

Bioremediation of Soil Pollution Based on Fungal Immobilization Technology

Muhamad Hamame*

American University of Afghanistan, Afghanistan

**corresponding author*

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Abstract: Fluxes of lead into the natural environment have been increasing in many regions of the world due to human activities, and lead fluxes have been reported to exceed those from natural processes. In recent years, due to the rapid development of industry in my country, the demand for lead has gradually increased. The large-scale mining and smelting of lead ore, the processing of lead-containing materials and other industries have vigorously developed and sharply increased lead-containing wastewater, waste gas, and waste to the natural world medium emissions. The main purpose of this paper is to study the bioremediation of soil pollution (SP) based on fungal solidification technology. The effects of different time, pH, temperature and initial lead concentration on the adsorption conditions of heavy metal lead were studied using the screened high lead-tolerant G strains. Experiments show that the heavy metal lead will hinder the germination of rapeseed seeds to a certain extent, but in the presence of microorganisms resistant to heavy metals, the phenomenon of inhibiting seed germination will be slowed down, and the final performance effect is that the germination rate of seeds is increased. However, when the concentration of heavy metal lead is too high, it will have a certain inhibitory effect on microorganisms and seeds. If it exceeds the tolerance range, it will be suppressed by heavy metals, and the higher concentration will lead to the death of both.

1. Introduction

There are two main sources of soil lead: natural occurrence and man-made pollution. Rock minerals in nature contain different heavy metal elements, among which lead element enters the soil through the diagenetic parent material, the weathering of the bedrock and the transportation of external natural forces. And man-made economic activities make the content of lead in the soil exceed the standard value, resulting in a decline in the quality of the soil ecological environment. The burning of leaded gasoline, coal (thermal power plants), and ore smelting in other high-temperature industries are the main sources of anthropogenic lead in the environment, and

agricultural soils are exacerbated by livestock manure, atmospheric deposition, fertilizer application, sewage irrigation, and solid waste deposition lead content in [1-2].

In a related study, Gazka et al. mentioned that fungi have increased tolerance to environmental stress. To evaluate the microbial community and functional diversity of fungi in long-term crude oil-contaminated soils as potential bioremediation agents for oil-contaminated sites [3]. Samples were taken from three historical oil wells, and next-generation sequencing (targeting the ITS region) was accompanied by functional fungal community determinations based on BiologFFPlates, glomalin-related soil protein (GRSP) content, trace elements, and PAHs concentrations. This study hypothesizes that long-term natural bioremediation of crude oil-contaminated soils can promote the intensive development of unique fungal communities adapted to the contaminated conditions. Alobaydy mentioned that bioremediation is a successful method for dealing with petroleum hydrocarbon contamination [4]. The study involved two processing steps, the first involving the isolation and diagnosis of the most widespread fungi in soil samples. The second step was to investigate the ability of the isolated fungi to decompose polycyclic aromatic hydrocarbons (PAHs). Analysis of the samples by GC equipment (Gas Chromatography) showed that the concentration of PAHs was significantly reduced compared to soil samples that were not injected with fungal genera (*Penicillium*, *Aspergillus*).

In this paper, the bioremediation of SP is mainly based on fungal solidification technology. In this paper, the materials and methods of immobilized microorganisms are firstly analyzed, and the mechanism of microorganisms is discussed. The release mechanism model of carbon-based inoculants in soil is studied. In this paper, the screened high lead-tolerant G strains were used to study the effect of different time, pH, temperature and initial lead concentration on the adsorption conditions of heavy metal lead. Plant pot experiments were carried out, and a microbial-phytoremediation system was constructed. The plant rape was used as the potted object to measure and analyze the plant height, plant fresh weight, plant dry weight, total lead content in the plant, and plant leaf width. The effect of heavy metal lead concentration on the germination of rapeseed seeds was studied accordingly.

2. Design and Research

2.1. Materials for Immobilizing Microorganisms

The material for immobilizing microorganisms can provide a stable place for the growth and reproduction of microorganisms, and different microorganisms and different immobilization methods have corresponding suitable carriers. A suitable carrier can protect the microorganisms from the external environment as much as possible, and can also reduce the loss of microorganisms during immobilization. At present, the most widely used materials for immobilized microorganisms are divided into inorganic materials, natural organic polymer materials, synthetic organic polymer materials and composite materials [5-6].

