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*Original Article*

# Phytotoxicity and Germination of Four Plant Species in Lake Junín Contaminated Soils, Peru

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## Abstract

This study evaluated the phytotoxicity and germination responses of four plant species, Neltuma glandulosa, Parkinsonia aculeata, Leucaena leucocephala, and Albizia lebbeck, in soils collected from Lake Junín, Peru. Soil samples were obtained using a composite sampling approach from contaminated sites and characterized for texture, organic matter, pH, electrical conductivity, and potentially toxic elements (PTEs). The experiment was conducted under controlled laboratory conditions using a completely randomized design with four replicates per treatment. Germination responses were assessed through germination percentage, germination index (IG), mean germination time (TMG), and radicle elongation. Data were analyzed using multivariate analysis of variance (MANOVA) and principal component analysis (PCA) to evaluate species-specific responses and relationships with soil properties. Soil characterization revealed a sandy clay loam texture with low organic matter content (1.1% ± 0.07). The soil from Site 2 (T2) exhibited the highest toxicity, linked to elevated concentrations of PTEs, increased pH and EC, and lower adsorption capacity due to soil texture and organic matter content. Phytotoxicity was assessed using the IG: values above 80% indicated low phytotoxicity, 50–80% moderate, and below 50% severe toxicity. N. glandulosa and P. aculeata showed greater tolerance, whereas L. leucocephala and A. lebbeck were more susceptible. Germination percentage was generally not severely impacted by PTEs, often remaining above 75%, but radicle elongation was more sensitive, showing clear inhibitory effects. Fertility parameters such as total nitrogen (N), phosphorus (P), and potassium (K), along with substrate moisture and composition, also influenced germination outcomes and species-specific responses. These results highlight the complex interactions between soil physical-chemical properties and toxic element bioavailability that regulate germination success. Species native to the Lake Junín area (N. glandulosa and P. aculeata) demonstrated greater tolerance, while exotic species such as A. lebbeck were more susceptible to phytotoxic effects. This work provides valuable insights for ecological risk assessment and revegetation strategies in contaminated Andean soils under similar environmental conditions.

**Keywords:** Fabaceae, Germination index, Phytotoxicity, Lake Junín.

## 1. Introduction

According to Ochoa Ochoa et al. (2009), one of the most effective strategies for mitigating and preventing the impacts associated with land use change and deforestation is the establishment of Protected Natural Areas (PNAs).

In industrialized urban regions, the accelerated expansion of metropolitan infrastructure, along with the intensification of petrochemical and manufacturing processes, has increasingly contributed to a range of environmental challenges. Among the most significant drivers of these impacts is the release and atmospheric or soil dispersion of potentially toxic elements (PTEs), including As, Be, Cd, Cu, Cr, Hg, Pb, and V, which are monitored and regulated under internationally recognized environmental standards. Numerous studies have underscored the ecological and public health risks associated with these contaminants (González Dávila et al. 2019; Cavazos Castillo 2021; Covarrubias & Peña Cabriales 2017; Wong Argüelles et al. 2021; Keramati et al., 2026).

Across protected wetland ecosystems, water quality is commonly compromised by the influx of domestic, municipal, and industrial discharges, as well as by the buildup of solid waste. Seasonal rainfall further exacerbates this condition by mobilizing sediments and various solid pollutants, leading to localized flooding within the wetland's perimeter. This hydrological disturbance accelerates siltation processes and fosters the accumulation of anthropogenic materials—such as glass, steel, and plastics—ultimately degrading adjacent soil properties and ecological integrity (GOBTAM & SEA 2014).

Environmental deterioration in wetland environments is frequently linked to contamination by potentially toxic elements, including As, Cr, Cd, Hg, and Pb (Herrera & Lombardi 2021). When present at elevated levels, these metals pose substantial hazards to biological systems, as they can disrupt physiological pathways and metabolic processes in aquatic organisms (Argota et al. 2012; González Chávez et al. 2017; Pabón et al. 2020; Gutiérrez Jaime et al. 2024). Additionally, their accumulation alters fundamental ecosystem properties and functional dynamics, thereby contributing to broader environmental degradation (Mazhari et al., 2019; Pouresmaieli et al. 2022; Thalassinos et al. 2023; Iannacone et al. 2024). These PTEs can percolate through soil layers to reach groundwater reservoirs or be transported across surface water bodies (Bhat et al. 2022).

Phytoremediation strategies often emphasize the use of plant species capable of tolerating potentially toxic elements (PTEs) as a means to support ecological restoration and conservation. Such an approach is considered sustainable given that these species possess genetic characteristics that enable growth and physiological functioning under contamination stress (López Hernández & Morales Hernández 2022; Huslina et al. 2024; Abhari, 2026). Numerous members of the Fabaceae family have been reported as tolerant to various PTEs, including species within the genera Neltuma, Acacia, and Clitoria (Noguez Inesta et al. 2017; Duarte Zaragoza et al. 2020; Chan Quijano et al. 2021). Certain Neltuma species, for instance, have demonstrated the ability to grow in Cu contaminated substrates without exhibiting detrimental effects on photosynthetic activity (González Mendoza et al. 2017). Furthermore, several Fabaceae taxa occurring in industrialized regions have shown considerable tolerance to heavy metals such as Cd, Pb, Cu, Ni, and Zn. These include Lupinus sp. and Neltuma laevigata, the latter of which also shows resistance to As. This combination of traits underscores their potential use in ecological restoration programs and in phytoremediation of metal-contaminated soils (Chan Quijano et al. 2021).

