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Phytoremediation of soil contaminated with hexavalent chromium using *Portulacaria afra* and *Cynodon dactylon* and microbial reduction.

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ABSTRACT: *The phytoremediation of an industrial soil contaminated with hexavalent chromium was evaluated, using the plant species Portulacaria afra and Cynodon dactylon for 192 days. The initial concentration of Cr in the soil was 5,782 mg/kg (2,683 mg/kg Cr (VI) and 3099 mg/kg Cr (III)). The concentration in the root and aerial part was monitored for total Cr. The bioconcentration factor (BCF) and translocation factor (TF) were 0.87 and 0.02, for P. afra, and 1.66 and 0.01 for C. dactylon. Both C. dactylon and P. afra removed Cr (VI) with an efficiency of 98.4%. The concentration of Cr (VI) in the soil at the end of treatment with both species was lower than the limits established by Mexican regulations for soils for agricultural, domestic, commercial, and industrial use. The possible contribution of two strains isolated from the contaminated soil, identified as Bacillus sp. and Aspergillus sp., in the reduction of Cr (VI) to Cr (III) was assessed. The highest Cr (VI) reduction efficiencies were observed with Aspergillus sp., 99.96% (50 ppm), 97.28% (100 ppm), and 54.29% (200 ppm). With the strain of Bacillus sp., reductions of 44.75% (50 ppm), 26.89% (100 ppm) and 27.79% (200 ppm) were obtained.*

KEYWORDS – *Cynodon dactylon, hexavalent chromium, microbial reduction, phytoremediation, Portulacaria afra.*

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I. INTRODUCTION

Soil contamination represents a major environmental problem and has turned vast tracts of land into non-arable areas that are dangerous for both wildlife and human populations [1]. Among the different pollutants, heavy metals stand out, with a high level of toxicity [2]. Although they are found naturally in soil, anthropogenic activities such as mining, manufacturing, agriculture, and waste disposal have promoted their availability, their dispersal into the environment, and their biomagnification through food chains [3].

Chromium (Cr) contamination affects both soil and water [2]. Its most stable forms in soil are trivalent chromium (Cr (III)) and hexavalent chromium (Cr (VI)), the former being an essential micronutrient and the latter a highly toxic and carcinogenic persistent chemical species [4]. The oxidation/reduction processes of Cr (VI) and Cr (III) in soil are thermodynamically spontaneous and can take place simultaneously [5]. The speciation of Cr in soil depends on factors such as pH, organic matter content, biological/microbial conditions, and metal concentration [6].

Phytoremediation of soils contaminated with heavy metals consists of their removal, transfer, and stabilization with plant species [7, 8]. It is an economical, aesthetically pleasing, and passive proposition. Bioconcentration, and translocation factors (BCF and TF) are two important parameters used to evaluate the effectiveness of plant species in the phytoremediation process [9]. A BCF > 1 indicates that the plant has phytoremediation potential [10]. A TF > 1 implies that the plant has the potential to transfer the metal from the root to the aerial part, and when it is close to zero, it signifies that the root accumulates the pollutant [9].

Different plants, *v. g. Pluchea indica*, *Phyllanthus reticulatus*, *Echinochloa colonum*, *Vetiveria nemoralis*, and *Amaranthus viridis* [11], have been proposed as phytoremediators of soils contaminated with chromium but little research has combined the role of the plants and the microorganisms in the rhizosphere in the phytoextraction and/or reduction of the hexavalent chromium. In this study, we investigated the phytoremediation capacity of Cr (VI) in a contaminated industrial soil using two different plant species: *Cynodon dactylon* (Fig. 1) and Portulacaria afra (Fig. 2). *C. dactylon* is widely distributed throughout the world, which is why it is considered the most important species of grasses [12]. It belongs to the *Poaceae* family and is known as Bermuda grass. It is a short-leaved perennial species, with creeping stolons and rhizomes, and possesses several thin spikes on top of its upright stems [13]. The genus Portulacaria includes succulent shrubs or small trees with branched trunks, green

sessile leaves, inconspicuous pink flowers and pink fruits 2 to 6 mm in diameter. *P. afra* is a specimen that has high importance in arid environments, as it is tolerant to drought. [14].

Figure 1. Cynodon dactylon

Figure 2. Portulacaria afra

Soil microorganisms may be involved in the regulation of the biogeochemical behavior of heavy metals [15]. Many of them adapt and reproduce in contaminated environments and develop both resistance and remediation mechanisms, such as biosorption and biotransformation, either by specific enzymes or by cellular metabolites [16, 17]. Numerous bacteria have the capability to reduce Cr(VI) to Cr(III) as a mechanism of resistance to Cr(VI), including *Escherichia coli*, *Pseudomonas putida*, and *Bacillus sp.* [18]. We also studied the role of microorganisms found in the soil and associated with the plant's rhizosphere, in the reduction of hexavalent chromium.

II. METHODOLOGY

2.1 Soil characterization

A 25 kg-sample of industrial soil contaminated with Cr (VI) was obtained from a tannery located in the city of León, Guanajuato, Mexico. The physicochemical analyses regulated by NOM-021-SEMARNAT-2000 official standard were performed [19]: pH, humidity, real density, bulk density, organic matter, inorganic nitrogen, and cation exchange capacity (CEC). Texture, humidity at field capacity, pore space, and extractable phosphorus, and potassium were also analyzed [20].