2.1.1. Inorganic Materials

Commonly used inorganic carrier materials for immobilizing microorganisms are ceramics, clay, molecular sieves, microporous glass, etc. The advantages of these materials are high mechanical strength, not easy to be decomposed by microorganisms, non-toxic, low cost, and high durability. Firm and easy to fall off.

2.1.2M Natural Organic Polymer Materials

There are many kinds of natural organic materials, such as alginate, chitosan, resin, starch, gelatin, agar, etc. for microbial immobilization. The advantages of using these materials are that the materials are easy to obtain, the price is low, they are non-toxic to microorganisms, the mass transfer performance is good, and they are biodegradable. Decomposed by microorganisms, etc.

2.1.3. Synthesis of Organic Polymer Materials

Synthetic organic polymer materials can overcome some disadvantages of natural organic materials, have high mechanical strength, and have good resistance to microbial decomposition, but such materials have a certain impact on cell activity when used for microbial immobilization. Poor permeability.

2.1.4. Composite Materials

The composite material is a combination of organic carrier materials and inorganic materials, retaining the advantages of the two types of materials, and the biocompatibility, stability and mechanical strength of the composite materials are improved compared to the two types of materials. Composite materials have good application prospects in the curing of biocatalysts and the preparation of biosensors. In general, the application range of composite materials can be wider.

2.2. Microbial Immobilization Method

When using microorganisms for experiments directly, due to the constant changes of external environmental conditions and the influence of the metabolic activities of the microorganisms, a large number of microorganisms are often lost, which makes the efficiency of the microbial reaction relatively low, and it is difficult to achieve the expected results. Immobilized microbial technology can increase the concentration of microorganisms and improve their anti-toxicity and tolerance [7-8]. The commonly used basic methods are shown in Table 1.

Table 1. Microbial immobilization methods

Microbial immobilization method	Commonly used main technology
Adsorption method	physical adsorption, chemical adsorption
Embedding method	Gel embedding, prefabricated material embedding, microcapsule embedding, emulsion film embedding
Cross-Linking method	Glutaraldehyde cross-linking, adsorption cross-linking, carrier cross-linking
Covalent binding method	Diazonation method, aromatic alkylation, metal coupling, azide reaction, cyanogen bromide method
Carrier-free immobilization	microbial cell flocculation
System trapping method	Hollow fiber: ultrafiltration, backflushing, reverse circulation

It is generally believed that the microbial immobilization method should have the characteristics of simple operation, easy availability of carrier materials, stable and non-toxic carrier, uniform

distribution of microorganisms in the carrier, uniform and controllable particle size of the immobilized microspheres, and good mass transfer performance of the immobilized microspheres [9-10]. Common microbial immobilization methods are as follows (Figure 1):

2.2.1. Adsorption Method

Adsorption is a method that utilizes the physical interaction between microorganisms and the surface of a carrier material to adsorb microorganisms onto slowly moving materials. Adsorption methods are generally reversible. The advantages of this method are simple operation, low cost, only need to mix the microorganism and the carrier uniformly, and the reproducibility is low, and its application is limited.

2.2.2. Cross-linking Method

Generally speaking, amino groups, hydroxyl groups, imidazole groups and other groups on the surface of microorganisms can withstand cross-linking reactions with reagents with similar functional groups, thereby forming a biological network system between microorganisms and achieving the reason that microorganisms are indistinguishable. Its way is interconnected. The cross-linking method has good stability, but due to the large reaction process, the great influence on the activity of microorganisms, and the high cost, it has great limitations in practical applications.

2.2.3. Embedding Method (Microencapsulation Method)

The embedding method is to use the embedding material to form an ionic network or polymerize to fix the microorganisms in the gel network space. The network structure of the gel can not only fix the microorganisms, but also allow the infiltration of the substrate and the diffusion of the metabolites of the microorganisms. The advantages of the embedding method are very obvious, the operation is simple and convenient, the reaction process is mild, and there are many types of materials used. The appropriate carrier can be selected according to the needs of microorganisms. The embedded microorganisms can be evenly distributed in the body and are less affected by the environment. Although some colloids have poor mass transfer effect and poor permeability when encapsulating microorganisms, the encapsulation method that does not affect the immobilization of microorganisms is currently the most widely used method for microbial immobilization [11-12].