Plant species exhibit diverse physiological and metabolic responses to environmental contaminants, and these responses are strongly influenced by both the nature of the pollutant and its concentration. Consequently, the selection of species for restoration initiatives must incorporate criteria such as tolerance to PTEs, resilience to hydric or saline stress, and the capacity to acclimate to thermal variability (Guzmán Castro et al. 2022). Additionally, several resistance mechanisms should be evaluated. These include the biosynthesis of organic ligands such as glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs), as well as the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase. Together, these mechanisms mitigate oxidative stress and enhance survival under contaminant exposure (Thalassinos et al. 2023).

Since PTEs can impair plant metabolism and inhibit germination, evaluating species with tolerance to these contaminants is crucial (Altonar Gómez et al. 2021). This study assessed the germination tolerance of leguminous plants grown in soils containing As, Cd, Cr, and Pb from a contaminated wetland. The objective of this study was to quantify and compare the phytotoxic effects of contaminated soils from Lake Junín on the germination and early seedling responses of four plant species (Neltuma glandulosa, Parkinsonia aculeata, Leucaena leucocephala, and Albizia lebbeck) by evaluating germination percentage, germination index (IG), mean germination time (TMG), and radicle elongation under controlled laboratory conditions.

The four species selected for this study represent different ecological strategies and potential applications in the restoration of degraded soils. Neltuma glandulosa and Parkinsonia aculeata are species adapted to arid and semi-arid environments and are commonly associated with tolerance to stressful soil conditions, including nutrient limitations and elevated salinity. In contrast, Leucaena leucocephala and Albizia lebbeck are widely used leguminous trees recognized for their rapid growth and capacity to improve soil fertility through nitrogen fixation. Evaluating these species under the same contaminated soil conditions allows for a comparative assessment of their sensitivity to phytotoxic effects and their potential suitability for revegetation programs in contaminated Andean environments such as the Lake Junín region.

## 2. Methodology and Data

### 2.1 Study area

This investigation was conducted at Lake Junín (Laguna Chinchaycocha), situated in the central highlands of Peru at approximately 10°53′ S latitude and 75°58′ W longitude (Fig. 1). The lake covers an estimated 530 km² and is characterized by shallow water columns, with average depths ranging from 8 to 12 m across most sectors. Climatic conditions in the basin correspond to a cold Andean regime, featuring mean annual temperatures of 6 to 8 °C and precipitation levels between 700 and 900 mm per year. Hydrological inputs derive from direct rainfall, upland surface runoff, and inflow from multiple tributary streams. The lake's catchment area is geographically linked to the Cerro de Pasco industrial mining region—one of Peru's primary centers for metal extraction and processing—making it environmentally relevant for assessing metal-related pollution pressures.

According to climatic characterizations for the central Peruvian Andes, the Lake Junín region corresponds to a cold high Andean climatic regime with pronounced wet and dry seasons. The area receives the majority of its annual precipitation during the austral summer, while winter remains relatively dry, though winter rainfall contributions may surpass 10% of the annual total. These conditions promote the dominance of puna grasslands, perennial herbs, and xerophytic plants like high Andean shrubs adapted to low atmospheric pressure, intense radiation, and frost events. Historically, the native vegetation was dominated by extensive puna ecosystems, which have undergone substantial reduction and fragmentation due to anthropogenic activities such as overgrazing, peat extraction, and land conversion. This environmental context favors the germination and persistence of Fabaceae species—particularly Lupinus, Adesmia, and Trifolium—which are frequently recorded in the Junín basin and exhibit known tolerance to potentially toxic elements (PTEs) associated with the regional mining industry.

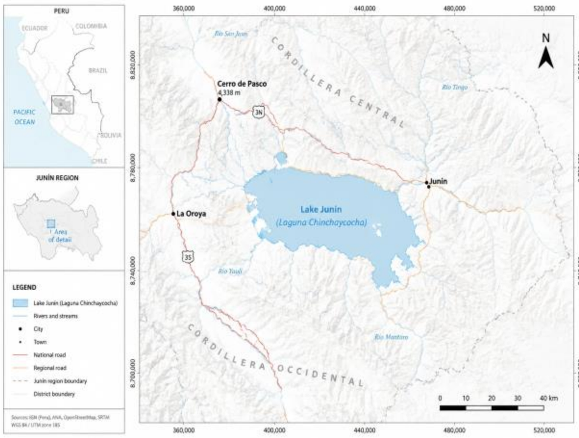


**Figure 1.** Location map of Lake Junín (Laguna Chinchaycocha) in the central Andes of Peru. The map illustrates the position of the lake within the Junín region and highlights nearby urban and mining areas, including Cerro de Pasco, a major historical mining center. Major transportation routes, surrounding settlements, and key geographic features of the high Andean plateau are indicated to provide spatial context for the study area.

### 2.2 Soil sampling and seed collection

For the present study, two soil sampling locations were established around Lake Junín, Peru, through random spatial selection. Sampling Site 1 was positioned along the southwestern perimeter of the lake, whereas Sampling Site 2 was located in proximity to a discharge zone receiving intermittent wastewater flows. This design was intended to capture contrasting contamination conditions within the lake basin and to support the comparative assessment of phytotoxic responses in the selected plant species.

