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# Germination of *Bouteloua dactyloides* and *Cynodon dactylon* in a Multi-Polluted Soil

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**Abstract:** Mining wastes generate high environmental impacts, and population exposure to metals and metalloids. Phytoremediation is a technology that uses plants to remediate polluted sites, but one of its limitations is seed germination in soil with a high content of metals and metalloids. *Bouteloua dactyloides* (former *Buchloe dactyloides*) is a native species from semiarid regions, while *Cynodon dactylon* is an invasive species; both are tolerant to harsh soil conditions. The objective of this research was to evaluate the germination of both species, exposed to a multi-polluted soil with As, Cd, Pb, and Zn of a mining site, considering different pH conditions (from 5.0 to 9.0). The study considered four repetitions by type of seed and soil pH. The highest germination of *B. dactyloides* was 83% at pH 7.8, while the greatest germination of *C. dactylon* was 34% at pH 6.0. These percentages are similar to those obtained in a standard germination test, which are 82.5% for *B. dactyloides* and 35% for *C. dactylon*. Germination was not reached in either species with soil at pH 5, owing to the fact that metals are more bioavailable in acid environments. *B. dactyloides* and *C. dactylon* had a high potential to germinate in multi-polluted soil at neutral pH, but further experiments are needed.

**Keywords:** sustainable technology; phytoremediation; germination; *Bouteloua dactyloides*; *Cynodon dactylon*

## 1. Introduction

The wastes generated by mining are a high-impact environmental problem because the population can be exposed to metals and metalloids. A change of pH and oxidation state can increase or decrease the potential bioavailability of metals in soil [1]. Metals such as Zn, Cu, and Mn are essential for living organisms at low concentrations, but become toxic at increasing concentrations. Other metals like Hg, Pb, and Cd have never been shown to be essential for organism development and are toxic at low concentrations [2]. Environment pollution with heavy metals by increased industrialization and geochemical activities is a major environmental and human health problem [3] due to these metals' non-biodegradability and high toxicity [4].

Potentially toxic elements (PTE) in Mexico, such as Pb, Cd, Zn, As, Se, and Hg, are commonly derived from mining processes [5]. From these elements, Pb and Cd are the pollutants most frequently found in the mining zones of the country [6]. Moreover, in soil, the metals of most concern are Cd and Zn, as they show the greatest mobility [3]. The environmental impact caused by the pollution in mining sites depends on the interaction capacity of metals with soil and water [1]. The degree of metal toxicity hangs on the element and its bioavailability, which it is controlled by abiotic factors such as metal concentration, soil pH, and biotic factors such as the presence of metal-liberating microflora [2]. If metals are bioavailable in the soil, they can affect the fertility and/or later land use, as well as induce population exposure [7].

Phytoremediation is an emerging technology that uses plants and their rhizosphere to extract, detoxify, and sequester pollutants (organic and inorganic) from soil, sediments, and water [8]. This technology can be considered a lower cost–benefit alternative compared to mechanic or physic-chemical processes [9]. The pollutant uptake by plants depends on pH, organic substances, metal content, and other elements in the rhizosphere [2]. Seed germination is the first step of a plant's life; it is one of the most sensitive processes in their physiology. Germination can be affected by hormonal interactions and environmental factors, both biotic and abiotic, such as a metal presence [10]. Seed germination studies are requisite groundwork to determine if plants are suitable for growth in polluted soils for phytoremediation applications [8]. Unfortunately, predicting the response of organisms simultaneously exposed to more than one PTE is a difficult task in environmental toxicology [4].

Different wild plant species have been used for phytoremediation [11]. Several studies have been developed with *B. dactyloides* and *C. dactylon* on phytoremediation for metal removal [12–15]. In Mexico, *B. dactyloides*, which is commonly called "Buffalograss", is a perennial native grass from semi-arid regions. Buffalograss requires low amounts of water, is drought-resistant, tolerates high salinity and low temperatures, has a low nutrient demand, and grows in argillaceous soil [16]. Another wild plant is *C. dactylon*, a perennial native plant from Africa commonly called "Bermudagrass". It grows from argillaceous to sandy soils with a pH of 5.5 to 5.7 and tolerates salinity as well as dry and warm summers. The sod growth of this species and their tolerance to halophyte soils are important characteristics for the development of material for covering mining wastes. Therefore, the first step is to evaluate their germination exposed to soil polluted with As, Cd, Pb, and Zn at a mining site, considering different soil pH conditions.