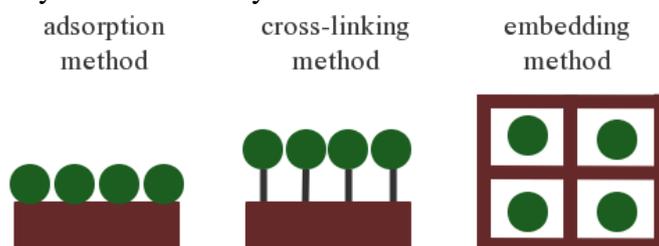


Figure 1. Schematic diagram of the immobilization method

This study is to immobilize biocontrol strains by embedding method for the biological control of aflatoxin. Therefore, the process of immobilizing microorganisms should have the following characteristics: easy to obtain embedding materials, low cost, non-toxic materials, and biocompatibility. It has good properties, is not easy to be decomposed, the operation process is convenient, the mechanical strength is good, and the application potential is wide [13-14].

2.3. Mechanism of Microorganisms

2.3.1. Mechanism of Microbial Resistance/Tolerance to Heavy Metals

The tolerance of microorganisms to heavy metals is a prerequisite for the use of microorganisms to facilitate plant extraction. Although toxic heavy metals were found to lead to reduced bacterial diversity, some bacteria showed resistance to heavy metals in the environments in which they lived, such as soil, industrial and municipal wastewater [15-16].

The seven known mechanisms of microbial resistance to heavy metals include: (1) repulsion of heavy metals by cell-permeable barriers; (2) extracellular sequestration of heavy metals by protein and chelation; (3) sequestration of heavy metals by protein and chelation. Intracellular sequestration; (4) Enzymatic detoxification; (5) Transport of heavy metals; (6) Passive tolerance; (7) Reduced cellular sensitivity to heavy metals. Microorganisms can have one or more resistance mechanisms.

2.3.2. Growth-promoting Effect of Microorganisms on Plants

The interaction between microorganisms and plants plays an important role in the adaptation and survival of both parties under the stress environment. Microorganisms with genetic and metabolic abilities can alleviate environmental stress in plants. Under heavy metal stress, some of the most basic properties of PGPRs to promote plant growth include: (1) The production of heavy metal chelates, such as metal carriers, organic acids, siderophores, and biosurfactants; (2) production of hormones, such as indoleacetic acid, cytokinin, and gibberellin; (3) production of biological mitigation agents, such as ACC deaminase; (4) for plant uptake of N, P, Nutrient elements such as K, Ca, Fe, Mn, Cu, and Zn provide convenience; (5) promote plant root development; (6) eliminate the harmful effects of plant pathogens. Heavy metal-tolerant microorganisms can utilize one or more of the above-mentioned mechanisms to regulate and increase plant biomass [17-18].

3. Experimental Research

3.1. The Release Mechanism of Carbon-Based Inoculants in Soil

3.1.1. Model Research

Carbon-based inoculants belong to polymer-embedded bioactive substances, and the release process of carbon-based inoculants in soil belongs to the diffusion process of biocontrol bacteria from high concentration to low concentration, which conforms to Fick's second law, namely:

$$\frac{\partial c}{\partial t} = D\nabla^2 c \quad (1)$$

where c is the diffusion concentration, t is the time, and D is the diffusion coefficient.

During the release process of carbon-based inoculants in the soil, the center is the core position of the biocontrol bacteria in the initial inoculum, and the outer layer is the wrapping structure of the outer layer of the inoculum, and the outermost layer can be regarded as being in contact with the inoculum. In the external soil part, inoculants in the soil can be seen as a three-layer release model. And the biocontrol bacteria concentration distribution and release rate of each layer are related to other layers.