2.2 Assembly of phytoremediation and sampling reactors

P. afra specimens were transplanted individually into plastic containers with 400 g of contaminated soil. For *C. dactylon*, seeds were sown in plastic boxes with 3 kg of contaminated soil. The boxes were lined with high-density black polyethylene to prevent leachates. Controls were also planted in soil without Cr for each species. The experimentation lasted 192 days. Samplings of soil, roots, and aerial parts were conducted in triplicate every 24 days. The plant samples were dehydrated in the oven at 50 C to determine total Cr.

2.3 Determination of Cr (VI) and total Cr

The concentration of Cr (VI) in the soil was obtained from the alkaline digestion method and colorimetric test established by NOM-147-SEMARNAT/SSAI-2004 official standard [21], using a UV-VIS Lambda BIO 2.0 spectrophotometer at 540 nm. For total Cr in soil and plant tissues, the extraction of the metal was conducted through the EPA 3051A method of assisted acid digestion [22], using a MARS-X microwave and the digestions were analyzed in a SpectrAA-200 VARIAN atomic absorption spectrophotometer.

2.4 Evaluation of the phytoremediation process

The BCF and TF were obtained from the concentrations of total Cr using the following equations [10]: BCF = concentration in plant tissues / concentration in the substrate; TF = concentration in the aerial part / concentration in the roots. The removal efficiency of Cr (VI) was obtained from the initial (Ci) and final (Cf) concentrations of the pollutant: Removal % = [(Ci - Cf] / Ci] x 100.

2.5 Bacterial population growth

The total count of plate colony-forming units (CFU) was performed to determine the tolerance and growth of viable microorganisms present in the rhizosphere both in clean soil and in contaminated soil in each sampling [23].

2.6 Culture and identification of microorganisms

The microorganisms associated with the rhizosphere of both plants after the phytoremediation process were investigated. A surface planting of contaminated soil samples was conducted using soybean trypticasein agar

(TSA) with 100 g/mL of Cr (VI) as a culture medium. Petri dishes were placed in an incubator at 37 C for 48 hours until colonies of microorganisms tolerant to Cr (VI) were obtained. The two most frequent colonies were selected according to their morphology and isolated by striated seeding. A sample was taken from each colony and inoculated in TSA and Sabouraud agar (SDA) plates with 100 g/mL of Cr (VI) until pure cultures were obtained. Plate reseeding was conducted three times. The samples were incubated at 37 C for 48 hours. Pure cultures (bacteria and fungus) were characterized macro and microscopically by their morphology.

2.7 Reduction of Cr (VI) by bacteria

Isolated bacteria, standardized to 6.29 x 10^6 CFU/mL were inoculated in reactors with 100 mL of minimum M9 salt medium (composition (g/L): 0.246, $MgSO_4 \cdot 7H_2O$; 0.01, $CaCl_2$; 3, KH_2PO_4; 6, Na_2HPO_4; 1, NH_4Cl; 0.5, NaCl; and 0.5% (weight/volume) of glucose as a carbon source [24], and 50, 100 and 200 mg/L of Cr (VI) at 21 C and 200 rpm for 14 days. 2 mL aliquots were taken from each reactor on days 1, 4, 6, 8 and 14 of experimentation and centrifuged at 6000 rpm for 10 minutes. 1 mL of the supernatant was taken from each sample and the concentration of Cr (VI) was determined from the colorimetric method of diphenyl carbazide for water samples described in NMX-AA-044-SCFI-2014 official standard [25], and absorbance readings were obtained at 540 nm. Bacterial growth was obtained by optical density at 600 nm and by cell count using a Neubauer chamber.

2.8 Reduction of Cr (VI) by fungi

Spores of the isolated fungal strain were incubated in a reactor with 100 mL of minimal modified Lee medium [26], (composition (%): 0.25, KH_2PO_4; 0.20, $MgSO_4$; 0.5, $(NH_4)_2SO_4$; 0.5, NaCl; and 0.25, glucose), with a pH of 5.3 and 100 ppm Cr (VI) at 25 C and 200 rpm, for 9 days until the formation of biomass pellets. The mycelium pellets were washed three times with sterile distilled water and centrifuged at 3000 rpm for 10 minutes. 1 g of pellets were placed in reactors with 100 mL of LMM and 50, 100, and 200 mg/L of Cr (VI) at 25 C and 200 rpm for 14 days. 2 mL aliquots were taken from each reactor on days 1, 4, 6, 8 and 14 and centrifuged at 6000 rpm for 10 minutes. 1 mL of the supernatant was taken from each sample and the concentration of Cr (VI) was calorimetrically determined. The growth of the fungi in each sampling was obtained from the determination of biomass on a dry basis.

III. RESULTS AND DISCUSSION

3.1 Soil characterization

The results of the physicochemical analyses of the soil contaminated with Cr (VI) and the control soil are presented in Table 1. The soil has a clay-loam texture. The high content of organic matter in the soil favors fertility and water retention [27]. The strongly acidic pH of the soil is attributed to the pollutant, the Cr (VI) species in solution are in an acidic range (3.57 - 4.04) [28]. Field capacity was obtained to define the volume of water necessary to maintain humidity in the experiments. As for the control soil, texture and organic matter content were taken as the main criteria to select it, since it is similar to the soil under study.