Field sampling took place in January 2024. From each site, 4 kg of soil were obtained for subsequent physical and chemical analyses, using duplicate samples collected from the 0–25 cm soil horizon. All samples were placed in hermetically sealed polyethylene bags to preserve their integrity during transport.

Four leguminous species were chosen based on their presence in the regional flora and their relevance for phytoremediation: Parkinsonia aculeata, Neltuma glandulosa, Leucaena leucocephala, and the restoration-oriented species Albizia lebbeck. From each taxon, mature pods were collected for seed extraction and later germination testing.

### 2.3 Physical and chemical characterization of soils

The determination of arsenic, cadmium, chromium, and lead was conducted in accordance with ISO 1995-aligned methods, incorporating analytical blanks and certified standards for quality assurance. Each sample was analyzed in duplicate. Soil samples were air dried, homogenized, and sieved before extraction.

A subsample of 10 g was treated with 20–30 mL of 0.005 M DTPA (Merck) under constant shaking for 2 h. Extracts were filtered through Whatman No. 42 filters, and metal concentrations were quantified using atomic absorption spectrophotometry (Perkin Elmer Model 3110, USA).

Physical and chemical soil parameters—including texture, available P and K, cation exchange capacity, total N (micro Kjeldahl), pH, and electrical conductivity—were evaluated following internationally established methodologies for soil analysis. Organic matter content was determined following the loss on ignition protocol of Nelson and Sommers (1982).

### 2.4 Preparation of aqueous soil extracts

Aqueous soil extracts were prepared to assess the effects of the water-soluble fraction of contaminated soils on seed germination under controlled laboratory conditions. Soil samples were air-dried and sieved through a No. 16 mesh (1 mm) before extraction. Distilled water was added at a ratio of 13.5 mL g$^{-1}$ soil, according to the procedure reported by Sánchez-Puente et al. (2020). This relatively high water-to-soil ratio was selected as a standardized extraction approach to recover the soluble fraction of the soil matrix and to obtain a sufficient volume of extract for replicated germination bioassays. Therefore, the aqueous extracts were used as laboratory test media for phytotoxicity screening rather than as a direct simulation of in situ soil solution chemistry. The soil–water suspensions were shaken for 30 min at 500 rpm at 18–37 °C, allowed to settle, and the supernatants were collected for use in the germination assays. Extracts from Site 1 and Site 2 were designated as T1 and T2, respectively; distilled water was used as the control treatment T0...

**2.5 Seed morphological characteristics**

Seeds of Parkinsonia aculeata are ovoid to ellipsoidal and slightly compressed, with a dark greenish coloration often marked by brown or purplish mottling. Their hard seed coat typically necessitates pregerminative treatment to overcome dormancy and promote germination (Sanz Elorza et al., 2004).

In Leucaena leucocephala, the seeds are flattened and slightly elliptical, exhibiting a shiny brown surface. A waxy layer within the seed coat limits water permeability, thereby delaying imbibition and germination. As a result, pretreatment is generally recommended to ensure uniform germination (Bakewell Stone, 2023).

Seeds of Albizia lebbeck are oblong and flattened with a chestnut coloration. They present physical dormancy associated with a thick, impermeable testa (Vázquez Yanes et al., 1999).

Likewise, Neltuma glandulosa produces ovoid, somewhat flattened seeds with a yellowish-brown color. These seeds are surrounded by a hard endocarp and possess an impermeable seed coat, both of which contribute to their physical dormancy (Illescas Gallegos et al., 2021).

**2.6 Seed pregerminative treatments**

Exploratory experiments were undertaken to optimize pregerminative treatments for each species. A total of 100 seeds per treatment were evaluated, with ten replicates for each species, following a modified protocol based on Sánchez Puente et al. (2020). Treatments included chemical, mechanical, and thermal scarification, selected according to species-specific dormancy characteristics (Sánchez et al., 2023; Ruiz Carranza et al., 2024).

For P. aculeata, L. leucocephala, and A. lebbeck, seeds were immersed in 50% sulfuric acid (v/v) for 10–30 min, depending on testa hardness. After thorough rinsing with distilled water, additional mechanical scarification was applied at the micropyle when required to ensure permeability. Surface disinfection was performed using 5% sodium hypochlorite (v/v) for 3 min, followed by soaking in distilled water for 24 h to facilitate water uptake.

In the case of N. glandulosa, mechanical scarification at the micropyle was followed by thermal treatment in distilled water at 50 °C for 30 min. Seeds were subsequently disinfected and soaked for 24 h before germination testing.

**2.7 Germination assays**

The germination experiment included three treatments: a control (T0) using distilled water and two treatments using aqueous soil extracts obtained from Site 1 (T1) and Site 2 (T2). Each treatment consisted of ten replicates with ten seeds per replicate, placed on absorbent paper in sterile Petri dishes. For Neltuma glandulosa, Parkinsonia aculeata, and Leucaena leucocephala, 13.5 mL of the corresponding aqueous extract was applied to each Petri dish, whereas for Albizia lebbeck the volume was reduced to 7.5 mL because higher volumes negatively affected seed viability (Sánchez Puente et al., 2020). The Petri dishes were incubated in darkness at a constant temperature of 28 °C under controlled laboratory conditions during the germination period. Germination was recorded daily, and a seed was considered germinated when radicle emergence was observed..

