
Al	158 ± 34.56	235.67 ± 23.23	7487.76 ± 23.54

honest significant difference (HSD) test with a significant level of 95% ($\alpha = 0.05$). All the analyses were carried out with the software SPSS (V. 22).

3. Results

3.1. Physical and chemical characterization of rhizosphere soil

The results of the physicochemical characterization of the mine tailing showed that the pH of the rhizosphere samples was slightly acid (pH 6.34 ± 0.02), the electrical conductivity was 4.01 ± 0.35 $\mu\text{S cm}^{-1}$, the organic matter percent was 57.6% ± 1.57, the texture corresponded to loamy sand, the saturation percentage was 52.5% ± 3.93, the cationic exchange capacity was 0.56 ± 0.14 $\text{Cmol}(+) \text{Kg}^{-1}$ and the extractable acidity was 4.3 ± 0.68 $\text{Cmol}(+) \text{Kg}^{-1}$. The found concentrations of soluble, extractable and total metals founded are shown in Table 1. The soluble and extractable fractions of metals have higher availability for the plants.

The XRD technique was used to establish and study the structural properties of the phases present in the soil samples. In Fig. 2, the XRD pattern of the soil treated with bacteria is presented. The soil is a mixture of compounds and contains magnesium aluminum hydroxide carbonate hydrate (hydrocalcite) (JCPDS-PDF 66-0802), microcline, sodian (JCPDS-PDF 04-011-0527), quartz (JCPDS-PDF 5-0490) and calcium magnesium carbonate (dolomite) (JCPDS-PDF 04-012-6929). The XRD pattern shows major peaks of magnesium aluminum hydroxide carbonate hydrate at $2\theta = 11.7$, 23.5 and 39.5°. In the figure, other major peaks corresponding to microcline sodian are shown at $2\theta = 25.7$, 27.1 and 27.6°. The middle-angle region of the pattern shows three diffraction lines attributed to quartz, a weak line at 20.9°, a more intense reflection found at 26.7 and other at 50.2° (2 θ). The reflections observed at 23.3, 29.3, 39.5, 43.2, 47.7 and 48.4° (2 θ)

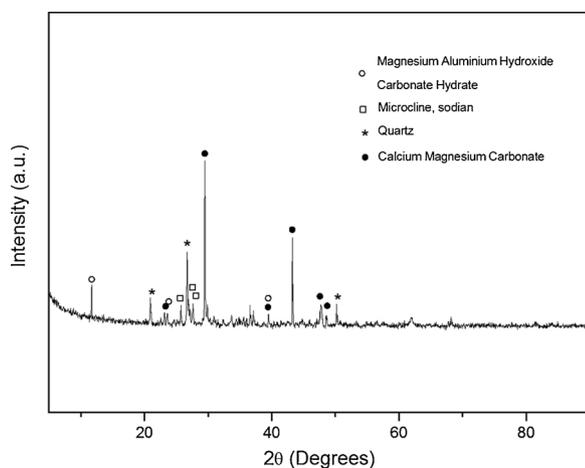


Fig. 2. XRD pattern of soil treated with bacteria.

correspond to calcium magnesium carbonate. The XRD results show that the soil comes from mine trails, which displays a different composition from farming soil whose main composition consists of mineral phases such as quartz, hematite, biotite and andesine. However, the mining trail keeps a composition of crystalline phases suitable for the growth of vegetative species, and then, for this reason, this type of substrates with this crystalline phase composition does not exert negative effects on the plant growth when plant growth-promoting bacteria are used, as shown by the potting test results.

