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## Effect of lead toxicity on seed germination in wild plant species of Southern Kazakhstan

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### Abstract

This study evaluates the potential of four wild plant species (*Centaurea pseudosquarrosa*, *Plantago lanceolata*, *Lamium purpureum*, and *Poa annua*) for phytoremediation of lead-contaminated soils in Southern Kazakhstan, specifically assessing their germination vigor and tolerance to varying lead concentrations. Soil samples were collected from lead-contaminated areas in Southern Kazakhstan's industrial and mining regions. Seeds of the four selected wild plant species were cultivated in laboratory conditions on soils containing different lead (Pb) concentrations ranging from 0.01% to 10%. Germination rates and early growth parameters were systematically measured to determine toxicity thresholds and species-specific responses. Seed germination of *Centaurea pseudosquarrosa* and *Plantago lanceolata* decreased significantly with increasing lead concentrations, with a lethal dose determined at 7.0% Pb salt concentration. However, these two perennial species exhibited remarkable resilience during early growth stages compared to the annual species *Poa annua* and *Lamium purpureum*. Significant variations in plant responses were observed across all treatments, indicating species-specific tolerance mechanisms to lead toxicity. The study demonstrates differential lead tolerance among the four wild plant species, with perennial species showing greater potential for survival in lead-contaminated environments than annual species. The established toxicity thresholds provide baseline parameters for understanding plant responses to heavy metal stress, particularly in the context of Southern Kazakhstan's contaminated industrial landscapes. These findings serve as a scientific reference for developing phytoremediation strategies specific to lead-contaminated soils in industrial regions. The identified resilient species, particularly *Centaurea pseudosquarrosa* and *Plantago lanceolata*, could be candidates for further investigation in field-based phytoremediation applications, offering environmentally sustainable and cost-effective solutions for soil remediation in mining-affected areas of Kazakhstan.

**Keywords:** *Centaurea pseudosquarrosa*, lead, phytotoxicity, *Plantago lanceolata*, seed germination, soil pollution.

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**Transparency:** The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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## 1. Introduction

Soil serves as a natural medium for the germination, growth, and development of plants. However, these processes can be influenced by various physicochemical factors. Alongside natural abiotic elements, numerous ecotoxicants, including heavy metals (HMs), adversely affect the survival of plants. Heavy metals, such as those resulting from industrialization and urbanization processes [1], find their way into the soil and plants, ultimately accumulating within the bodies of living organisms. Consequently, elevated concentrations of HMs in the environment, soil, and plants have toxic effects on organisms. It is revealed that the order of heavy metal toxicity for plants is  $Hg > Cd > Pb > Cu$  [2]. The accumulation of HMs in the soil can disrupt various biochemical processes within plants, leading to significant changes at the molecular, cellular, and organismal levels [3]. Several studies have demonstrated that such processes in organic life can contribute to the degradation of plant community composition in contaminated areas [4-6].

The Southern Kazakhstan Province is among the country's most industrially developed regions. The long-standing mining, metallurgical, and chemical activities in this area have had a substantial impact on the environment. Soil and water pollution primarily stems from waste products and emissions generated during polymetallic production processes. Lead (Pb), cadmium (Cd), and nickel (Ni) are the predominant types of soil pollutants in these industrial zones [7]. Currently, these areas have become additional sources of environmental contamination. Consequently, the detoxification of soils contaminated with high concentrations of HMs is a crucial task. Phytoremediation, utilizing the inherent capabilities of hyperaccumulator plants, represents one approach to address this issue.