## 2. Materials and Methods

### *2.1. Soil Collection*

The soil was collected from an area southwest of Chihuahua City, Mexico, in a zone with mining residues (tailing dams) from a former foundry. The sampling coordinates were X = 402,288.6431, Y = 3,166,594.5405, in 13 zones; at 1578 m.a.s.l. The climate is predominantly dry-tempered, and the precipitation range is around 200–400 mm.

Five different soil samples were collected superficially (at 5 cm), extracted with a stainless steel shovel, and classified as A, B, C, D, and E. A non-polluted soil sample was collected as a reference, called F. According to the Universal Soil Classification System (USCS), the soil samples were cataloged as poorly graded sand.

### *2.2. Soil pH*

The pH determination of the soil was performed for each collected soil sample (A, B, C, D, E, F) with previously calibrated Thermo ORION 3 STAR equipment. The samples were prepared according to the method from the Mexican Regulation NOM-147 [17].

The pH of Soil Sample D was chemically modified to generate strongly acid conditions, adding 200 mL of hydrochloric acid (HCl) 3 N to 500 g of soil.

Soil Sample E was modified to preserve strongly alkaline conditions (pH 9). To 500 g of soil, 15 g of sodium carbonate ($Na_2CO_3$), 8 mL of sodium hydroxide (NaOH) 0.1 N, and 3 mL of NaOH 1 N were added.

### 2.3. Total Metals

Prior to the analysis, soil samples of each type were dried at 60 °C for 24 h. To determinate total metal concentration in soil, samples were digested in a microwave MARSx CEM, under stated conditions in the SW 846-3051 method [18].

Soil concentration of As, Cd, and Pb were compared to the reference concentration (Rc) in NOM 147, stated for residential and industrial use. The Zn concentration was compared with the reference level of ecological and human health risk indicated in the Federal Attorney for Environmental Protection of Mexico [19].

### 2.4. Soluble Metals

The extraction and analysis of soluble metals in each soil sample were based on the procedures in NOM-147, where the soil reacts with water liberating the PTE.

An extracting agent solution ($H_2O$–$CO_2$ at pH = 5.5 ± 0.2) was prepared, 500 mL of the extracting agent solution were added to 25 g of soil. The mix was agitated for 18 ± 0.25 h on constant rotation equipment (Environmental Express). After agitation, the mixture was filtrated, and the extract was digested according to the SW 846.3015A method [20]. The analysis of the total and soluble metals (As, Cd, Pb, Zn) was performed with Optic Plasma Equipment ICP Thermo Jarrel Ash IRIS/APDIV and with an Atomic Absorption Spectrophotometer with Generation Hydride GBC Avanta (Sigma-Mexico).

### 2.5. Seed Germination

Prior to the setup of the experiment, a standard germination test was done to evaluate seed quality; it was performed in four repetitions with non-contaminated sand for each species, 30 seeds for each Petri dishes, and irrigated with faucet water.

The germination experiment of the two grass species, *B. dactyloides* and *C. dactylon*, was prepared in the collected Soil Samples A, B, C, D, E, and in the reference F. Four repetitions with 30 seeds each were prepared by the type of soil and grass species. The Petri dishes were irrigated every three days with the type of water corresponding to the pH of each soil (Table 1). Soil Sample A was irrigated with distilled water (pH 5.4); Soil Samples B, C, and F with faucet water (pH 7.9). For Soil Sample D, 2 L of distilled water were prepared and 0.5 mL of HCl 1 N added to obtain acid water conditions (pH of 4.6). For Soil Sample E, 2 L of distilled water were prepared and 5.0 mL of NaOH 0.1 N added to obtain alkaline conditions (pH of 9.0).