**2.8 Determination of germination variables**

Germination performance was quantified through several indicators. The germination percentage (GP) is the initial capacity of seeds to break dormancy and initiate radicle emergence, representing the first developmental phase of the life cycle rather than sustained seedling growth, calculated as:

GP=("number of germinated seeds" /"total number of seeds" )×100 (1)

The mean germination time (MGT), an indicator of seed vigor and emergence quality, was computed following:

MGT=(∑[("number of seeds germinated at time " t)×("time of observation" )])/(∑"total germinated seeds") (2)

The modal germination time (MoGT) corresponds to the day with the highest frequency of germinated seeds, reflecting the main germination peak (Ranal & Santana, 2006).

The root growth rate (RGR), expressed in cm/day, was determined from the slope (m) of the linear model y=mx+b, fitted to root length measurements over time.

Relative germination percentage (RGP), relative root length (RRL), and the Zucconi germination index (GI) (Carbó et al., 2023; Zapata González et al., 2023) were calculated using:

RGP=("germinated seeds in extract" /"germinated seeds in control")×100 (3)

RRL=("mean root length in extract" /"mean root length in control" )×100 (4)

IG=(RGP×RRL)/100 (5)

The Relative Germination Percentage (RGP) represents the proportion of seeds that germinate under exposure to a contaminant source compared with the germination observed in the control. The Relative Root Length (RRL) expresses the percentage of root growth tolerance, while the Zucconi Germination Index (GI) integrates both variables to assess the sensitivity of plants to contaminants in phytotoxicity assays (Carbó et al., 2023), following the method of Zucconi et al. (1981).

According to this scale, $GI \leq 50\%$ indicates severe phytotoxicity, values between $50\% < GI < 80\%$ indicate moderate toxicity, and $GI > 80\%$ reflects low or negligible phytotoxicity (Varnero et al., 2007; Carroza et al., 2020; Zapata González et al., 2023).

**2.9 Statistical Analysis**

Data processing was conducted in R version 4.3.1 (2023). Descriptive metrics, including the mean and standard deviation, were calculated to summarize central tendency and variability.

Differences between the physical and chemical soil parameters from Site 1 and Site 2 were evaluated using one-way ANOVA. Before model evaluation, ANOVA assumptions were tested by verifying residual normality via the Shapiro–Wilk test ($p > 0.05$) and variance homogeneity through Levene's test ($p > 0.05$).

Significant differences were further examined using Tukey's multiple comparison test with a significance threshold of $p < 0.05$.

A MANOVA was conducted to determine whether germination responses differed significantly among treatments corresponding to aqueous soil extracts from Site 1, Site 2, and the control. The analysis considered the set of germination variables measured for each plant species simultaneously.

When significant differences were identified, Tukey's multiple comparison test was employed to separate treatment means and determine pairwise differences ($p < 0.05$).

The average of each variable was used as input for a Principal Component Analysis (PCA) to characterize the multivariate structure of the dataset. PCA provides a reduced set of components that explain a substantial proportion of the variance contained in the original variables (Everitt & Hothorn, 2011). The analysis and visual representations were generated using FactoMineR and Factoextra. This approach allowed the exploration of associations among physicochemical soil traits, morphological parameters, and germination performance.

Selection of principal components was based on eigenvalue magnitude and the accumulated explained variance, following established recommendations (Kaiser, 1961; Kassambara, 2017b).

A complementary clustering analysis was carried out to classify treatments with similar profiles. The k-means algorithm was applied, and the optimal number of clusters was determined using multiple validation indices from the NbClust package. Indices including "kl", "mcclain", "gamma", "gplus", "dunn", "sdindex", and "ptbiserial" converged on two clusters, further supported by the Elbow and Silhouette diagnostic plots.

## 3. Results

The soils collected from the Lake Junín area exhibited a sandy–clay–loam texture. Chemical analyses revealed significant differences in physicochemical properties between the two sampling sites ($p \leq 0.05$, Tukey's test, one-way ANOVA; Table 1). Higher physical and chemical parameter values were recorded at Site 1, whereas potentially toxic element (PTE) concentrations were higher at Site 2. Soil pH at both sites was classified as moderately alkaline, with measurements of 7.7 at Site 1 and 7.3 ± 0.1 at Site 2.

**Table 1.** Physical and Chemical Properties of Soils from Lake Junín, Peru. Heavy metal values correspond to total concentrations measured in bulk soil samples.