The analysis of metals for stems and aerial part of *Brassica juncea* L. shows the highest concentrations of Al, Cu, Pb, Cr, Cd, K, Mn, Fe and As for *Serratia K120*; for Na and Mg with *Serratia MC107*, while the lowest concentrations of Pb, K, Na, Mn, Fe, Mg and As correspond to *Enterobacter MC156*; Al, Cr and Cd for *Enterobacter K125* and finally in Cu with *Serratia MC107*. The plant stems show a significant difference ($p < 0.05$) in the absorption of all the analyzed metals for *Serratia K120* with respect to the testigo. On the other hand, in the absorption of Al, Mg and As, there is a significant difference among all the strains and the testigo whereas the behavior pattern of Cu, Pb, Fe and Mn displays a significant difference between *Serratia K120*, *Serratia MC119* and the testigo, notwithstanding, for the other strains, the absorption was lower than that of the testigo. In the absorption of Zn, there is a significant difference between *Serratia K120*, *Serratia MC107* and *Serratia MC119* and the testigo; as for Cr, only a significant difference between *Serratia K120* and the testigo was found. For Cd, there was a significant difference between the testigo and *Serratia K120*, *Serratia MC119* and *Enterobacter MC156*, and for K, the significant difference was between *Serratia K120* and the testigo; regarding Na, the significant difference occurred between *Serratia K120* and *Serratia MC119* and the testigo as shown in Table 3.

The highest concentrations of Al, Pb, Cr, Cd, Mn and As in roots corresponded to *Serratia K120*; K, Na and Mg for *Enterobacter K125*; and Cu for *Serratia MC119*. The lowest concentrations of Al, As and Cd were found in *Enterobacter K125*; Pb, Cr and Fe for *Enterobacter MC156*; Cu, K and Mn for *Serratia MC107*; in the case of Na and Mg for *Serratia MC119*. The absorption analysis of the roots of the plants inoculated with PGPB and those of the testigo showed a significant difference of $p < 0.05$ in all the strains tested with Al, Cu, Pb, Zn, Cr, K, Mn, Mg and As; for Cd, the differences occurred with *Serratia K120* and *Serratia MC119*; for Na, the significant differences were for *Serratia K120*, *Enterobacter K125* and *Serratia MC107* and for Fe, the significant difference occurred with *Serratia K120* and *Enterobacter K125* as shown in Table 4.

3.2. Pot trial

The comparison between the stem height and the length of the roots showed a significant difference ($p < 0.05$) in all the strains with respect to the testigo as shown in Table 2 and Fig. 3.

3.3. SEM

In the surface samples of the testigo plant roots analyzed by SEM, the absence of adhered bacterium colonies can be observed (Fig. 4a).

Table 2

Statistical analysis of the stem height and length of roots in the *Brassica juncea* L. plants inoculated with different bacterial treatments.

Strain	Stem height	Length of roots (cm)
Testigo	9.33 ± 236	7.26 ± 1.99
<i>Serratia K120</i>	15.81 ± 2.02	23.83 ± 1.68
<i>Enterobacter K125</i>	16.54 ± 2.76	31.74 ± 2.51
<i>Serratia MC107</i>	18.73 ± 1.33	38.19 ± 6.68
<i>Serratia MC119</i>	14.31 ± 1.81	24.46 ± 3.40
<i>Enterobacter MC156</i>	14.74 ± 2.98	19.71 ± 3.79

Table 3
Concentrations of metals in stems and aerial part of *Brassica juncea* L.