When the biocontrol bacteria spreads from the inside to the outside, it will move in multiple

directions. In order to simplify the three-layer release model, the following assumptions are made in this study:

- (1) Biocontrol bacteria move in one direction in each layer, that is, a single radial diffusion;
- (2) The release medium of each layer is uniform;
- (3) The diffusion coefficient of each layer is the same;
- (4) The whole release process is dominated by diffusion.

The release process of carbon-based inoculants in soil satisfies the following relationship:

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (2)$$

where x represents the radial distance, t represents the time, and $c(x, t)$ represents the diffusion concentration at the radial distance x at time t .

3.1.2. Study on Release Kinetics

In order to better evaluate the release of biocontrol bacteria in the immobilized microspheres, the release kinetics and release mechanism of the immobilized microspheres in the simulated environment can be studied, and the release law of the embedded substances can be known.

The release result of the embedded substance is expressed as the release ratio, and the calculation method is as follows:

$$R_s = r_t / r_0 \quad (3)$$

Among them, R_s represents the release ratio of the embedded substance, r_t represents the amount released at time t , and r_0 represents the total amount of biocontrol bacteria embedded in the release sample.

According to the Korsmeyer-Peppas model, different release mechanisms can be distinguished by the following formula:

$$R_s = kt^n \quad (4)$$

Among them, R_s represents the release ratio of the embedded substance, k is the kinetic characteristic constant, n is the release index representing the release mechanism, and t is the release time.

In order to more accurately fit the release kinetics of biocontrol agents, the parameter z was added to the Korsmeyer-Peppas model. The improved model is as follows:

$$R_s = z + kt^n \quad (5)$$

3.2. Experimental Study on the Release of Carbon-Based Inoculants in Soil

The diffusion coefficients of biocontrol bacteria in different release media were determined, including the coating layer of bacterial agent and soil media. The measuring device is shown in Figure 2. The sterile container on the left is filled with a certain concentration of bacterial suspension, and the sterile container on the right is filled with sterile deionized water of the same volume. The connection between the two containers is sterilized. Medium, the diameter of the medium is 4cm and the thickness is about 0.1cm. The concentrations of biocontrol bacteria in the containers on both sides were measured for a certain period of time, and their diffusion coefficients

in the release medium were calculated. The experiments were carried out at 25 °C and 35 °C, respectively.

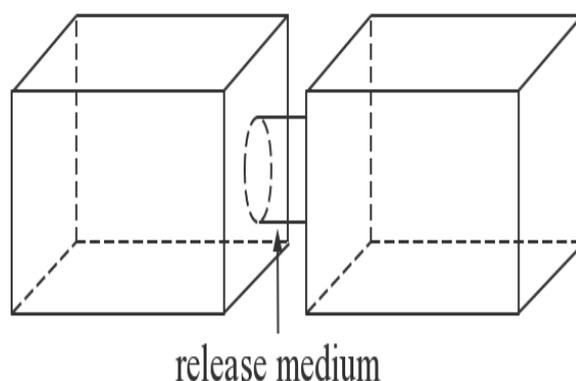


Figure 2. Schematic diagram of the measurement of the diffusion coefficient of biocontrol bacteria in the release medium

In this device, the volume of the medium is much smaller than that of the containers on both sides, so it can be assumed that the flux through the medium can be stabilized in a short period of time, and the flux of biocontrol bacteria through the medium is the same as that of diffusion.

In order to determine the range of the third layer of release medium soil, the biocontrol agent particles were buried 5cm below the surface of the sterilized soil, watered every 3 days at room temperature after sealing, and the soil relative humidity was maintained above 90%. After 60 days, soil samples were taken from different positions of the same level and the same radial direction of the inoculant particles to detect the content of biocontrol bacteria.

3.3. Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration, also known as MIC value. A single colony of the isolated strain was inoculated into PDA liquid medium for expansion culture (28 °C, 180r/min, cultured for 12h). The prepared heavy metal culture medium (Pb²⁺, Co²⁺, Mn²⁺, Hg²⁺, Cr²⁺) was divided into test tubes, and then 100 mL of bacterial liquid was added to each test tube for heavy metal lead gradient culture (180r/min, 28°C). After culturing for 48 hours, the mother liquor and PDA medium of lead metal were used in the sterile operation table to prepare a medium plate containing heavy metal lead with different concentrations, and 0.1 mL of bacteria was dropped with a pipette gun and added to the heavy metal solid medium for coating. Cultivate, wrap the parafilm separately in the incubator at 28 °C for upside-down culture, and then even observe whether the colony grows, so as to verify the maximum MIC value of the experimental strain.