| Parameters | Site 1 | Site 2 |
|---|---|---|
| **Textural class** | Sandy clay-loam | Sandy clay-loam |
| **Sand (%)** | 52.9 ± 0.0 | 56.2 ± 1.2 |
| **Clay (%)** | 31.1 ± 0.0 | 29.1 ± 0.0 |
| **Silt (%)** | 16.0 ± 0.0 | 14.7 ± 1.2 |
| **pH** | 7.7 ± 0.1$^{a}$ | 7.3 ± 0. 1$^{b}$ |
| **EC (dS/m)** | 0.4 ± 0.0$^{b}$ | 1.6 ± 0.04$^{a}$ |
| **P (mg/kg)** | 20.0 ± 0.0$^{a}$ | 10.0 ± 0.0$^{b}$ |
| **OM (%)** | 2.8 ± 0.1$^{a}$ | 1.1 ± 0.1$^{b}$ |
| **Organic C (%)** | 1.6 ± 0.1$^{a}$ | 0.7 ± 0.1$^{b}$ |
| **Total N (%)** | 0.11 ± 0.01$^{a}$ | 0.05 ± 0.0$^{b}$ |
| **Exchangeable K (%)** | 3.4 ± 0.1$^{a}$ | 1.2 ± 0.1$^{b}$ |
| **CEC (cmol/kg)** | 14.5 ± 0.1$^{a}$ | 9.8 ± 0.1$^{b}$ |
| **PTE (mg/kg)** | | |
| **As** | Nd | 40.1 ± 1.0$^{a}$ |
| **Cd** | 0.5 ± 0.0$^{b}$ | 1.5 ± 0.0$^{a}$ |
| **Cr** | 6.3 ± 0.2$^{b}$ | 7.9 ± 0.01$^{a}$ |
| **Pb** | 1.0 ± 0.0$^{b}$ | 4.6 ± 0.3$^{a}$ |

**Notes**

Values represent the mean of three replicates (n = 3) ± standard deviation.

Different letters within a row indicate statistically significant differences ($p \leq 0.05$, Tukey).

PTE = Potentially Toxic Elements.

EC: Electrical conductivity; P: phosphorus; OM: organic matter; Organic C: organic carbon; Total N: total nitrogen; Exchangeable K: exchangeable potassium; CEC: cation exchange capacity; As: arsenic; Cr: chromium; Cd: cadmium; Pb: lead; Nd: not detected.

The germination test revealed significant differences among species and treatments according to MANOVA with Wilks' lambda ($p \leq 0.05$). Neltuma glandulosa and Parkinsonia aculeata showed the highest germination percentages (PG), with values ranging from 95–99% ± 3.2 and from 92 ± 8.7 to 95% ± 8.2, respectively. These values were significantly higher than those observed for Leucaena leucocephala, which ranged from 40 ± 17.7 to 44% ± 14.5, and Albizia lebbeck, which ranged from 75 ± 9.7 to 94% ± 5.2 (Table 2).

Significant differences were also observed for the total root growth rate (TCR) ($p \leq 0.05$). The highest TCR values were recorded in P. aculeata (1.9 ± 0.7 cm $d^{-1}$) and N. glandulosa (1.4 ± 0.4 cm $d^{-1}$), whereas lower values were found in L. leucocephala (1.0 ± 1.3 cm $d^{-1}$) and A. lebbeck (0.9 ± 0.5 cm $d^{-1}$). The longest mean germination time was observed in P. aculeata (2.8 ± 1.3 days), followed by A. lebbeck (1.1 ± 0.3 days) (Table 2).

**Table 2**. Germination test analysis of legumes in soil from Lake Junín, Peru

| Species | Treatment | PG (%) | TCR (cm/d) | TMoG (d) | TMG (d) |
|---|---|---|---|---|---|
| *Neltuma glandulosa* | T0 | $99 \pm 3.16^{a}$ | $1.00 \pm 0.29^{bdf}$ | $1.0 \pm 0.01^{b}$ | $4.00 \pm 0.01^{b}$ |
| | T1 | $95 \pm 7.24^{a}$ | $1.37 \pm 0.45^{bcd}$ | $1.2 \pm 0.42^{be}$ | $4.01 \pm 0.02^{b}$ |
| | T2 | $97 \pm 6.78^{a}$ | $1.03 \pm 0.48^{bdf}$ | $1.1 \pm 0.32^{b}$ | $4.00 \pm 0.01^{b}$ |
| *Parkinsonia aculeata* | T0 | $92 \pm 8.74^{ac}$ | $1.45 \pm 0.74^{ad}$ | $1.6 \pm 0.70^{bcde}$ | $4.04 \pm 0.04^{b}$ |
| | T1 | $95 \pm 8.22^{a}$ | $1.88 \pm 0.67^{a}$ | $2.2 \pm 0.42^{ac}$ | $4.30 \pm 0.06^{a}$ |
| | T2 | $92 \pm 13.66^{ac}$ | $1.52 \pm 0.86^{ac}$ | $2.7 \pm 0.68^{a}$ | $4.39 \pm 0.02^{a}$ |
| *Leucaena leucocephala* | T0 | $40 \pm 17.74^{d}$ | $0.75 \pm 1.06^{efgh}$ | $2.8 \pm 1.32^{a}$ | $4.48 \pm 0.05^{a}$ |
| | T1 | $44 \pm 14.50^{d}$ | $0.98 \pm 1.28^{bdg}$ | $2.2 \pm 0.63^{ad}$ | $4.29 \pm 0.02^{a}$ |
| | T2 | $42 \pm 16.26^{d}$ | $0.83 \pm 1.18^{efgh}$ | $2.0 \pm 0.47^{ac}$ | $4.31 \pm 0.02^{a}$ |
| *Albizia lebbeck* | T0 | $94 \pm 5.16^{a}$ | $0.91 \pm 0.55^{bg}$ | $1.0 \pm 0.01^{b}$ | $4.00 \pm 0.01^{b}$ |
| | T1 | $77 \pm 11.59^{bc}$ | $0.90 \pm 0.76^{bh}$ | $1.0 \pm 0.01^{b}$ | $4.00 \pm 0.01^{b}$ |
| | T2 | $75 \pm 9.71^{b}$ | $0.67 \pm 0.74^{efgh}$ | $1.1 \pm 0.32^{b}$ | $4.01 \pm 0.02^{b}$ |

Notes

Data represent the mean of 10 replicates (n = 10) ± standard deviation. Different letters within a column indicate statistically significant differences among species and treatments according to the MANOVA test ($p \leq 0.05$).