The initial concentration of Cr (VI) was 2,683 mg/kg and exceeded the total reference concentration (TRC) stipulated in the Mexican standard for both agricultural/domestic/commercial (280 mg/kg) and industrial (510 mg/kg) soils. The concentration of total Cr was 5,782 mg/kg and that of Cr (III) was 3,099 mg/kg. In the control soil, the concentration of total Cr was 1.3 mg/kg. Since Cr (VI) was non-detectable, it was assumed that Cr was in trivalent form [21].

Table 1. Characterization of contaminated soil and control soil

Parameter	Contaminated soil	Interpretation	Control soil	Interpretation
pH	4.60 ± 0.07	Strongly acidic	6.55 ± 0.03	Moderately acidic
Real density (g/cm^3)	2.37 ± 0.001	-	1.58 ± 0.002	-
Apparent density (g/cm^3)	1.32 ± 0.02	Loamy mineral soil	1.09 ± 0.01	Clay mineral soil
Pore space (%)	44.13	-	31.01	-
Field capacity (%)	28.45 ± 0.25	-	34.26 ± 0.31	-
Texture (%)	Sand: 34.50 ± 0.01 Silt: 27.59 ± 0.02 Clay: 37.92 ±0.01	Clay loam	Sand: 18.31 ± 0.03 Silt: 46.20 ± 0.01 Clay: 35.52 ±0.01	Silty loam
Organic material (%)	6.14 ± 0.36	High	6.35 ± 0.24	Remarkably high
CEC (Cmol(+)/kg)	15.04 ± 0.06	Half	26.18 ± 0.13	High
Inorganic nitrogen (mg/kg)	20.23 ± 3.2	Half	42.20 ± 2.4	High
Extractable phosphorus (mg/kg)	22.31 ± 2.1	Half	31.45 ± 1.6	High
Extractable potassium (mg/kg)	5.85 ± 0.06	High	6.24 ± 0.09	High

3.2 Biomass generation

To analyze the growth of both plant species in the presence of the pollutant, the generation of biomass on a dry basis for the roots and the aerial part was determined in each sampling. Both species demonstrated tolerance to Cr (VI). For *P. afra*, the generation of root and aerial biomass was determined to be higher than that of *C. dactylon* (Figure 1) since the species has woody stems, thick roots, and the limited loss of its succulent leaves. On the other hand, germination of *C. dactylon* from seed slowed biomass generation. However, the roots of the grass consist of multiple thin and long filaments with a high capacity for extension in the medium, which occupied a large volume of the container during the treatment.

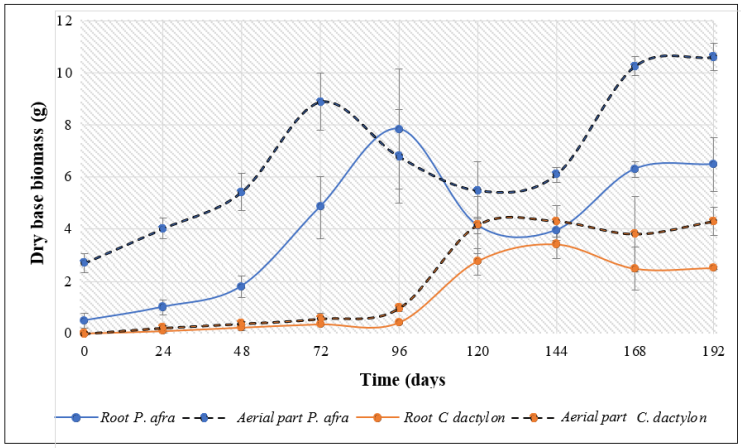


Figure 1. Biomass generation of *P. afra* and *C. dactylon*

3.3 Removal of Cr in the soil with plant species

In the first 24 days of treatment with *P. afra*, there was a removal of Cr (VI) in the soil of 51.87% (Figure 2a). However, only 1.7% of initial total Cr was removed (Figure 2b), which shows the reduction of Cr (VI) to Cr (III). This trend occurred until day ninety-six, obtaining a concentration of 199.8 mg/kg of Cr (VI) in soil, which is below the TRC. The concentration of Cr (VI) was maintained within the TRC until the end of treatment. The maximum percentage reduction of Cr (VI) was 98.1% and was obtained after 120 days of treatment (Figure 2a), leaving a remaining non-toxic concentration of Cr (III) (4,936.5 mg/kg) (Figure 2b). The predominant form of Cr until the end of treatment was Cr (III). The reduction in Cr (VI) can be attributed both to the excretion of root exudates of P. *afra* and to microbial action in the rhizosphere. The concentration of total Cr decreased at extremely low rates until day 120 of treatment (Figure 2b), and a significant removal by the plant could be observed once Cr (III) predominated in the soil from day 144 (38.5%).