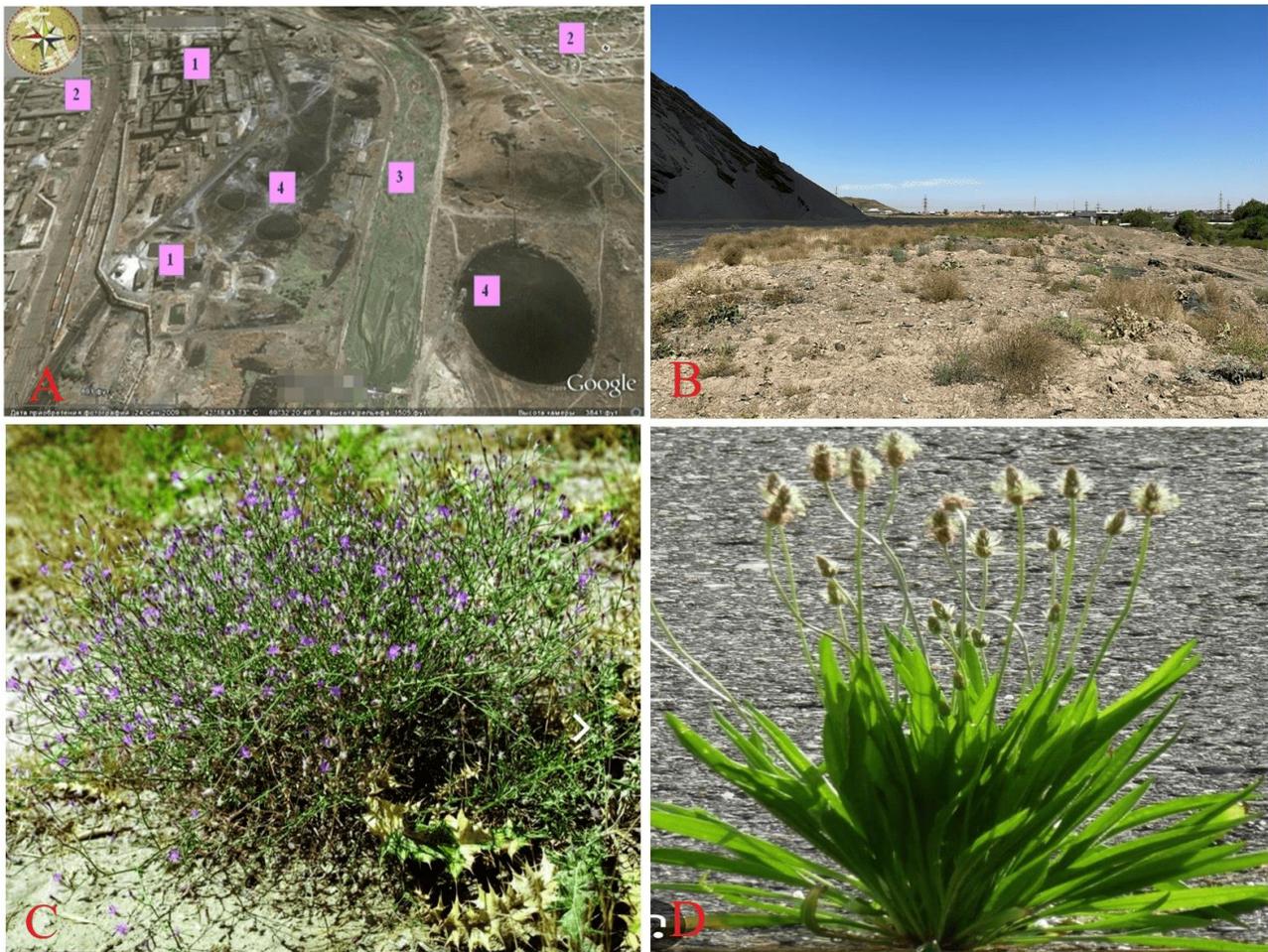
It is well documented that certain plant species can accumulate heavy metal (HM) ions in their biomass while remaining highly resistant to their toxic effects. Cultivated species like Sarepta mustard (*Brassica juncea* L.), perennial ryegrass (*Lolium perenne* L.), and maize (*Zea mays* L.) have been widely used in phytoremediation [8]. However, more information on wild hyperaccumulator plant species, which hold practical relevance for remediating heavy metal-contaminated soils, is needed. The mechanisms underlying the resistance of hyperaccumulator plants have also received insufficient attention. Within the scientific literature, researchers highlight the existence of various forms of plant resistance. Juvenile resistance, associated with genetic characteristics, is one such type observed during the initial stage of plant life. Mechanical barriers or the formation of insoluble organic complexes through chelation processes are some of the ways in which many plant species protect themselves from excessive heavy metal ions.