**Table 1.** Germination conditions for each type of soil.

| Soil Sample | Soil pH | Irrigation Water pH |
|---|---|---|
| **A** | 6.0 | 5.4 |
| **B** | 7.8 | 7.9 |
| **C** | 7.0 | 7.9 |
| **D** | 5.0 | 4.6 |
| **E** | 9.0 | 9.0 |
| **F** | 8.0 | 7.9 |

The prepared boxes were placed in a drying stove at 28 ± 2 °C during the germination period. Every third day, the germinated seeds of each box were counted, so the germination percentage and variation coefficient were compared by grass species.

A Bartlett's test of homogeneity variance and an ANOVA analysis were done using Minitab 16, considering a 95% confidence for both grasses regarding the type of soil, the mean, the standard deviation, and the variation coefficient ($V_c$). Additionally, a general linear model (PROC GLM) in SAS 9.1.3 (2009, SAS Institute Inc., Cary, NC, USA) was considered to compare germination between grasses, and a test of least significant difference (LSD) was performed to contrast germination among soils and grasses.

## 3. Results

### 3.1. Total Metals

Metal concentration (As, Cd, Pb) of the collected soil exceeded the Rc for residential use in the Mexican Regulation NOM-147 at 22 mg of As $kg^{-1}$, 400 mg of Pb $kg^{-1}$, and 37 mg of Cd $kg^{-1}$. It also exceeded the Rc for industrial use at 260 mg of As $kg^{-1}$ and 800 mg of Pb $kg^{-1}$ (Table 2).

The amount of Zn in the analyzed soil exceeded the Rc established in Mexico for ecological risk at 300 mg of Zn $kg^{-1}$ and human health risk at 800 mg of Zn $kg^{-1}$ [19] (Table 2).

**Table 2.** Total metal in soil.

| | | Concentration ($mg \cdot kg^{-1}$) | | | |
|---|---|---|---|---|---|
| **Soil Sample** | **pH** | **As** | **Cd** | **Pb** | **Zn** |
| A | 6.0 | 2153.30 | 82.58 | 6340.38 | 8082.77 |
| B | 7.8 | 2447.15 | 98.72 | 6227.56 | 11,441.56 |
| C | 7.0 | 1171.16 | 200.52 | 9172.16 | 9506.29 |
| D | 5.0 | 2956.18 | 126.89 | 5455.38 | 14,502.99 |
| E | 9.0 | 2549.33 | 142.91 | 5516.44 | 15,309.95 |
| F | 8.0 | 27.61 | 4.60 | 401.56 | 229.49 |

### 3.2. Soluble Metals

Table 3 shows the results of soluble-metal concentration in $mg \cdot L^{-1}$. As varied from 0.07 to 0.13, Cd from 0.004 to 0.36, Pb from 0.02 to 0.10, and Zn from 0.05 to 29.51. Only Cd exceeded the Rc of 0.10 $mg \cdot L^{-1}$ indicated in NOM-147 (Soil Sample D). The toxicity of PTE is proportional to the solubility of the solid stage in which they are associated [21].

**Table 3.** Soluble metal in soil.

| | | Concentration ($mg \cdot L^{-1}$) | | | |
|---|---|---|---|---|---|
| **Soil Sample** | **pH** | **As** | **Cd** | **Pb** | **Zn** |
| A | 6.0 | 0.09 | 0.04 | 0.03 | 1.28 |
| B | 7.8 | 0.10 | 0.05 | 0.06 | 1.62 |
| C | 7.0 | 0.08 | 0.10 | 0.05 | 0.17 |
| D | 5.0 | 0.07 | 0.36 | 0.02 | 29.51 |
| E | 9.0 | 0.10 | 0.007 | 0.10 | 0.51 |
| F | 8.0 | 0.13 | 0.004 | 0.04 | 0.05 |

### 3.3. Seeds Germination

The standard germination test demonstrated that *B. dactyloides* germinated at 82.5% and *C. dactylon* at 35%.

The germination time with the contaminated soil for both seeds was from 3 to 21 days. Figures 1 and 2 show the behavior over 21 days of seed germination for *B. dactyloides* and *C. dactylon* by the type of soil. *B. dactyloides* presented a greater germination percentage from the fifth day in Soil Samples A, B, C, and F.