Strains	Al (mgKg ⁻¹)	Cu (mgKg ⁻¹)	Pb (mgKg ⁻¹)	Cr (mgKg ⁻¹)	Cd (mgKg ⁻¹)	K (mgKg ⁻¹)	Na (mgKg ⁻¹)	Mn (mgKg ⁻¹)	Fe (mgKg ⁻¹)	Mg (mgKg ⁻¹)	As (mgKg ⁻¹)
Testigo	1023.4 ± 22.7	12.0 ± 0.3	12.0 ± 3.2	12.3 ± 3.2	10.8 ± 3.4	90.3 ± 0.3	260.4 ± 25.3	18.6 ± 1.0	1,187.0 ± 100.0	765.0 ± 143.2	30.5 ± 1.5
Serratia K120	5413.4 ± 24.3	165.4 ± 2.4	1,072.0 ± 192.0	99.2 ± 2.3	83.1 ± 1.5	444.0 ± 24.8	386.0 ± 25.0	123.0 ± 10.3	2,588.0 ± 106.0	2,369.0 ± 106.5	363.8 ± 2.1
Enterobacter K125	3648.0 ± 27.7	6.0 ± 0.1	4.0 ± 1.7	8.0 ± 2.3	12.0 ± 1.0	2.7 ± 0.1	257.5 ± 29.3	5.6 ± 1.2	190.3 ± 35.2	2,588.0 ± 35.9	65.6 ± 1.9
Serratia MC107	3846.0 ± 23.0	3.8 ± 0.1	6.6 ± 1.1	40.1 ± 2.9	15.0 ± 1.3	6.6 ± 0.1	946.1 ± 25.8	17.9 ± 5.4	439.0 ± 84.3	10,532.8 ± 84.6	123.2 ± 1.1
Serratia MC119	3806.0 ± 14.4	27.4 ± 2.9	40.4 ± 1.9	9.3 ± 2.8	21.0 ± 1.8	3.5 ± 0.3	273.9 ± 23.2	42.2 ± 9.8	1,688.0 ± 134.6	3,993.0 ± 134.3	112.3 ± 1.7
Enterobacter MC156	3798.0 ± 21.3	6.7 ± 0.2	1.2 ± 0.2	9.5 ± 3.1	34.0 ± 2.4	2.6 ± 0.2	232.9 ± 16.8	5.4 ± 1.3	163.3 ± 42.7	1,527.0 ± 42.4	65.4 ± 1.2

* Significant differences p < 0.05.

Table 4
Concentrations of metals in the roots of *Brassica juncea* L.

Strains	Al (mgKg ⁻¹)	Cu (mgKg ⁻¹)	Pb (mgKg ⁻¹)	Cr (mgKg ⁻¹)	Cd (mgKg ⁻¹)	K (mgKg ⁻¹)	Na (mgKg ⁻¹)	Mn (mgKg ⁻¹)	Fe (mgKg ⁻¹)	Mg (mgKg ⁻¹)	As (mgKg ⁻¹)
Testigo	1174.5 ± 14.9	13.5 ± 0.3	27.0 ± 3.3	15.7 ± 3.1	45.0 ± 3.4	3.7 ± 0.2	679.0 ± 12.8	25.0 ± 1.7	2765.0 ± 135.0	1024.0 ± 141.0	45.0 ± 1.2
Serratia K120	5959.0 ± 33.9	299.3 ± 19.2	1,815.7 ± 195.0	135.6 ± 2.8	134.0 ± 1.3	23.3 ± 0.3	1688.3 ± 14.7	342.5 ± 13.8	12513.1 ± 104.0	18184.1 ± 137.0	398.0 ± 1.8
Enterobacter K125	3909.0 ± 13.5	23.0 ± 0.2	52.6 ± 1.1	91.4 ± 3.4	23.0 ± 1.1	34.3 ± 0.3	3128.2 ± 18.8	64.0 ± 10.1	4635.5 ± 122.0	30288.0 ± 101.0	104.0 ± 1.1
Serratia MC107	4010.0 ± 19.7	18.4 ± 0.3	90.1 ± 2.7	62.3 ± 3.3	45.0 ± 2.7	8.3 ± 0.2	1000.4 ± 24.1	55.4 ± 7.9	2861.9 ± 145.0	9737.9 ± 142.0	245.0 ± 1.9
Serratia MC119	3957.0 ± 30.1	394.2 ± 2.9	498.1 ± 18.0	71.8 ± 1.2	76.0 ± 2.1	12.4 ± 0.3	283.7 ± 20.9	253.1 ± 12.9	2399.0 ± 131.0	3365.0 ± 139.0	124.7 ± 2.4
Enterobacter MC156	3959.0 ± 15.3	33.0 ± 0.3	48.4 ± 3.2	58.8 ± 3.3	56.0 ± 2.8	12.7 ± 0.1	1447.3 ± 21.1	55.6 ± 5.9	796.0 ± 126.0	14536.3 ± 108.0	176.7 ± 1.8

* Significant differences p < 0.05.