Identifying such indigenous hyperaccumulator plant species is of significant practical importance for phytoremediation efforts. Therefore, this research aims to investigate the juvenile resistance of wild plant species, *Centaurea pseudosquarrosa* and *Plantago lanceolata*, from the Southern Kazakhstan region against Pb toxicity.

## 2. Materials and Methods

### 2.1. Sample Collection

The samples were collected from the Shymkent area of Southern Kazakhstan, which is the most developed area of Kazakhstan in terms of industry, mining, and other anthropogenic activities. This area is badly contaminated with HMs. The site for sample collection was in the coordinates of  $42^{\circ}18'43.7''N$  and  $69^{\circ}32'05.8''E$  as per Google Maps (Figure 1A). Soil sampling contaminated with lead was performed according to the protocol of GOST-a (17.4.3.01-83) of the Republic of Kazakhstan. The concentration of acid-soluble lead ions in the soil was determined using atomic absorption spectrometry with electrothermal sample atomization and a tungsten spiral atomizer. The method's resolution ranges from 5 to 10ng [9].



A: Google map of experimental site,  
 B: Pb-contaminated soil area,  
 C: *Centaurea pseudosquarrosa* observed on Pb-contaminated soil area and  
 D: *Plantago lanceolata* observed on Pb-contaminated soil area  
**Figure 1.**  
 Site of sample collection.

There are no agricultural and other related activities in this area; however, the site is full of natural vegetation and wild plants (Figure 1B). Two major plant species, *C. pseudosquarrosa* (Figure 1C) and *P. lanceolata* (Figure 1D), were widely observed in the contaminated soil.

2.2. Germination of Plant Species on Pb-Contaminated Soil

Germination of four wild species was analyzed on soil collected from Pb-contaminated soil from the Southern Kazakhstan region. The soil was collected from the Pb-waste warehouse for the experiment. Samples were taken from the top 25cm soil layer. Soil samples taken at a distance of 35km to the north from the lead production zone were used as a control. The atomic absorption method for analyzing soil samples showed the content of heavy metals in the collected samples (Table 1). The experiment was conducted in six replicates.

**Table 1-**  
 Concentration of heavy metals in contaminated soil.

S. No.	Heavy metal	Pb-contaminated soil (mg/kg)	Control soil (mg/kg)
1.	Lead (Pb)	14400.60±35.75	1.83±0.038
2.	Cadmium (Cd)	112.39±7.35	0.17±0.150
3.	Zinc (Zn)	5176.85±127.90	2.49±0.032

2.3. Analysis of Pb-Toxicity on Germination Ability

The experiment was conducted under laboratory conditions in four replicates, where plant seeds were germinated in petri dishes on ash-free filter paper dampened with a Pb salt solution to test the toxicity range on seed germination. Different concentrations of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] were used in this study, ranging from 0.01% to 10% (0.01%, 0.03%, 0.05%, 0.07%, 0.09%, 0.15%, 0.20%, 0.40%, 0.60%, 0.80%, 1.00%, 2.00%, 4.00%, 5.00%, 7.00%, and 10.00%). In the control group, seeds were germinated on ashless filter paper moistened only with distilled water. Seed germination energy was assessed on the 5th day of germination for *P. lanceolata*, *Poa annua*, and *Lamium purpureum*, and on the 10th day for *C. pseudosquarrosa*.

Germination, germination energy determination, and seed germination of the studied plant species followed the protocols of GOST-s of the Republic of Kazakhstan (GOST:12038-84, GOST:12039-82, and GOST:24983.2-81).

### 2.3. Statistical Analysis

Statistical analysis of the obtained results involved calculating the arithmetic mean and standard deviation at a confidence level of  $0.95 > P > 0.80$ . All measurements were performed in 3 to 5 replications. Data processing was carried out using an IBM Pentium personal computer with the Excel application software package.

## 3. Results and Discussion

The experiment was carried out with the seeds of four plant species, *C. pseudosquarrosa*, *P. lanceolata*, *P. annua*, and *L. purpureum*, for germination purposes. The percentage germination of four species showed significant variation in the soils taken from Pb-contaminated and control soils (Table 2).