The grass *B. dactyloides* presented a germination percentage from 68% to 83% in soils with pH between 7 and 8 (B, C, and F). In the soil with a pH of 6 (Soil Sample A), germination declined to 50%. The soil with an increased pH of 9 (Soil Sample E) caused a germination reduction to 13%, and the soil with acid conditions (pH of 5, Soil Sample D) did not germinate (Figure 1). The germination percentage of *C. dactylon* was from 28% to 34% in soils with pH of 6 and 7 (Soil Samples A and C). In the soil with pH > 7 (Soil Samples B and F), a germination reduction to 17% and 18% was presented. In the soil with pH values of 5.0 (Soil Sample D) and 9.0 (Soil Sample E), germination was not reached (Figure 2).

Despite the differences between germination percentages, both species showed a high germination compared with the standard germination test.

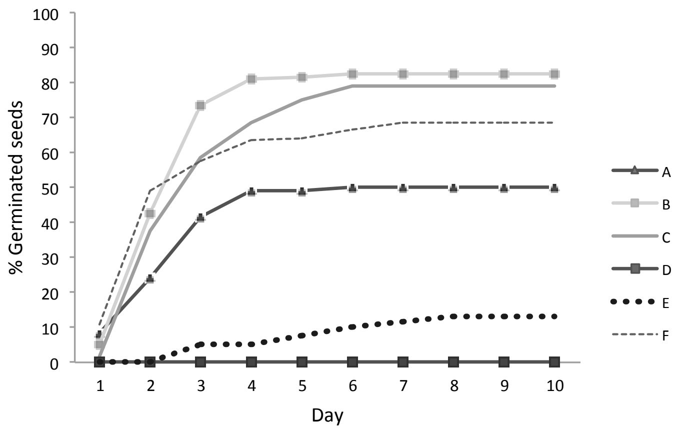


**Figure 1.** Germination percentage of *Bouteloua dactyloides*.

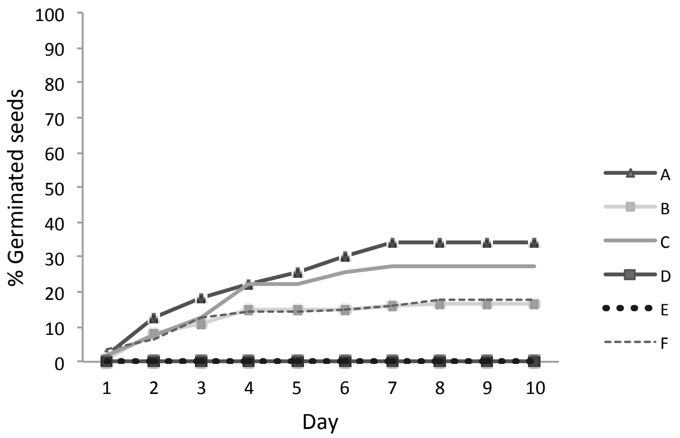


**Figure 2.** Germination percentage of *Cynodon dactylon*.

According to Bartlett's test, the data exhibit variance homogeneity ($p > 0.296$). PROC GLM model demonstrated that *B. dactyloides* and *C. dactylon* germination were significantly different ($R^2 = 0.9315$, $p < 0.05$).

In the LSD test ($p < 0.05$) the least significant difference was 3.9473. For *B. dactyloides* demonstrated that germination in Soil Samples B and C was not significantly different (pH of 7.8 and 7.0, respectively), nor was that of Soil Samples C and F (pH of 7.0 and pH of 8.0, respectively) (Table 4), so Soil Sample C soil had similar germination development than the uncontaminated soil. Soil Sample A was not similar to any other; and Soil Samples B, C, and F had a higher germination mean than A.

For *C. dactylon*, germination in Soil Samples A and C (pH 6 and 7, respectively) was not significantly different (soils with the higher germination for this species). Additionally, Soil Samples B and F soils (pH >7) were not significantly different, so Soil Sample B had similar germination development than that of the uncontaminated soil (Table 4).