4. Experimental Analysis

4.1. Determination of Minimum Inhibitory Concentration

The measurement was carried out according to the method, and the results are shown in Table 2. Finally, the comparison of the tolerance of three experimental strains to different heavy metals was carried out, and the microorganism G that had certain tolerance to other heavy metals and the strongest tolerance to heavy metal lead was selected for follow-up experimental operation research.

Table 2. Minimum inhibitory concentrations (MIC, mg/L) of strains G, R, Y

Heavy metal (mg/L)	Pb ²⁺	Co ²⁺	Mn ²⁺	Hg ²⁺	Cr ²⁺
MIC(G)	2000	350	200	200	50
MIC(R)	1750	100	200	0	0
MIC(Y)	1750	300	350	200	50

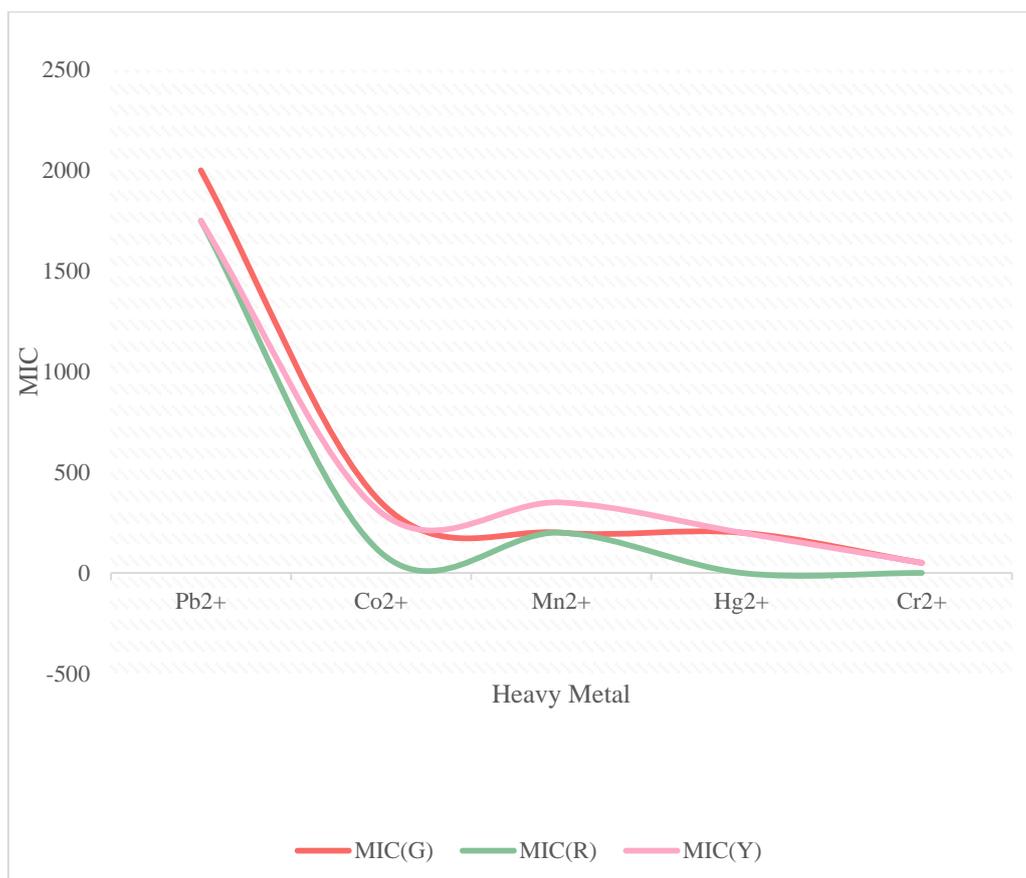


Figure 3. Minimum inhibitory concentration analysis of G, R, Y strains

As can be seen from the figure, strain Y and strain G have certain resistance to heavy metal ions Pb²⁺, Co²⁺, Mn²⁺, Hg²⁺, Cr²⁺, and strain R has resistance to heavy metal ions Pb²⁺, Co²⁺, Mn²⁺. Among them, three strains, especially strain G, have the highest resistance to heavy metal Pb²⁺, and the other two strains have higher resistance to heavy metal Pb²⁺ than other heavy metal ions. The MIC values of strain G, strain R, and strain Y for heavy metal Pb²⁺ respectively up to 2000mg/L, 1750mg/L and 1750mg/L, much higher than the tolerance value of common strains to Pb²⁺.