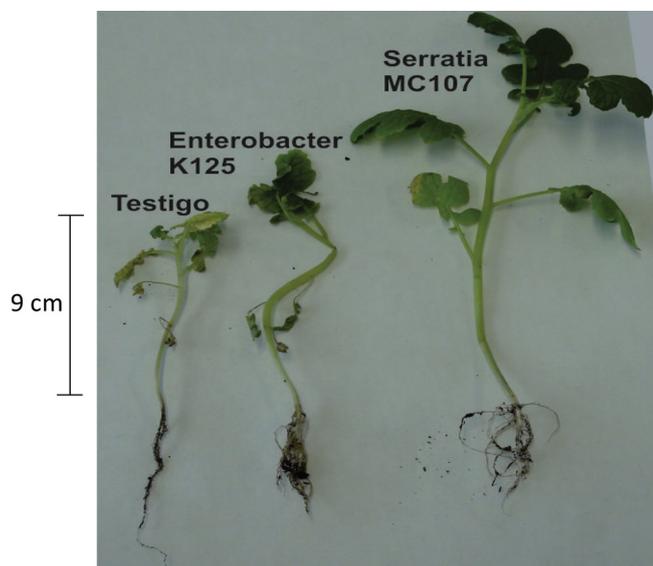


Fig. 3. Growth comparison between *Brassica juncea* L. inoculated with *Enterobacter K125* and *Serratia MC107* strains and non-inoculated *Brassica juncea* L.

Likewise, the surface of the sample inoculated with *Serratia MC119* (Fig. 4b) shows a slight colonization with colony sizes in the order of 1–4 microns whereas in Fig. 4c), corresponding to *Serratia K120*, an increase in the number of colonies of these bacteria (in the order of 1–3 microns) is observed, which displays a higher colonization capacity inside the plant root and finally, in Fig. 4d), *Enterobacter MC156* showed the highest root colonization with an uncountable number of colonies, which indicates a better microorganism-plant interaction.

3.4. Transfer and translocation factors

The obtained transfer factors of the metals in the plant aerial part correspond to $Al > Fe > Pb > Cd > Cu > Cr > Mn > As$, where the bacteria favored the transfer of metals toward the plant aerial part as shown in Table 5. *Serratia K120* favored the transfer of all the metals analyzed in the plant aerial part with a significant difference of $p < 0.05$. For Al, all the bacteria showed significant differences of $p < 0.05$, and only for Fe, *Serratia K120*, *Enterobacter K125* and *Enterobacter MC156* showed significant differences of $p < 0.05$. The lowest transfer factors corresponded to As and the highest to Al.

The transfer factors in the plant roots occurred in the order $Al > Cr > As > Cd > Pb > Cu > Fe > Mn$ without any significant difference with respect to the testigo. For Cu, Cr, Pb, Mn and Fe, the concentrations were higher in the testigo than in the bacterium inoculated plants whereas Cd, Al and As, in most bacteria, had higher concentrations than those in the testigo, but without significant differences.

The plants inoculated with *Serratia K120* showed the highest concentrations of metals in roots in comparison with those inoculated with other strains. The concentration of Al in the roots was similar in the inoculated plants and the testigo whereas for Cu, the bacterium that allowed a lower metal concentration in roots was *Serratia MC119*; for Pb, it was *Serratia MC107*; for Cr, Mn and Fe, it was *Enterobacter K125* and for As, it was *Enterobacter MC156* as shown in Table 6.

The translocation factors showed a decreasing order: $Fe > Al > Cu > Pb > Cd > Cr > Mn > As$. A significant difference with respect to the testigo of $p < 0.05$ for Al with all the bacteria was found; also for Cu, Pb and Mn with *Serratia K120* and *Serratia MC119*; for Cr, Cd and Fe with *Serratia K120* and for As with *Serratia K120* and *Serratia MC107* as shown in Table 7.

The bioaccumulation factor of the metals in the plants displayed the

following order: $Fe > Al > Pb > Cd > Cu > Cr > Mn > As$. Significant differences of $p < 0.05$ with respect to the testigo for Cu, Pb and Mn using *Serratia K120* and *Serratia MC119* were found; for Cr and Cd with *Serratia K120*, for Fe with *Serratia K120* and *Enterobacter MC156* and finally for As with *Serratia K120* and *Serratia MC107*. It is worth emphasizing that the accumulation factor > 1 was observed in not essential metals such as Al and Pb and in essential metals such as Fe. The bacterium that allowed lower bioaccumulation of metals was *Enterobacter K125* (Table 8).