**Table 2.**  
Germination of seeds on Pb-contaminated soil.

S. No.	Plant species	Seed germination Pb-contaminated soil (%)	Seed germination in Control soil (%)	Increase in germination (%)
1.	<i>Centaurea pseudosquarrosa</i>	18.50±0.84	78.68±1.46	325.29
2.	<i>Plantago lanceolata</i>	14.28±0.20	68.87±1.41	382.28
3.	<i>Lamium purpureum</i>	12.44±0.19	76.51±1.25	515.03
4.	<i>Poa annua</i>	6.22±0.10	63.85±1.09	926.52

The wild-growing species *C. pseudosquarrosa* and *P. lanceolata* were examined in this study against high concentrations of Pb in soil, along with the widely distributed ephemeral annual plants *Poa annua* L. and *Lamium purpureum* L. The first two species represent the dominant flora group in the Pb-contaminated production area of the "Yuzhpolimetal plant," while the ephemeral annual plant species were included in the experiment for comparative analysis.

### 3.1. Determination of Germination Energy

The species, *P. lanceolata* and *C. pseudosquarrosa*, demonstrated a noticeable increase in the germination energy of seeds when exposed to Pb toxicity. At a Pb concentration of 0.01%, the germination energy for *P. lanceolata* was 46.5±2.8%, and for *C. pseudosquarrosa*, it was 48.9±2.1%. In the control group, the germination energy was 42.6% ± 2.2% for *P. lanceolata* and 45.4±2.3% for *C. pseudosquarrosa*. The same trend was observed for seed germination, with values of 54.4±3.3% and 55.5±2.4% in the experimental group, and 46.2±2.6% and 48.4±3.1% in the control group. As the concentration of Pb increased, both the germination energy and seed germination of the tested plant species decreased. The threshold concentration of Pb salt inhibiting the growth processes of both plant species was 7.0% (Figure-1A&B). However, the responses of the two species to the increasing Pb concentrations in the solution differed significantly. For *P. lanceolata*, the germination energy decreased from 46.5±2.8% at 0.01% concentration to 2.3±0.01% at 7.0% concentration. A similar trend was observed for *C. pseudosquarrosa*, with the germination energy decreasing from 48.9±2.1% at 0.01% concentration to 2.4±0.01% in subsequent concentrations. The seeds lost their viability at a Pb concentration of 7.0% or higher.