4.2. Seed Germination Results

The experiment was carried out according to the method, and the results of the seeds after

germination were recorded within the specified date. The statistical results of root length, shoot height, germination potential and germination rate of germinating seeds are shown in the table.

Table 3. Seed germination under different bacterial concentrations

Different bacteria (pcs/mL)	Root length (cm)	Bud height (cm)	Germination potential (%)	Germination rate(%)
0	1.38±0.14	1.78±0.12	26	90
10 ³	1.44±0.11	1.83±0.17	30	90
10 ⁴	1.51±0.12	1.85±0.12	33	93
10 ⁵	1.63±0.13	1.89±0.18	33	93
10 ⁶	1.46±0.12	1.85±0.19	17	83
10 ⁷	1.31±0.15	1.74±0.14	10	77
10 ⁸	1.22±0.15	1.72±0.12	10	70

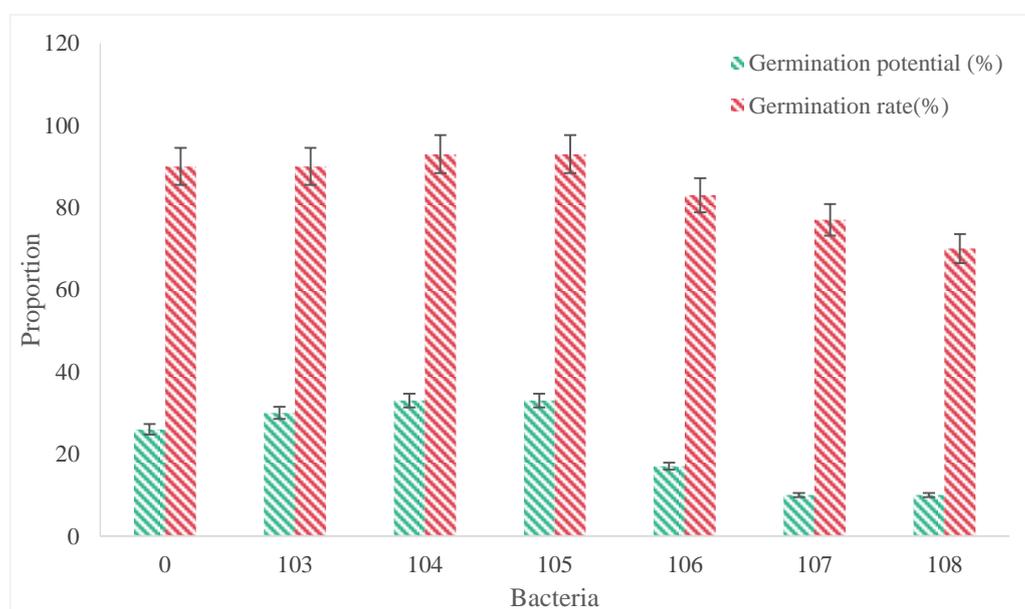


Figure 4. Analysis of seed germination under different bacterial concentrations

It can be seen from the figure that under the conditions of different bacterial concentrations, the root length, bud height, germination potential, and germination rate of rapeseed seeds first increased and then decreased, indicating that microorganisms have a certain role in promoting the germination of rapeseed seeds. Finally, the inhibitory effect at high concentrations may be because microorganisms can stimulate the content of some germination-related substances in rapeseed seeds to increase within a certain concentration range, such as various enzymes required for seed germination, and enzymes that promote internal metabolic activities of seeds. Similarly, if the concentration of microorganisms is too low, there will be no such effect, and if the concentration is too high, harmful substances or related metabolic activities that inhibit the germination of seeds will be produced, which will eventually lead to a decrease in the germination rate of rapeseed seeds.