4. Discussion

The sampled mine tailings had concentrations of soluble, extractable and total metals such as Cu, As, Ni, Pb, Cr, Cd and Al above the limits allowed by the Mexican standard NOM-147-SEMARNAT, which establishes the following total metal concentrations for farming soil.

In the present study, it was found that the inoculation of *Brassica juncea* L. with strains of PGPB such as *Serratia K120*, *Enterobacter K125*, *Serratia MC107*, *Serratia MC119* and *Enterobacter MC156* showed a significant increase in biomass and root growth with respect to the testigo, which is in good agreement with what was stated by Román-Ponce et al. (2017) and Houda et al. (2017), where *Brassica nigra* and *Brassica napus*, inoculated with PGPB, showed significant difference in the root growth, which is in contrast with what was reported by Aung et al. (2015), where *Brassica*, in soils contaminated with Cs, was inoculated with PGPB without showing significant differences in plant growth; this effect could be due to the fact that bacteria, in the presence of metals, increase the production of IAA and the activity of the enzyme ACC deaminase (Mendoza-Hernández et al., 2016).

The ability of *Brassica juncea* L. to tolerate and accumulate metals can be exploited in phytoremediation processes of mine tailings (Chandra et al., 2017). In the present work, the content of metals and micronutrients in the plant displayed significant differences in the stem and roots when it was inoculated with *Serratia K120*, which is in good agreement with the works by Wu et al. (2006); Román-Ponce et al. (2017) and Aung et al. (2015), where the plants inoculated with PGPB increased the concentration of metals, although these works were carried out by using an artificially polluted medium. The plant transfer factors indicate that the accumulation of metals such as Al, Pb, Cd and As and that of essential ones such as Fe was higher in roots than in leaves, which is in good agreement with what was reported by Chen et al. (2016), Zhang et al. (2018), Chandra et al. (2017); Papaioannou et al. (2018) and Shen et al. (2017), and being always higher in the plants inoculated with PGPB, which shows that the transfer of metals can be due to the bacteria and to any of the multifactorial effects that affect it as stated by Papaioannou et al. (2018). The bacteria also promoted the translocation of metals in the plants from the roots to the aerial parts with significant differences with respect to the non-inoculated plants. The previous processes led to the bioaccumulation of the metals in the plants having factors above 1 in metals such as Al, Pb, Cd and Fe. The metal with higher accumulation in the presence of bacteria was Al, for it can be available, which can be corroborated by XRD, helping to perform a phytoextraction of Al to recover it, which coincides with the studies performed for bioremediation systems using enzymes such as *Trametes versicolor* (Wu et al., 2006) and *Aspergillus niger* (accumulation), which due to the production of citric exudates such as the citric, oxalic and gluconic, are capable of contributing to the extraction and mobility of Al in polluted soils (Boriov et al., 2016). On the other hand, the mobility of Al depends on the soil pH, where this element is normally static at values below 6 (Gonçalves et al., 2017), but for our system, the pH values are above 6 and then, soluble compounds of this element are formed, thus favoring the phytoextraction process.

The SEM results confirmed that the bacteria not only colonized the external part of the roots, but also colonized them inside, working as endophytic bacteria, where *Enterobacter MC156* was the strain with