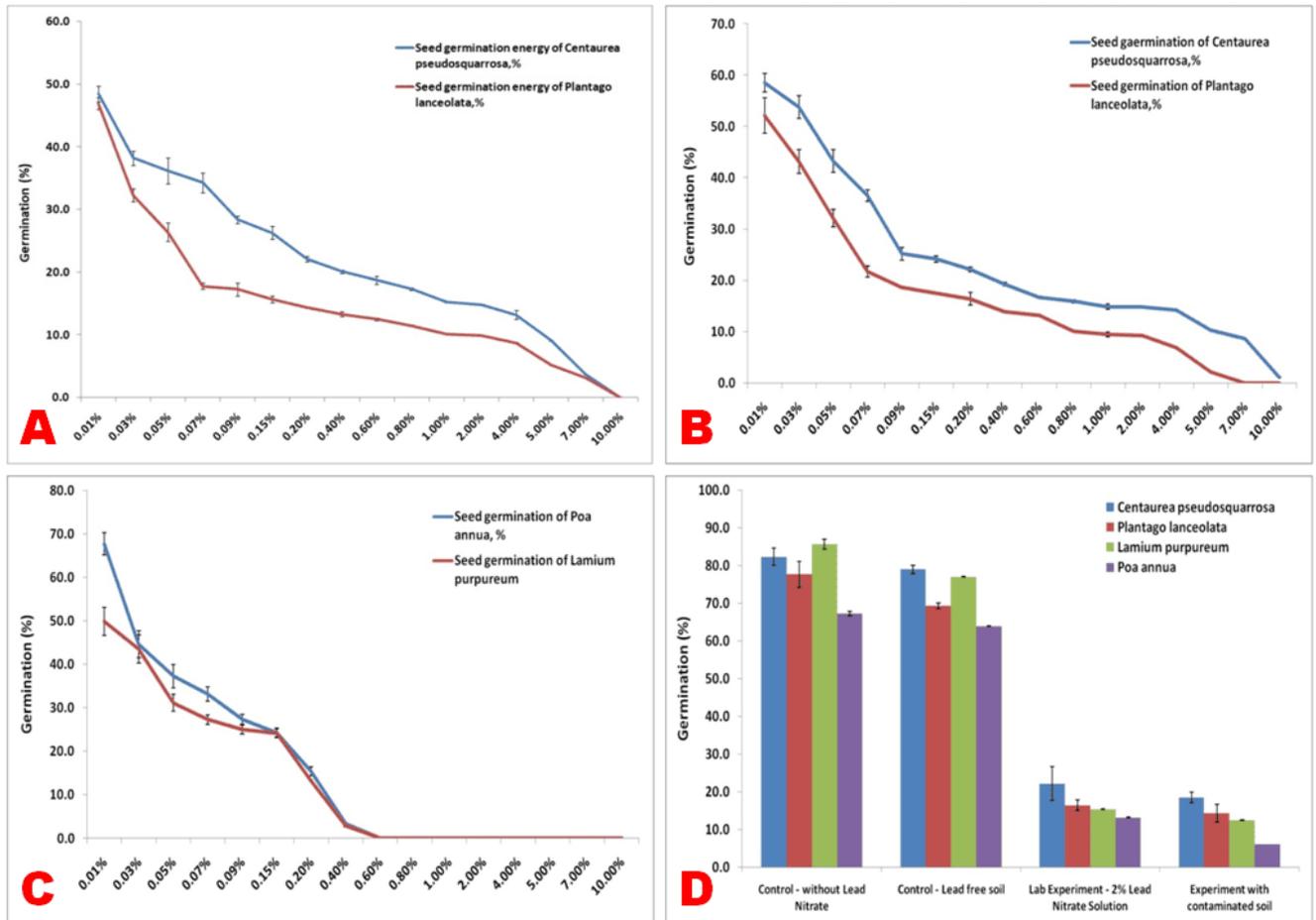
It is worth noting that *P. lanceolata* exhibited higher sensitivity compared to *C. pseudosquarrosa*. The germination energy of *P. lanceolata* seeds was consistently lower across all concentrations (Figure 2A). In the control group, the germination energy for these plant species was 46.9±2.2% and 48.5±2.4%, respectively, which did not differ significantly from the lowest concentration treatment in the experiment.

### 3.2. Determination of Seed Germination

Seed germination rates were slightly higher than germination energy rates at low concentrations of Pb (Figure 2B). However, as the Pb concentration increased to 3% and beyond, there was a significant decline in seed germination of the plant species. This trend was observed across all concentrations of the Pb that were studied.

Upon analyzing the experiment's results involving annual plant species, it was observed that the germination energy and seed germination of *P. annua* in the control group were 46.8±3.0% and 49.5±3.3%, respectively. For *L. purpureum*, the corresponding values were 59.9±3.4% and 67.7±3.5%. The lowest concentration of Pb in the solution also stimulated both germination energy and germination rates, with average values being 12.3±0.11% and 14.6±0.12% higher than those of the control group. However, further increases in the concentration resulted in an acute toxic effect, leading to a significant decline in seed germination. A Pb concentration of 7.0% proved lethal for both of the studied plant species (Figure 2C).

The plant species examined in our study exhibited significant variations in their resistance to the toxic effects of Pb ions. At the lowest concentration of 0.01% of the toxicant, all four plant species showed increased germination energy and seed germination. However, as the concentration increased, there was a sharp decline in these growth parameters. Additionally, there were notable differences in the plants' susceptibility to Pb during their early stages of development. For instance, while *P. lanceolata* and *C. pseudosquarrosa* had a lethal concentration limit of 7.0%, *P. annua* L. and *L. purpureum* had a limit of 4.0% of Pb concentration (Figure 2D).



A. The impact of increasing Lead concentrations on the germination energy of seeds from the test plants *Centaurea pseudosquarrosa* and *Plantago lanceolata* L.  
 B. The impact of increasing the Lead concentrations on the seed germination of the test plants *Centaurea pseudosquarrosa* and *Plantago lanceolata* L.  
 C. The impact of increasing the Lead concentrations on the seed germination of the test plants *Poa annua* L. and *Lamium purpureum* L.  
 D. Comparative evaluation of the influence of the same concentration of Lead Nitrate and acid-soluble Lead salts in contaminated soil on the germination of plant seeds.