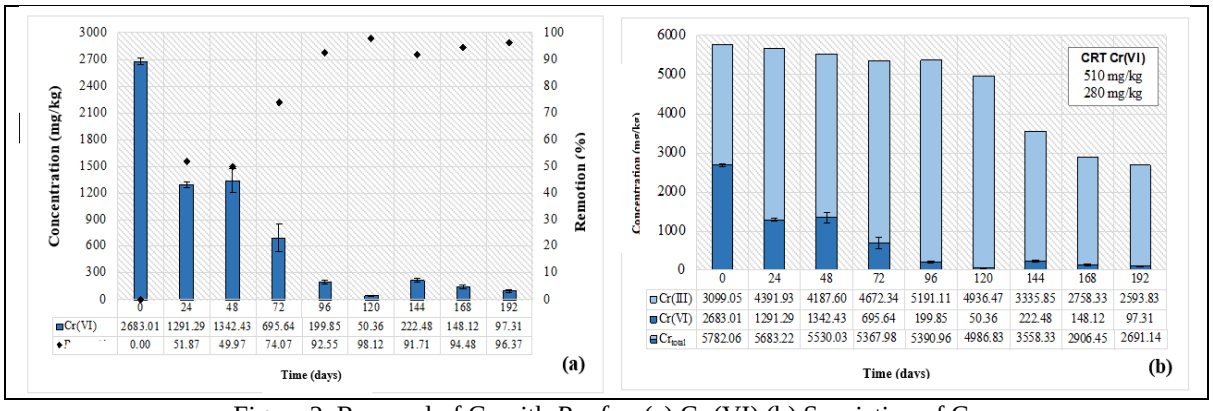


Figure 2. Removal of Cr with *P. afra*. (a) Cr (VI) (b) Speciation of Cr

Chromium was removed in 61.33% after 24 days of treatment with *C. dactylon*, when the grass was still in an early stage of development (Figure 3a). The maximum percentage of removal of Cr (VI) was 98.41% (192 days). The removal of Cr (VI) throughout treatment was due both to the absorption of *C. dactylon* and to the reduction of Cr (VI) to Cr (III) (Figure 3b), stimulated by root exudates and by microbiological action in the rhizosphere. The concentration of Cr (VI) decreased to 421.9 mg/kg after 48 days of treatment, which is below the TRC for industrial soils. At 96 days (73.5 ± mg/kg) definitive compliance with both TRCs was achieved. Total Cr removal could be observed from the beginning of treatment, due to the rapid development of the C. *dactylon*

root system. From day ninety-six until the end of treatment, the residual Cr in the soil remained mostly in its trivalent form.

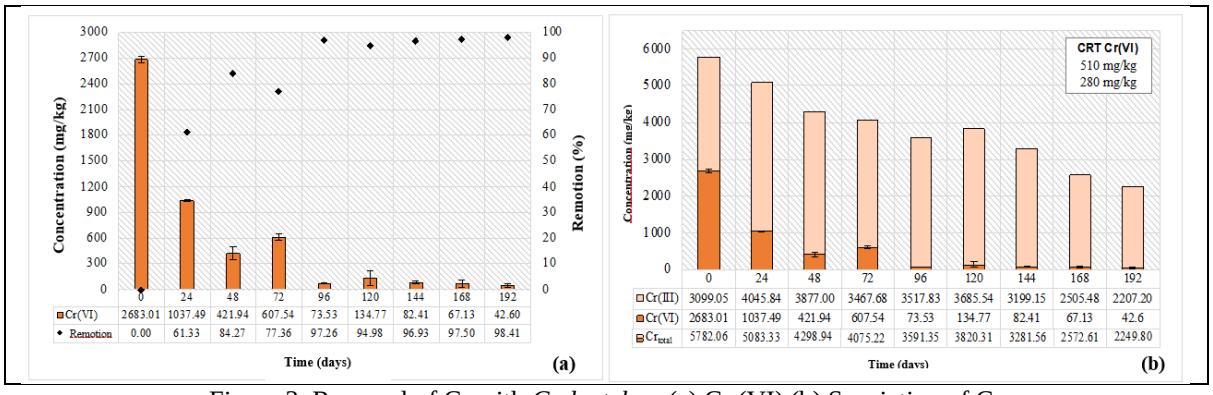


Figure 3**.** Removal of Cr with *C. dactylon*. (a) Cr (VI) (b) Speciation of Cr

After 24 days of treatment, removal percentages greater than 50% were observed using both plant species (*P. afra*: 51.87%, *C. dactylon*: 61.33%). With both plant species, it was possible to maintain Cr (VI) removal/reduction rates above 90% from day ninety-six of treatment and the maximum removal percentages occurred when the Cr in the soil was mostly in the form of Cr (III).

3.4 Accumulation of Cr in roots and aerial part

The concentration of total Cr in the roots and aerial part of both species is shown in Figure 4. With *P. afra*, the greatest accumulation of Cr was obtained at the root, reaching 2,293.0 mg/kg on the last day of treatment. The accumulation in the aerial part was not significant obtaining a maximum concentration of 37.43 mg/kg, so we concluded that *P. afra* is a phyto-stabilizer plant. The high generation of biomass of *P. afra* is another relevant factor in phyto-stabilization, since the thick and woody root system of the species results in a wide surface area that promotes the absorption of the contaminant, as well as the production of root exudates that regulate its speciation and immobilization [29].