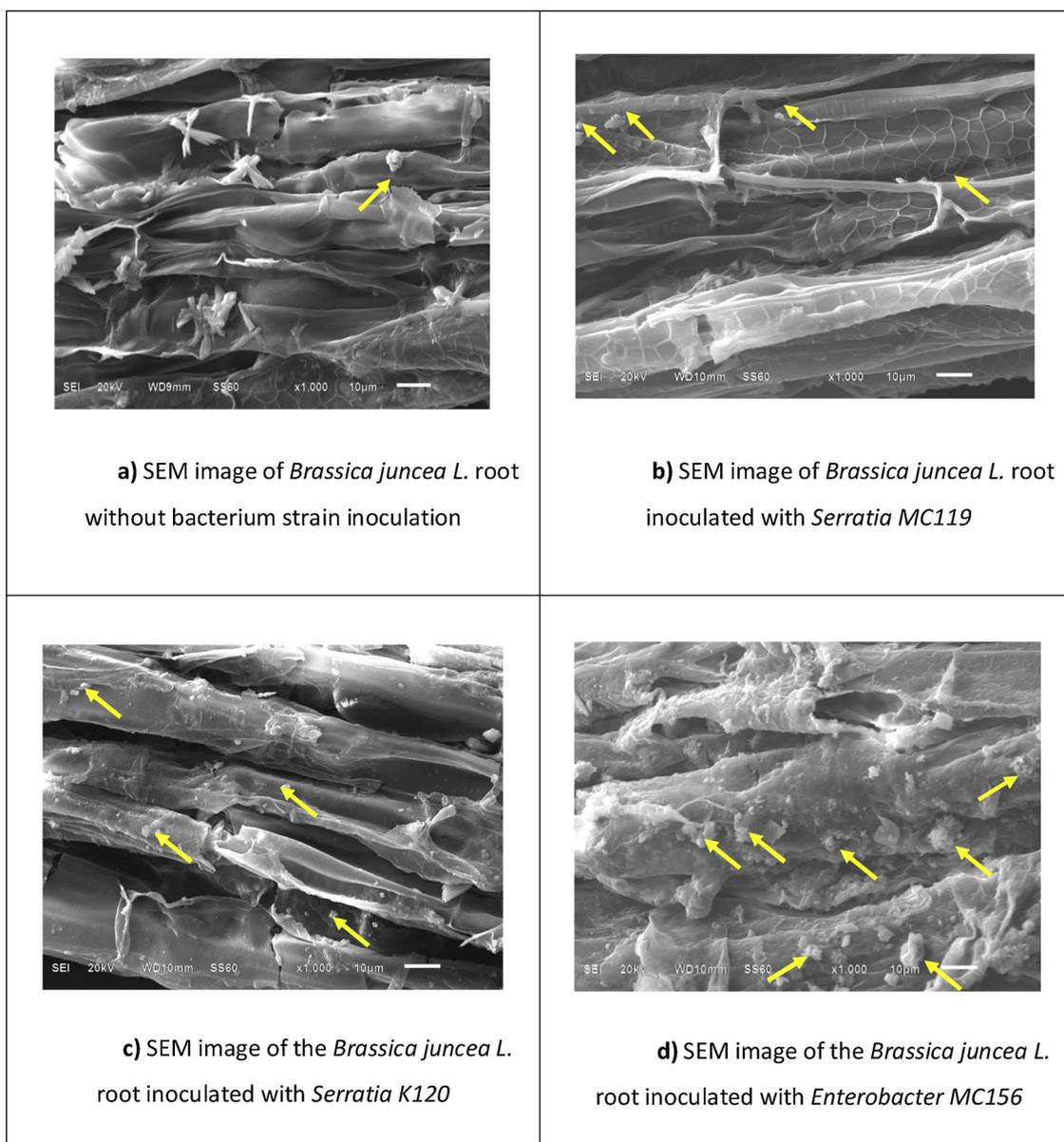


Fig. 4. a) SEM image of *Brassica juncea* L. root without bacterium strain inoculation. b) SEM image of *Brassica juncea* L. root inoculated with *Serratia MC119*. c) SEM image of the *Brassica juncea* L. root inoculated with *Serratia K120*. d) SEM image of the *Brassica juncea* L. root inoculated with *Enterobacter MC156*.

Table 5
Transfer factors of the metals in the plant aerial part.