PG: germination percentage; TCR: radicle growth rate; TMoG: modal time of germination; TMG: mean time of germination; T0: control; T1: soil extract from site 1; T2: soil extract from site 2; d: days.

The phytotoxicity indicators showed significant differences among species and treatments. According to Table 3, N. glandulosa and P. aculeata presented the highest values of percentage of relative germination (PGR), relative root length (LRR), and germination index (IG). The PGR values ranged from 92 ± 11.3 to 96 ± 7.0 for these species, with no significant differences observed between treatments T1 and T2. For LRR, the highest values were recorded for N. glandulosa and P. aculeata, ranging from 96.9 ± 11.6 to 100 ± 13.7, whereas lower values were observed in L. leucocephala and A. lebbeck, ranging from 60.6 ± 28.9 to 75.9 ± 15.7. In addition, the germination index of N. glandulosa and P. aculeata exceeded 80%, while lower IG values were recorded for L. leucocephala and A. lebbeck (Table 3).

Principal component analysis showed a clear association between germination variables and soil physicochemical characteristics (Fig. 2). The first two principal components explained 86.9% of the total variance, with Dim 1 accounting for 66.8% and Dim 2 for 20.1%. The variables As, Cd, Cr, Pb, and electrical conductivity were negatively associated with Dim 1, whereas CEC, Corg, K, OM, Nt, P, and pH were positively associated with this component. The PCA also showed a separation between T1 and T2, indicating differences in the physicochemical composition of the soil extracts and their relationship with germination responses.

Cluster analysis further separated the legume species according to their responses to the soil extracts contaminated with PTEs (Fig. 3). N. glandulosa and P. aculeata were grouped according to their higher germination and phytotoxicity indicator values, whereas L. leucocephala and A. lebbeck were separated from these species due to their lower performance, particularly in root-related parameters. This grouping was consistent with the differences observed in PG, PGR, LRR, and IG values reported in Tables 2 and 3.

**Table 3.** Phytotoxic indicators evaluated in legumes in soil from Lake Junín, Peru

| Species | Treatment | PGR (%) | LRR (%) | IG (%) |
|---|---|---|---|---|
| *Neltuma glandulosa* | T1 | 95 ± 7.07<sup>ac</sup> | 100.00 ± 13.72<sup>a</sup> | 100.00 ± 18.81<sup>a</sup> |
| *Neltuma glandulosa* | T2 | 96 ± 6.99<sup>a</sup> | 85.60 ± 19.03<sup>ad</sup> | 82.40 ± 20.29<sup>ad</sup> |
| *Parkinsonia acule-ata* | T1 | 95 ± 7.07<sup>ac</sup> | 96.88 ± 11.58<sup>ac</sup> | 92.67 ± 16.90<sup>ac</sup> |
| *Parkinsonia acule-ata* | T2 | 92 ± 11.35<sup>ac</sup> | 77.12 ± 25.50<sup>bcd</sup> | 73.06 ± 29.76<sup>ae</sup> |
| *Leucaena leuco-cephala* | T1 | 73.33 ± 23.83<sup>bcd</sup> | 60.63 ± 28.87<sup>bd</sup> | 50.23 ± 36.07<sup>bde</sup> |
| *Leucaena leuco-cephala* | T2 | 69.99 ± 28.10<sup>bd</sup> | 51.24 ± 21.09<sup>b</sup> | 39.58 ± 23.12<sup>be</sup> |
| *Albizia lebbeck* | T1 | 85.57 ± 12.87<sup>ad</sup> | 75.92 ± 15.74<sup>bcd</sup> | 65.53 ± 19.55<sup>bcde</sup> |
| *Albizia lebbeck* | T2 | 82.24 ± 13.03<sup>ad</sup> | 57.44 ± 23.75<sup>bd</sup> | 48.09 ± 23.36<sup>be</sup> |

Note
Data represent the mean of 10 replicates (n = 10) ± standard deviation. Different letters in the same column indicate significant differences among species and treatments according to ANOVA ($p \leq 0.05$). PGR: percentage of relative germination; LRR: relative root length; IG: germination index; T1: soil extract from site 1; T2: soil extract from site 2.