**Figure 2.**

Seed germination on contaminated soil.

Plant resistance to pollutants is influenced by complex biochemical processes and genetic characteristics specific to each plant species [10-14]. The inhibitory effects of salts, including Pb, Cd, Cu, and Zn, on the germination energy of *Melilotus officinalis* L were studied, and significant inhibition in seed germination was found [15, 16]. Several studies have provided insights into the mechanism of Pb toxicity on plant vitality. They indicate that lead inhibits ATP synthesis, triggers lipid peroxidation, and causes DNA damage due to the excessive production of reactive oxygen species (ROS). Pb also hampers seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and reduces water and protein content [17-22]. Hence, it can be inferred that the resistance mechanism of different plant species to the toxic effects of heavy metals is associated with the aforementioned disruptions in biochemical processes. Different plant species have varying thresholds for lethal concentrations of toxicants, which, under natural conditions, lead to the elimination of sensitive species from plant communities and the emergence of a group of resistant species.

Several studies have documented information about HM hyperaccumulators. Currently, more than 400 hyperaccumulator plant species from 45 families have been identified. Approximately 75% of these species are Ni accumulators, while only 20-30 species accumulate cobalt, copper, and zinc. The list of zinc hyperaccumulator plants includes around 20 species, predominantly from the Brassicaceae family, including 11 species from the Thlaspi genus and one species from the Arabidopsis genus. Other families and genera, such as Silene, Armeria, and Viola, also contain zinc hyperaccumulators. Overaccumulation of cadmium and Pb is even rarer among higher plants. For instance, only zinc hyperaccumulators *Thlaspi caerulescens*, *Arabidopsis halleri*, and Ni hyperaccumulators *Thlaspi goesingense* are known to accumulate cadmium. Brassicaceae plants, particularly *Brassica juncea*, are capable of accumulating multiple heavy metals such as Cd, copper, Ni, Pb, selenium, and zinc. This versatility is crucial since soil pollution rarely involves only one metal.

Plants have various physiological barriers that prevent heavy metals (HMs) from entering cells, ensuring their resistance. At the cellular level, the cell wall and plasmalemma play a significant role by restricting the flow of metals into the protoplast. At the tissue level, the endoderm and cell membranes of the root prevent metal entry into the vascular system and,

consequently, its transfer to aboveground organs. However, these barriers are not universally applicable. For instance, cadmium (Cd) and lead (Pb) can move through the apoplast but have limited access to the central cylinder compared to nickel, which can freely penetrate through the symplast. The portion of heavy metals that enters the leaves predominantly accumulates in epidermal cells, thus preventing physiological disruptions in mesophyll cells, which are functionally more critical for plants. The stability of apical meristems, primarily responsible for plant resistance, relies on maintaining a constant cellular composition and average rates of cell division. Meristematic cells possess a specialized structural and functional organization, enabling them to divide rapidly under stressful conditions and ensure plant resistance to adverse environmental factors [23-25]. Several researchers have highlighted organism-level mechanisms of plant resistance to heavy metals, including delayed metal uptake by plant roots, the presence of barriers preventing metal transport to vital plant organs, regulation of metal transport from roots to shoots, participation of trichomes in removing excess metals, and shedding of leaves with high metal accumulation [26-28].

#### 4. Conclusion

Based on our findings, it can be concluded that Pb hinders the germination energy and decreases seed germination of *P. lanceolata* and *C. pseudosquarrosa*. Furthermore, *P. lanceolata* demonstrated greater sensitivity to the toxic effects of Pb compared to *C. pseudosquarrosa*. In our model experiment, the lethal dose for both plant species was determined to be 7.0% of Pb concentration. However, our experimental results highlighted the significant resilience of both species during their early stages of development in the presence of Pb, particularly when compared to the studied annual plant species. These observed patterns, to some extent, explain the natural vegetation composition in areas contaminated by Pb ions, where perennial ruderal plants dominate while annual ephemeral species are absent.

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