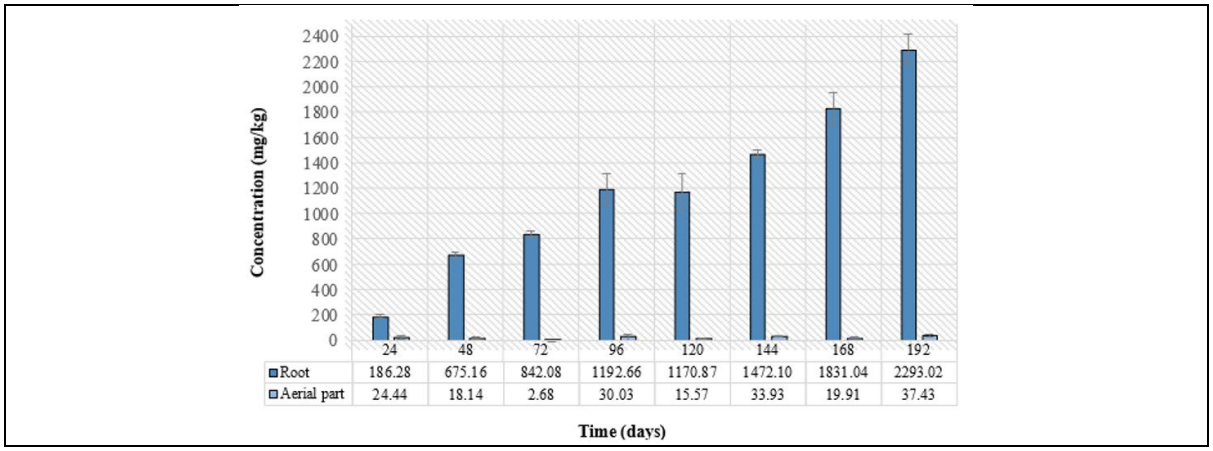


Figure 4**.** Total Cr concentration in the root and aerial part of P. *afra*

With *C. dactylon*, a similar trend in Cr accumulation was observed in the roots, obtaining a maximum concentration of 4,664.7 mg/kg at 120 days of treatment. The maximum concentration of Cr in the aerial part was 660.6 mg/kg. It can be concluded that *C. dactylon* is also a phyto-stabilizing species, as previously suggested by Sampanpanish *et al*. [11], and Nelushi *et al*.[30].

3.5 Evaluation of the phytoremediation process

The accumulation of Cr took place in the roots of both species. The BCF were 0.87 and 1.66 and the FT, 0.02 and 0.01, for *P. afra* and *C. dactylon*, respectively, since the translocation process to the aerial part was limited. Likewise, the elevated values of root BCFs confirm that Cr accumulated in the roots. This suggests that both *P. afra* and *C. dactylon* are phyto-stabilizing species of Cr. This accumulation of the metal in the roots decreases its mobility and bioavailability in the soil. Plants that have a BCF > 1 root and a TF < 1 have been considered as potential species for phyto-stabilization [31]. The results of *C. dactylon* meet both conditions. As for *P. afra*, the BCF obtained was 0.87.

Table 2. BCF and TF of plant species in soil contaminated with Cr.

Plant species	BCF	TF
P. afra	0.87	0.02
C. dactylon	1.66	0.01

3.6 Effects of pH on Soil

The existence of the two most stable forms of Cr in the environment, Cr (VI) and Cr (III), depends on the pH conditions present. The evolution of the pH of contaminated soil during phyto-stabilization with *P. afra* and *C. dactylon* is presented in Figure 5.

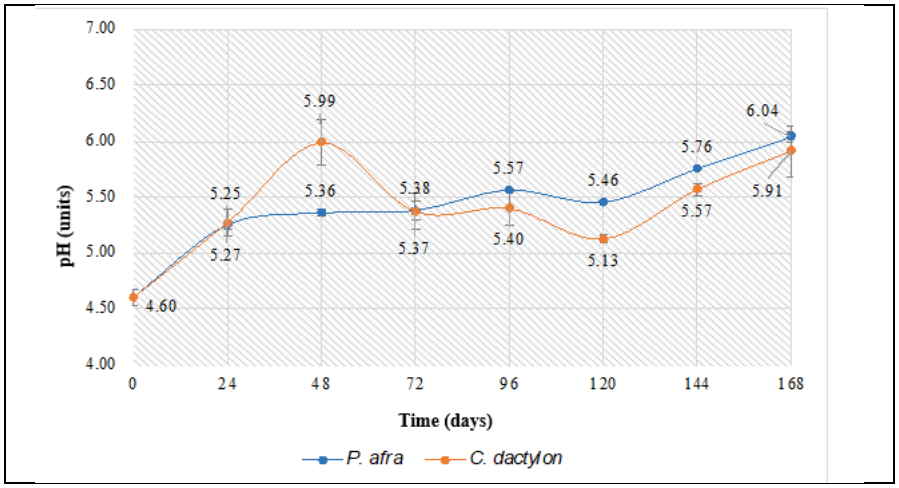


Figure 5. pH of contaminated soil

The initial pH of the contaminated soil was 4.6 ± 0.1 and reached moderately acidic conditions from the introduction of the plant species at the end of the treatment. The acidic pH conditions in the soil favored the of the Cr (III) species [32].