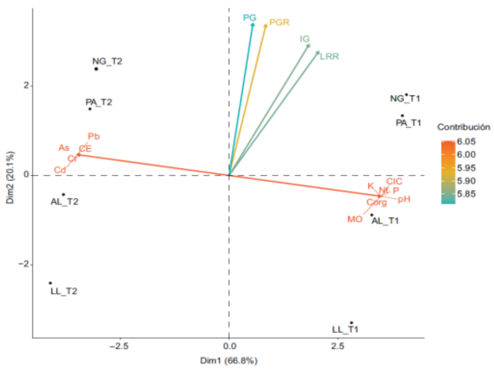


**Figure 2.** Principal Component Analysis (PCA) of germination variables and soil physical and chemical characteristics. AL: A. lebbeck; LL: L. leucocephala; PA: P. aculeata; NG: N. glandulosa; T1: aqueous extract of soil from site 1; T2: aqueous extract of soil from site 2; As: arsenic; Cd: cadmium; Cr: chromium; Pb: lead; EC: electrical conductivity; PG: germination percentage; PGR: relative germination percentage; LRR: relative root length; IG: germination index; Corg.: organic carbon; OM: organic matter; CEC: cation exchange capacity; Nt: total nitrogen; P: phosphorus; Int. K: exchangeable potassium.

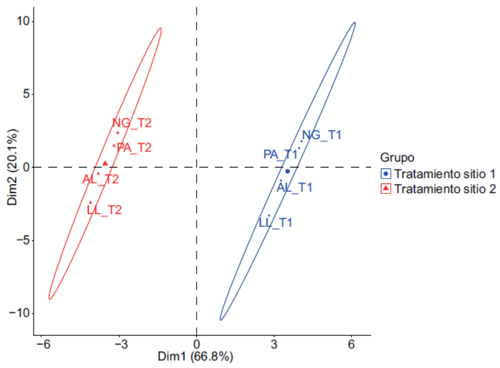


**Figure 3.** Cluster analysis of the legume species assessed in aqueous soil extracts contaminated with PTEs from Lake Junín, Peru. AL: A. lebbeck; LL: L. leucocephala; PA: P. aculeata; NG: N. glandulosa; T1: aqueous extract from site 1; T2: aqueous extract from site 2.

## 4. Discussion

The germination responses observed in this study indicate that the physicochemical properties of the soil extracts strongly influenced the performance of the tested legumes. Site 2 (T2) was identified as the most phytotoxic site for seed germination, mainly because of its elevated pH, electrical conductivity, and PTE concentrations. Previous studies have shown that pH plays an important role in regulating the mobility and bioavailability of heavy metals, and that EC values above 1.5 mS/cm may hinder plant growth. In the present study, pH emerged as a key factor in phytotoxicity because it significantly alters the availability of PTEs in soil; alkaline pH reduces the bioavailability of cationic elements (Thalassinos et al., 2023). Conversely, Pb and Cd tend to be more available under acidic pH conditions, whereas As and Cr become more available under alkaline pH, consistent with the values registered here (7.7 and 7.3). The increase in pH may also favor the formation of organometallic complexes with Pb and Cd in soil solution, limiting their uptake by plant roots. However, for As and Cr, alkaline pH may have enhanced their bioavailability (Van Herreweghe et al., 2002; Olaniran et al., 2013). In addition, the seed coats of L. leucocephala and A. lebbeck may contain chemical compounds that indirectly influence their toxicity response (Thalassinos et al., 2023).

Organic matter (OM) and soil texture may also have limited the bioavailability of PTEs because the large surface area of deprotonating functional groups (carboxylic, hydroxylic, phenolic, etc.) promotes adsorption of toxic elements (Strawn, 2021). In T2, the OM content was low (1.1% ± 0.07), reducing the soil's capacity to adsorb PTEs and thereby increasing their bioavailability. The sandy clay loam texture also corresponds to low CEC, which diminishes the soil's buffering capacity and its ability to retain Pb and Cd. Moreover, OM and texture influence soil water retention, affecting both seed moisture availability and substrate aeration (Andra et al., 2006; Saminathan et al., 2010). These conditions may have contributed to the differential responses observed among the tested species.

Site 2 (T2) showed high PTE concentrations according to national and international standards. Under these conditions, N. glandulosa and P. aculeata presented higher IG (73.06–100%), PGR (92–95%), and LRR (77.1–100%), indicating greater tolerance to PTEs (Table 3 and Fig. 3). This pattern may be explained by intraspecific variability and by the fact that N. glandulosa and P. aculeata grow naturally in the soils of Lake Junín, Peru, meaning they are within their natural distribution range (Molina-Guerra et al., 2019) and therefore may be better adapted to germinate under the tested conditions. In contrast, although A. lebbeck showed a PG of 75–94%, it was statistically different, probably because this species is not naturally present in the lake area and therefore does not have the same degree of tolerance to PTEs (Verma et al., 2013). Thus, this species appears to be more sensitive to phytotoxic substances, particularly in terms of root growth.

Additionally, N. glandulosa, P. aculeata, L. leucocephala, and A. lebbeck have hard-coated seeds, making pregermination treatments necessary to break dormancy and enhance germination (Kwakye and Blankson, 2022). This characteristic may partly explain why germination responses were not always directly proportional to the degree of contamination, since dormancy-breaking and seed-coat permeability can modulate exposure to toxic elements during imbibition and early embryo development.