Table 4. Seed germination under different lead concentrations with bacteria

Different lead concentrations in bacteria (mg/L)	Root length (cm)	Bud height (cm)	Germination potential (%)	Germination rate (%)
0	1.63 ± 0.14	1.89 ± 0.16	33	93
20	1.79 ± 0.13	1.93 ± 0.18	37	93
200	1.81 ± 0.13	1.93 ± 0.13	27	87
400	1.62 ± 0.18	1.47 ± 0.14	10	87
800	0.84 ± 0.15	1.18 ± 0.14	10	67
1500	0.27 ± 0.12	0.75 ± 0.14	7	50

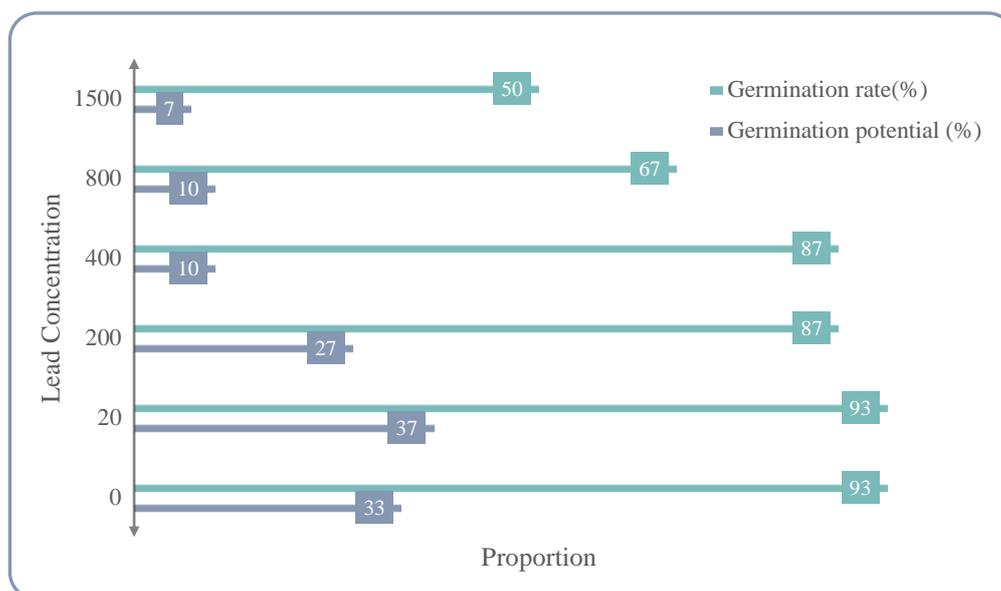


Figure 5. Analysis of seed germination under different lead concentrations containing bacteria

Under the condition of initially containing a certain concentration of microorganisms and different concentrations of heavy metal lead, the root length, shoot height, germination potential and germination rate of rapeseed seeds increased first and then decreased gradually. The downward trend of physiological indicators has slowed down, indicating that the presence of microorganisms can effectively alleviate the toxic effect of heavy metal lead on plant seeds, and can effectively promote rapeseed germination.

5. Conclusion

Heavy metal elements can occur naturally in the Earth's crust, depending on the geochemical composition of source rocks and soil formation processes such as weathering, deposition, and volcanic eruptions. At the same time, anthropogenic activities such as mining and smelting operations, oil and gas production, military practices and agricultural activities can also lead to the accumulation of heavy metals in soils. This paper studies the bioremediation of SP based on fungal solidification technology. The microbial-phytoremediation system was constructed, and the physiological and biochemical indicators of rapeseed under different experimental conditions were

also measured. It was found that the growth of rapeseed was poor in the soil with high heavy metal lead content, while strain G had a certain promotion of rapeseed. At the same time, it can improve the adsorption of rapeseed to heavy metal lead in soil. It can be seen from the seed germination test that low concentrations of lead can promote the germination of rapeseed seeds, while high concentrations have inhibitory effects. But in the case of heavy metal lead, strain G can obviously improve the germination rate of rapeseed, and has the effect of promoting growth.

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