Another factor that intervened to maintain an acidic pH was the initial high content of organic matter in the soil, associated with root development and microbial growth in the rhizosphere. Organic matter is an electron donor and promotes the reduction of Cr (VI) to Cr (III) [33]. Cr (III) has a low solubility at pH < 5.5, however, it can be absorbed by plants. Above that pH, it tends to adsorb on soil surfaces and can precipitate in the form of hydroxides, becoming stable and less mobile in natural environments [34]. Both effects could occur during the phytoremediation process with both plant species, since the pH stayed in a range of 5.25 to 6.04 from day twenty-four until the end of the treatment.

3.7 Bacterial population growth

The initial bacterial count for the contaminated soil was 5.31 x10^5 CFU/g d. s., with a higher amount for the initial control soil, of 5.39 x10^7 CFU/g d. s.; it is common for a clean soil to have a higher growth of microorganisms than a contaminated soil [34]. The plate count in CFU of bacterial population of the rhizosphere of contaminated and control soils during treatment with *P. afra* and *C. dactylon* can be observed in Figure 6.

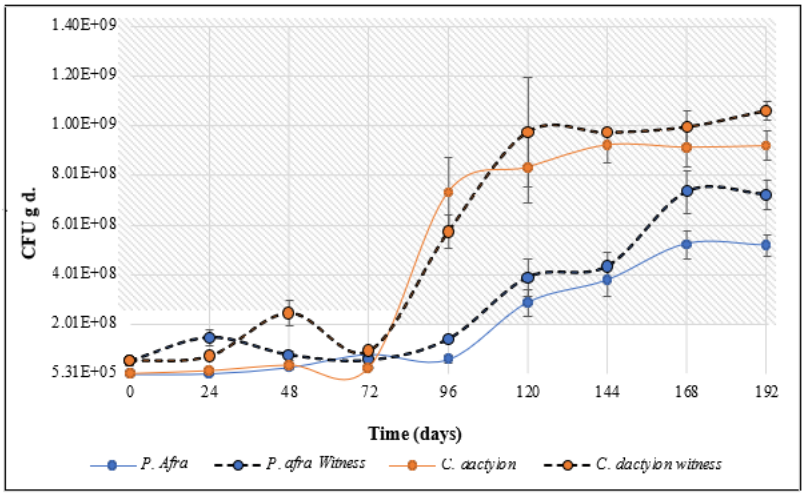


Figure 6. Growth curve of microorganisms with *P. afra* and *C. dactylon*

As the roots of both plant species began to interact with the soils (control and contaminated) and a rhizospheric environment was consolidated, the gradual increase in the microbial population began to be observed. The microbial growth in the control soil of both species was always higher than in the contaminated soil, however, the difference in CFU does not exceed 30% growth, so the microbial population demonstrated tolerance to Cr.

In treatment with *both species* in contaminated soil, we observed a dormancy phase in the growth of the microbial population during the first 48 days of treatment. However, with the grass, the exponential phase of growth of microorganisms occurred from day seventy-two of treatment, reaching a maximum number of 9.22 x 10^8 CFU/g of dry soil (day 144), and maintaining a stationary phase until the end of the treatment. With P. *afra*, the exponential phase occurred from day ninety-six of treatment and the maximum number of viable microorganisms was 5.23 x 10^8 CFU/g d. s. (day 168).

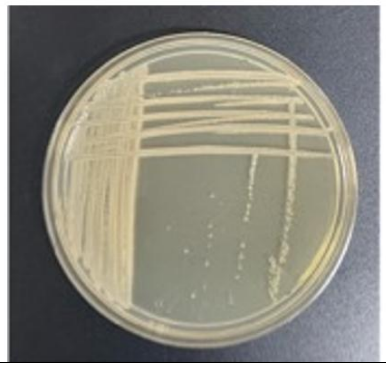
Perennials can be used for land management because they have a high permanence in the soil and have deep or woody roots that sequester carbon for years [35]. Both *C. dactylon* and *P. afra* are perennial species, however, the root system of *C. dactylon* was formed by multiple long and thin branches with a high capacity for extension in the soil, which could cause a higher amount of available carbon, and in turn, a greater microbial population than in the middle of P. *afra*, whose roots are thicker and woodier, but presented lower elongation and extension.

3.8 Bacterial reduction of Cr (VI)

Eight strains of indigenous microorganisms from the contaminated soil with different morphological characteristics that were tolerant to Cr (VI) were visually spotted, of which the two most constant in the dilutions made during plate counting were selected. The selected strains correspond to species of bacteria and fungi. Both were identified from their phenotype.

3.9 Bacterial strain

The illustration of the pure plate culture of the selected bacteria and the morphology of the corresponding colony based on the method suggested by Castañeda-Briones [23] are shown in Figure 7.