In the PG, TCR, TMoG, and TMG tests, one factor that contributed to the germination of the four species was the soil fertility characteristics related to Nt, P, and K (Table 1). These macronutrients are essential for seed development and germination (Fig. 2). Furthermore, according to Mantilla (2008), seed germination also depends on external conditions such as substrate composition—in this case, the aqueous extract—and moisture content (Caputo-Suarez et al., 2020). This is because the amount of water absorbed by a mature seed enables elongation of the embryo or radicle (Mantilla, 2008). Germination percentages above 75% for all study species were not influenced by PTE concentrations, as noted by Di Salvatore et al. (2008), who reported that when high metal concentrations are present in the substrate, they may be absorbed by the seed coat without affecting embryo growth. The parameters used to obtain germination percentage do not necessarily reflect growth through cell division (Soudek et al., 2010). Likewise, the chemical composition and salinity of the water saturating the aqueous extract may influence germination, as they can affect seed osmoregulation and also act as phytotoxic factors.

These results are consistent with previous studies. González-Villalobos et al. (2021) reported that the germination of P. aculeata is affected by the presence of Pb in solutions with concentrations up to 2.0 mM of PbCl2 and Pb(NO3)2, showing 82% and 74% germination, respectively. In the present study, however, the germination of P. aculeata was higher, with PG and PGR > 92%, considering that the aqueous extract concentration was 0.015 ppm Pb (data not reported in this study). Another study by Kabir et al. (2018) reported that the germination of L. leucocephala was 63.33% in the presence of Pb and Cd in Pb(NO3)2 and Cd(NO3)2 solutions at 100 ppm. The authors concluded that both Pb and Cd have toxic effects on the germination and growth of this species, supporting the results of the current study, which show that lower PTE concentrations affect GI in this species. On the other hand, L. leucocephala has been reported to have germination rates ranging from 8 to 35% in environments contaminated with Cd and Pb in aqueous solutions (25 to 100 ppm) (Shafiq et al., 2008). In this study, N. glandulosa and P. aculeata presented GI values of 80–100%, consistent with the reports by Abbas et al. (2019), González-Mendoza et al. (2017), and González-Villalobos et al. (2021), who mention that these species can grow in soils contaminated with As, Cd, and Pb.

The phytotoxicity indices further supported the differential tolerance among the studied legumes. N. glandulosa and P. aculeata exhibited low phytotoxicity indices, whereas L. leucocephala and A. lebbeck showed severe and moderate phytotoxicity, respectively, based on the classification by Zucconi et al. (1981). This is attributed to the toxic effects of the EPTs present in T2, which caused inhibition or delay in germination (Kranner and Colville, 2011; González-Villalobos et al., 2021). Another factor affecting the germination of L. leucocephala and A. lebbeck is seed dormancy due to their hard seed coat (Utello et al., 2023; Ruiz-Carranza et al., 2024). Overall, the phytotoxicity level of EPTs depends on the bioavailability of the specific element, plant species, and the physicochemical properties of the soil such as pH, electrical conductivity (EC), and OM (Antoniadis et al., 2019), and in some cases temperature (Fernández et al., 2023). Temperature had no effect here as it was maintained constant at 28 °C. The study was conducted in the Lake Junín region, Peru.

Taken together, these findings show that soil physicochemical properties profoundly affect seed germination responses by modulating the bioavailability of potentially toxic elements. The combined evidence from Table 1, Table 3, Fig. 2, and Fig. 3 indicates that T2 was the most phytotoxic site and that native species, particularly N. glandulosa and P. aculeata, exhibited greater tolerance under the conditions tested. Although germination percentages were often high, radicle elongation proved to be a more sensitive bioindicator of stress. Therefore, the interaction between soil fertility, contamination level, and species-specific tolerance should be considered when evaluating phytotoxic risk and selecting plant species for restoration in contaminated Andean ecosystems.

## 5. Conclusion

The study concludes that soil physicochemical properties profoundly impact seed germination responses through modulating the bioavailability of potentially toxic elements. Site 2 (T2) was identified as the most phytotoxic due to its elevated pH, high electrical conductivity, low organic matter, and sandy clay loam texture, which reduced the soil's capacity to adsorb toxic elements, increasing their bioavailability. These conditions, combined with higher concentrations of PTEs, caused variable phytotoxic effects on the tested species. Native species, particularly Neltuma glandulosa and Parkinsonia aculeata, showed considerable tolerance and adapted germination responses, whereas species such as Albizia lebbeck were more sensitive, exhibiting moderate to severe phytotoxicity. While germination rates often remained above 75%, radicle elongation was significantly inhibited in toxic environments, indicating its higher sensitivity as a bioindicator.

The findings underscore the importance of considering soil chemical and physical characteristics alongside contaminant levels to accurately assess phytotoxic risk and select appropriate species for restoration projects in contaminated Andean ecosystems like Lake Junín. Integrating soil fertility parameters (Nt, P, K) may mitigate some toxicity effects, although species-specific responses must guide revegetation efforts.

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**Declarations**

**Data and code availability**

All data generated or analyzed during this study are available from the corresponding author upon reasonable request. No computational code was used in this study.

**Declaration on Artificial Intelligence (AI) Use**

The authors declare that Artificial Intelligence (AI) tools were employed in the writing, editing, and language refinement processes of this manuscript. However, all data presented are original and collected by the authors. The research team independently performed the interpretation, analysis, and conclusions drawn from the data. The authors take full responsibility for the content and integrity of this publication.

**Conflicts of interest**

The authors announced that they have no known conflicts of interest or personal relationships that could have appeared to influence the work reported in this manuscript.

**Ethical approval**

The authors declare no ethical issues; the research was conducted in full accordance with ethical standards. Also, this paper is neither under Review nor published elsewhere.

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