**Table 4.** Groups by the LSD test.

| Group | | Mean | N | Treatment |
|---|---|---|---|---|
| | a | 24.75 | 4 | BDB |
| | a | | | |
| b | a | 23.75 | 4 | BDC |
| b | | | | |
| b | | 20.50 | 4 | BDF |
| | c | 15.00 | 4 | BDA |
| | d | 10.25 | 4 | CDA |
| | d | | | |
| e | d | 8.25 | 4 | CDC |
| e | | | | |
| e | f | 5.25 | 4 | CDF |
| e | f | | | |
| e | f | 5.00 | 4 | CDB |
| | f | | | |
| | f | 4.00 | 4 | BDE |
| | g | 0.00 | 4 | CDD |
| | g | | | |
| | g | 0.00 | 4 | CDE |
| | g | | | |
| | g | 0.00 | 4 | BDD |

Note: BD = *Bouteloua dactyloides*; CD = *Cynodon dactylon*; A, B, C, D, E, F = type of soil.

Tables 5 and 6 show the germination percentage, mean of germinated seeds, and variation coefficient ($V_c$) of each grass species and soil type.

**Table 5.** Germination analysis of *Bouteloua dactyloides* in each soil.

| Soil Sample | pH | % Germination | Germinated Seeds Mean | Variation Coefficient ($V_c$) |
|---|---|---|---|---|
| **A** | 6.0 | 50.0 | 15.0 ± 5.9 | 39.6 |
| **B** | 7.8 | 83 | 24.8 ± 3.4 | 13.8 |
| **C** | 7.0 | 79 | 23.8 ± 2.5 | 10.5 |
| **D** | 5.0 | 0 | 0 | - |
| **E** | 9.0 | 13 | 4.0 ± 3.6 | 89.0 |
| **F** | 8.0 | 68 | 20.5 ± 2.6 | 12.9 |

**Table 6.** Germination analysis of *Cynodon dactylon* in each soil.

| Soil Sample | pH | % Germination | Germinated Seeds Mean | Variation Coefficient ($V_c$) |
|---|---|---|---|---|
| **A** | 6.0 | 34 | 10.3 ± 3.0 | 29.1 |
| **B** | 7.8 | 17 | 5.0 ± 1.8 | 36.5 |
| **C** | 7.0 | 28 | 8.3 ± 1.0 | 11.6 |
| **D** | 5.0 | 0 | 0 | - |
| **E** | 9.0 | 0 | 0 | - |
| **F** | 8.0 | 18 | 5.3 ± 2.2 | 42.2 |

As far as the results of $V_c$, the greatest homogeneity on grass germination with *B. dactyloides* was in Soil Samples B and C with a $V_c$ of 13.8% and 10.5%, respectively. Meanwhile, with *C. dactylon*, it was Soil Sample C with a $V_c$ of 11.6% (Tables 4 and 5).

Grass germination reached a peak and stabilized, and this was determinate with a regression of orthogonal contrast, so the model proved being square ($p < 0.0001$).

### *3.4. Discussion*

*B. dactyloides* showed a better germination percentage than *C. dactylon*. Both species had higher germination percentages with soil with an approximately neutral pH, similar percentages than the standard test, and lower variation. In both grasses, there was no germination in Soil Sample D (pH of 5). Although Soil Sample D did not present a higher concentration of total metals than the other soils, it had a lower pH and a higher bioavailability of Cd and Zn; in fact, Cd content was higher than the Rc (Table 3). This metal solubility could cause a lack of development in both species, because soil pH has a strong influence on the bioavailability of metals and metalloids. Acidic soil pH makes metals more soluble; therefore, their bioavailability is greater for plants [22].

Cd has been known to interfere with the water uptake of plants, which is why germination does not occur [10]. Cd has also been reported to cause chromosomal aberration, with cytogenetic analysis of *Pisum sativum* root from seeds germinated on different concentrations of Cd, which demonstrated an increased frequency of abnormalities. The total number of aberrations increased with Cd concentration [8].

On the other hand, Zn is one of the essential micronutrients in plants; it is necessary for normal plant growth and development and is required in several metabolic processes. Nevertheless, high concentrations of Zn are toxic and hamper plant growth [3]. The excess of Zn affects the uptake of other nutrients, inhibiting seed germination, plant growth, and root development [10].