Size¤	1·mm¤
Surface¤	Smooth¤
Elevation¤	Pulvinate¤
Edge¤	Whole·or·continuous¤
Color¤	Beige¤
Opacity¤	Bright¤
Consistency¤	Mucous¤

Figure 7. Pure culture of the bacterial species isolated in TSA (left) and description of its morphology (right)

To microscopically visualize the bacteria and continue with its differentiation, Gram staining tests were performed, finding Gram-negative red bacilli (*Bacillus sp.*). Gram-negative bacteria can effectively eliminate Cr (VI) by biosorption, biotransformation, or both mechanisms [36]. The microscopies obtained are presented in Figure 8. Formerly, Cr resistant bacteria and bacterial consortia have been isolated from soils, effluents, and sludge from tanneries, such as *Lactobacillus*, *Bacillus sp.*, *Stenotrophomonas maltophilia*, *Staphylococcus sciuri, and Pseudomonas aeuruginosa*, among others [37].

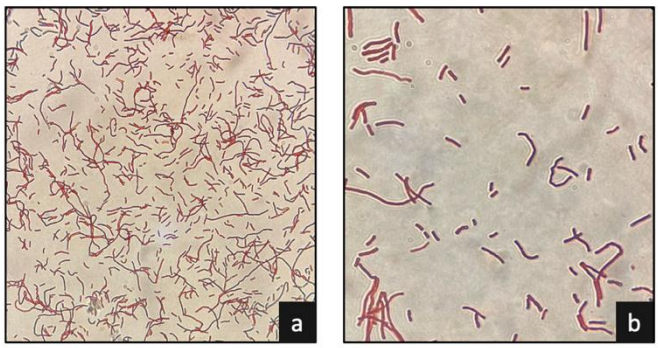


Figure 8. Gram stain in *Bacillus sp.* (a) forty times (b) one hundred times

3.10 Reduction of Cr (VI) by bacteria

The highest reduction rates of Cr (VI) by the identified strain of *Bacillus sp.* for 14 days of treatment. was observed in the reactor of 50 ppm (Figure 9). A 42.55% reduction was recorded on the first day, reaching 44.75% at the end of treatment. In the 100 and 200 ppm reactors, lower reduction rates were observed with similar trends from day six of treatment to the end of it with 26.89% and 27.59%, respectively.

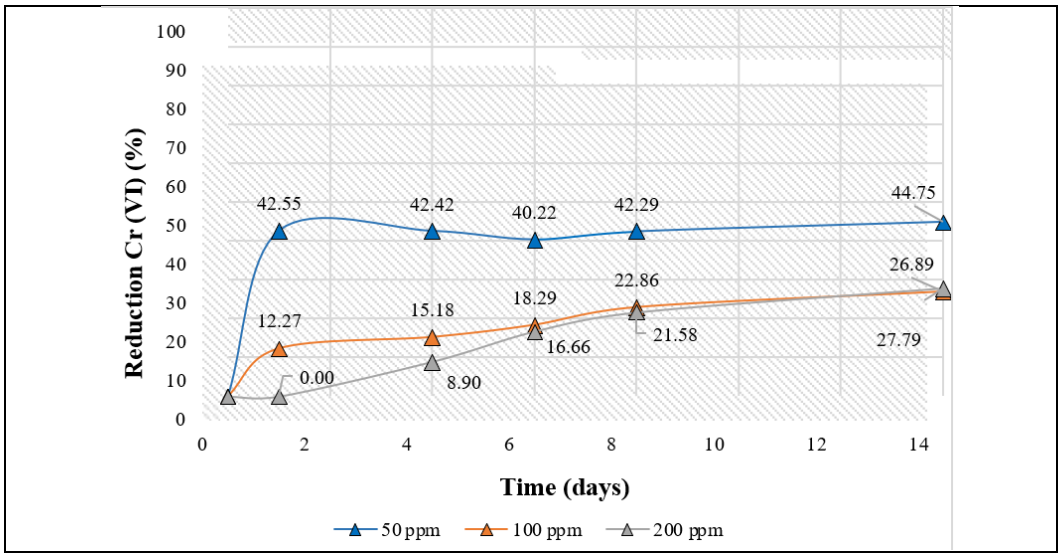


Figure 9. Reduction of Cr (VI) by *Bacillus sp.*

3.11 Fungal strain

The pure plate culture with SDA of the selected fungus is shown in Figure 10. Macroscopic features include dense, granular mycelial colonies with an initial white appearance that were subsequently covered with black spores (Figure 10 (a)). The reverse of the colony is yellow (Figure 10 (b)).

Figure 10. Pure culture of the isolated fungal species in SDA (a) Colony on obverse (b) Colony on reverse

As for the microscopic characteristics, it is a filamentous fungus, with long conidiophores and smooth-walled hyalines. It has a globose vesicle with biseriate phylalides around it and has black globose and rough conidia. It has been reported that filamentous fungi have a high tolerance to Cr (VI), especially those that inhabit contaminated sites. The macroscopic and microscopic characteristics of the isolated fungus suggest that it belongs to the genus *Aspergillus*. The characteristic conidiophore of *Aspergillus* was observed, as well as the structures that compose it: vesicle, stipe and foot cell [38]. Taking a morphological criterion to classify the species, it is highly probable that it was a strain of *Aspergillus niger* [39].

In general, *Aspergillus* is one of the genera that is frequently isolated in sites contaminated with Cr [26]. *Aspergillus* has been reported as tolerant of Cr (VI) at 600 ppm, as well as 5000 ppm for total Cr [40, 41].