Strains	Al	Cu	Pb	Cr	Cd	Mn	Fe	As
<i>Testigo</i>	0.14	0.02	0.01	0.03	0.06	0.02	0.26	0.004
<i>Serratia K120</i>	0.72	0.34	0.48	0.22	0.43	0.13	0.56	0.05
<i>Enterobacter K125</i>	0.49	0.01	0.002	0.02	0.06	0.01	0.04	0.01
<i>Serratia MC107</i>	0.51	0.01	0.003	0.09	0.08	0.02	0.10	0.02
<i>Serratia MC119</i>	0.51	0.06	0.02	0.02	0.11	0.05	0.37	0.01
<i>Enterobacter MC156</i>	0.51	0.01	0.001	0.02	0.18	0.01	0.04	0.01

higher colonization, followed by *Serratia K120*, which favored the accumulation of metals in *Brassica juncea* L., promoting its growth by diminishing the stress caused by the high concentrations of metals.

If the transfer, translocation and bioaccumulation factors of the metals are considered, it can be seen that bacteria exert different effects, for example, *Serratia K120* promoted the translocation and accumulation of metals in the plant aerial part, which could be explored in phytoextraction processes whereas *Enterobacter MC156* had the

Table 6
Transfer factors of metals in the roots.

Strains	Al	Cu	Pb	Cr	Cd	Mn	Fe	As
<i>Testigo</i>	0.87	0.89	0.44	0.78	0.24	0.74	0.43	0.68
<i>Serratia K120</i>	0.91	0.55	0.59	0.73	0.62	0.36	0.21	0.91
<i>Enterobacter K125</i>	0.93	0.26	0.08	0.09	0.52	0.09	0.04	0.63
<i>Serratia MC107</i>	0.96	0.21	0.07	0.64	0.33	0.32	0.15	0.50
<i>Serratia MC119</i>	0.96	0.07	0.08	0.13	0.28	0.17	0.70	0.90
<i>Enterobacter MC156</i>	0.96	0.20	0.02	0.16	0.61	0.10	0.21	0.37

highest colonization inside the roots, promoting phytostabilization processes, for it does not contribute much to the bioaccumulation of metals; on the other hand, the other studied bacteria promoted the accumulation of only some metals like Al, Cu and Fe.

5. Conclusions

The phytoremediation processes with *Brassica juncea* L. were

Table 7
Translocation factors in plants.

Strains	Al	Cu	Pb	Cr	Cd	Mn	Fe	As
<i>Testigo</i>	0.16	0.03	0.01	0.03	0.24	0.03	0.60	0.01
<i>Serratia K120</i>	0.80	0.61	0.82	0.30	0.70	0.37	2.73	0.05
<i>Enterobacter K125</i>	0.52	0.05	0.02	0.20	0.12	0.07	1.01	0.01
<i>Serratia MC107</i>	0.54	0.04	0.04	0.14	0.24	0.06	0.62	0.03
<i>Serratia MC119</i>	0.53	0.80	0.23	0.16	0.40	0.28	0.52	0.02
<i>Enterobacter MC156</i>	0.53	0.07	0.02	0.13	0.29	0.06	0.17	0.02

Table 8
Bioaccumulation factor of metals.

Strains	Al	Cu	Pb	Cr	Cd	Mn	Fe	As
<i>Testigo</i>	0.29	0.05	0.02	0.06	0.29	0.05	0.86	0.01
<i>Serratia K120</i>	1.52	0.94	1.31	0.51	1.13	0.51	3.29	0.10
<i>Enterobacter K125</i>	1.01	0.06	0.03	0.22	0.18	0.08	1.05	0.02
<i>Serratia MC107</i>	1.05	0.05	0.04	0.22	0.31	0.08	0.72	0.05
<i>Serratia MC119</i>	1.04	0.86	0.24	0.18	0.51	0.32	0.89	0.03
<i>Enterobacter MC156</i>	1.04	0.08	0.02	0.15	0.47	0.07	0.21	0.03

favoured by the presence of PGPB such as *Serratia K120* and *Enterobacter MC156*, where the first one promoted the phytoextraction process and the second one, the phytostabilization of metals, because in addition to promote the plant growth, they colonized the external and internal parts of the roots, diminishing the stress caused by the presence of heavy metals through the accumulation of metals in the plants; these strains can be used as microbial inoculants in biotechnological processes of phytoremediation.

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