Rapeseed exposed to 1.12 mM Zn caused plant growth inhibition and chlorosis in leaves. The contents of Fe, Cu, Mg, and Mn decreased in roots and leaves. Additionally, it was demonstrated that Mg and Cu content decreased with increased Zn concentrations in ryegrass. These results indicated that an excess of Zn might suppress the uptake of these elements due to the competition among metals [23]. Moreover, a study with sunflower determined that seeds germinated at high Zn concentrations, while germination decreased to below 50% after exposure to Cd, Cu, and Pb [8].

Marichali et al. (2014) achieved germination of *C. sativum* seedlings irrigated with high Zn concentrations at a pH of 6.8, but root length was reduced. They showed that, for *C. sativum*, Zn has low toxic effects on germination, and roots are more sensitive than seeds to metal stress [3]. This result does not agree with the present research possibly because the pH and the germination conditions were different.

The bioavailability of Cd, Pb, and Zn decreases while soil pH increases due to its precipitation as insoluble hydroxides, carbonates, and organic complexes; opposite As bioavailability increases at a basic pH [24]. This can explain why *B. dactyloides* and *C. dactylon* reduced germination in Soil Sample E (Table 4) with a similar metal pollution level compared with the other soils (Table 2), but a basic pH induced more As bioavailability (Table 3). The most common genotoxic effects reported for As exposure involve mitotic spindle disturbance. In *Hordeum vulgare* seeds, the frequency of chromosomal abnormalities is proportional to the concentration of As [8].

Metal tolerance is an important factor for the use of plants and seeds for phytoremediation, so the capacity of seeds to germinate in the presence of metals and metalloids could be a primary concern [8]. This research supported the hypothesis that metals generally inhibit germination and seedling growth [25], but the metal distribution in seeds and affectation degree differs depending on the metal involved, the plant species, and the seed anatomy. Additionally, the soil pH interferes in the bioavailability of the metals and, with it, in the germination response [24]. Thus, seeds of metal-tolerant plants may have a higher threshold for toxicity than non-tolerant ones [8]. After permeation through the seed coat, germination relies on the seed reserves for metabolite supply for respiration, Metals can cause stress and disrupt the process, and interfere with the enzymes involved in the germination [10].

## 4. Conclusions

The concentration of total and soluble metals in the used soils for germination supported the supposition that the site of collection is highly polluted with As, Cd, Pb, and Zn.

The germination of *Bouteloua dactyloides* was greater than *Cynodon dactylon*. Moreover, *B. dactyloides* reached the highest germination percentage sooner than *C. dactylon*. However, both species showed similar germination at an approximately neutral pH compared with the standard test.

In soil at pH 5, the germination of both species was not reached, owing to the fact that metals are more bioavailable in acid environments; thereby, Cd and Zn were more soluble. *C. dactylon* do not even germinate at basic conditions in soil (pH 9) where As was more bioavailable.

*B. dactyloides* and *C. dactylon* tolerate metal contained in soil where the pH is approximately neutral. These grasses grow at a maximum of 20 cm, which could allow for a covering of the surface of tailings dams with a vegetal coat.

Since this species has been shown to be tolerant, it is necessary to evaluate and determine its ability to retain and/or accumulate metals from soil. Nevertheless, in future research, it is required that the soil pH and soluble metals are evaluated before a phytoremediation process is begun. In addition, more studies are needed to investigate the effects of metals on seed germination and plant growth in situ.

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**Author Contributions:** María del Rosario Delgado-Caballero performed the experiment and all analyses conducted. María Teresa Alarcón-Herrera headed the experiment and the data analysis. María Cecilia Valles-Aragón analyzed data and discussed the results; she also organized the structure of this paper. Alicia Melgoza-Castillo was involved in the experimental performance as the assessor of the main author during her PhD program. Arwell Leyva-Chávez collaborated with the statistic analysis of the data. Dámaris Lepoldina Ojeda-Barrios was integrated in this project because of her expertise on plant physiology, so she assisted with the development of both grasses.

**Conflicts of Interest:** The authors declare no conflict of interest.

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