3.12 Reduction of Cr (VI) by fungi

Reduction of Cr (VI) by the strain of *Aspergillus sp.* for 14 days of treatment is presented in Figure 11. From the first day of treatment, there was a high Cr (VI) reduction, 58.84% in the 50-ppm bioreactor, reaching a reduction of 100% after 4 days. It is also observed that from day five until the end of treatment, the reduction rates remained above 99%. In the 100-ppm bioreactor, a reduction of 21.33% was obtained on the first day, satisfactorily ending with 97.28%.

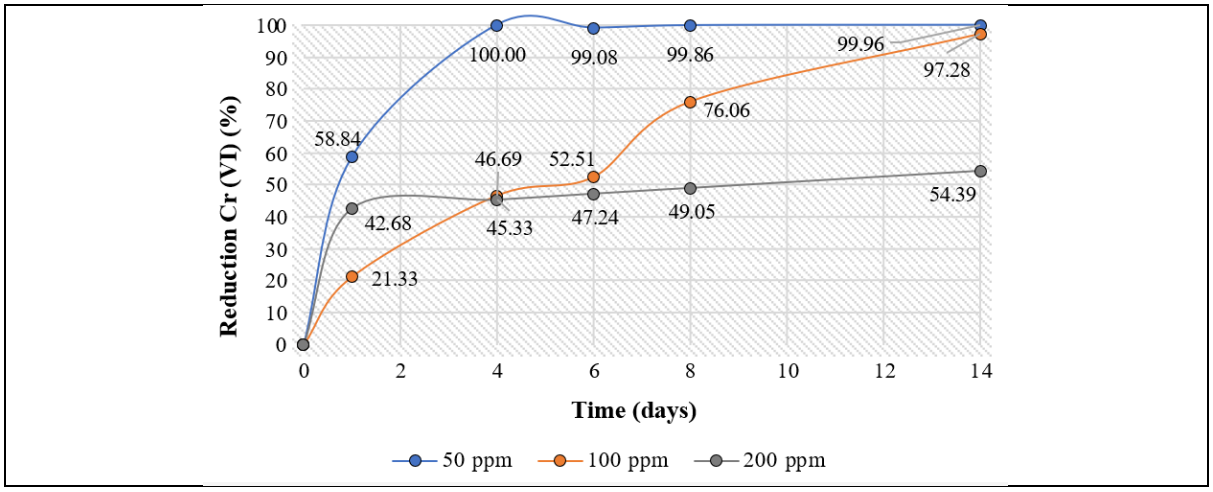


Figure 11. Reduction of Cr (VI) by *Aspergillus sp.*

As for the 200-ppm bioreactor, a reduction of 42.68% was registered on the first day. A higher initial concentration of metal ions provides a higher propulsive force that is capable of overcoming the mass transfer resistance of metal ions between the aqueous and solid phases, resulting in a higher probability of collision between Cr (VI) ions and the sorbent biomass. This also results in a higher metal sorption capacity (García-Hernández *et al.*, 2017). Likewise, in this bioreactor a reduction of 54.39% was recorded, being the lowest efficiency of the experiments at the end of the treatment.

IV. CONCLUSION

The phytoremediation process of Cr (VI) of a contaminated industrial soil using the plant species *P. afra* and *C. dactylon* was evaluated. Both plants were tolerant to Cr (VI). The maximum removal rate of Cr (VI) obtained was 98.41% at the end of the treatment using *C. dactylon*, however, with *P. afra* a similar removal rate

of 96.37% was obtained, which evidences its potential for phytoremediation of the heavy metal and to comply with the Mexican regulatory standards stipulated in NOM-147-SEMARNAT/SSA-2004 official standard for soils for agricultural. domestic, commercial, and industrial use. Treatment with *P. afra* was governed by the reduction of Cr (VI); the plant absorbed Cr once it was mostly in its trivalent form. Removal with *C. dactylon* was due to both absorption and reduction throughout treatment. The reduction in Cr (VI) in the two treatments can be attributed both to root exudates and to the growth of the microbial population in the rhizosphere. Likewise, the root development of the species and the moderately acidic conditions in the substrates stimulated the reduction process.

P. afra and *C. dactylon* accumulated Cr in the roots and their translocation to the aerial part was negligible, making them phyto-stabilizing species. The highest accumulation of Cr was obtained with *C. dactylon* with an FBC of 1.66. Using *P. afra*, an FBC of 0.87 was obtained at the end of the experiment, but the trend of Cr accumulation suggests that it could increase with more treatment time due to its high biomass generation and its woody root system that represents a large surface area for absorption.

Two families of indigenous microorganisms from the contaminated soil, namely *Bacillus sp.* and *Aspergillus sp.* Contributed to the reduction of Cr (VI). The highest reduction rates at different concentrations of Cr (VI) were recorded using the *Aspergillus sp*. strain due to the versatility of adaptation typical of fungi in hostile environments and their high capacity for penetration and expansion in the environment, obtaining 99.96% (50 ppm), 97.28% (100 ppm) and 54.39% (200 ppm) at the end of the experiment. In contrast, reduction rates using the *Bacillus sp.* were lower: 44.75% (50 ppm), 26.89% (100 ppm) and 27.79% (200 ppm).

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