KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

# QUALIFICATION THESIS

on the topic **Metagenomic analysis of the effect of RB2 on the bacterial community composition in the rhizosphere soil of Chinese cabbage under lead pollution**

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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**APPROVE**
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
_____
«___»_____2024

**ASSIGNMENTS**
**FOR THE QUALIFICATION THESIS**
**Jia Liyan**

1. Thesis topic **Metagenomic analysis of the effect of RB2 on the bacterial community composition in the rhizosphere soil of Chinese cabbage under lead pollution**
Scientific supervisor Olena Okhmat, Ph.D., Assoc. Prof.

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3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments_____

## EXECUTION SCHEDULE

| № | The name of the stages of the qualification thesis | Terms of performance of stage | Note on performance |
|---|---|---|---|
| 1 | Introduction | From 01 April 2024 to 11 April 2024 | |
| 2 | Chapter 1. Literature review | From 06 April 2024 to 20 April 2024 | |
| 3 | Chapter 2. Object, purpose and methods of the study | From 21 April 2024 to 30 April 2024 | |
| 4 | Chapter 3. Experimental part | From 01 May 2024 to 10 May 2024 | |
| 5 | Conclusions | From 07 May 2024 to 12 May 2024 | |
| 6 | Draw up a bachelor's thesis (final version) | From 20 May 2024 to 25 May 2024 | |
| 7 | Submission of qualification work to the supervisor for feedback | From 25 May 2024 to 27 May 2024 | |
| 8 | Submission of bachelor's thesis to the department for review (14 days before the defense) | 27 May 2024 | |
| 9 | Checking the bachelor's thesis for signs of plagiarism (10 days before the defense) | 31 May 2024 | |
| 10 | Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense) | 03 June 2024 | |

I am familiar with the task:

Student _____ Jia LIYAN

Scientific supervisor _____ Olena OKHMAT

## SUMMARY

**Jia Liyan. Metagenomic analysis of the effect of RB2 on the bacterial community composition in the rhizosphere soil of Chinese cabbage under lead pollution – Manuscript.**

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

With the development of industrialization, soil heavy metal pollution is a serious problem. Our country as a large agricultural country, the impact of soil heavy metal pollution on our country should not be underestimated, so we need to find green and efficient solutions to deal with the hazards brought by the industrialization process and reduce its own defects caused by social progress. With the discovery of more and more microorganisms, microbial remediation technology rises accordingly. In recent years, excellent results have been achieved in the application of high-yielding exopolypaccharides in Marine sewage treatment, while few studies on its application to soil heavy metal pollution in China. Therefore, this project conducted high-throughput sequencing of bacterial genome DNA in rhizosphere soil of cabbage by design of a pot experiment. The influence of Marine bacteria *Micrococcus antarcticus* RB2 with high exopolysaccharide yield under lead (Pb) stress on the microbial community structure of the rhizosphere soil of *Brassica chinensis L*. was analyzed. In this study, it was found that the relative abundance of Proteobacteria and cyanobacteria, which have the ability to remove heavy metals, increased in the rhizosphere soil under the lead contamination stress of the inoculated strain RB2. Meanwhile, after the inoculated strain RB2 in the rhizosphere soil of the inoculated strain RB2, the relative abundance of Bacillus and Pseudomonas which can promote plant growth increased. Their relative abundance was higher than that of unvaccinated control group. Therefore, RB2 promoted the relative abundance of heavy metal-clearing and growth-promoting bacteria in the rhizosphere soil community under lead stress.


**Key words:** *Marine bacteria; Soil lead pollution passivation; Chinese cabbage; High-throughput sequencing*

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# INTRODUCTION

In order to better observe the changes of bacterial community composition in rhizosphere soil by inoculated strain *M. antarcticus* RB2, NMDS analysis was carried out, and the stress coefficient Stress was 0.114, indicating that the analysis results were basically credible。 At the OTU level, the strain *M. antarcticus* RB2 had no significant effect on the number of OTUs in the rhizosphere soil microbial community without pollution and high concentration of $Pb^{2+}$. The number of OTUs of rhizosphere soil flora increased when inoculated with the strain *M. antarcticus* RB2 in soil contaminated with low concentration of $Pb^{2+}$. The results showed that inoculated strain *M. antarcticus* RB2 had a significant effect on the diversity of rhizosphere soil community of Chinese cabbage. *M. antarcticus* RB2 did not change the dominant bacterial species in the region, only the relative abundance of the region. These results showed that the strain significantly changed the bioavailability of $Pb^{2+}$ in rhizosphere soil and the distribution and abundance of indigenous microorganisms. The results showed that *M. antarcticus* RB2 posed a low ecological threat to heavy metal contaminated soil such as $Pb^{2+}$, and was a new microbial resource with great development potential.

**The relevance** of the topic is metagenomic analysis.

**The purpose of the** is to analyze the effect of RB2 on the biocommunity composition of bacteria in the rhizosphere soil under lead pollution by using Metagenomic analysis, so as to study the restoration mechanism of RB2 on lead-contaminated soil and whether the use of RB2 poses an ecological threat to the region, and to find a more green and effective restoration method for soil remediation of heavy metals.

**The objectives** of the study is to analyze the effect of RB2 on the biocommunity composition of bacteria in the rhizosphere soil under lead pollution by using Metagenomic analysis, so as to study the restoration mechanism of RB2 on lead-contaminated soil and whether the use of RB2 poses an ecological threat to the

region, and to find a more green and effective restoration method for soil remediation of heavy metals.

**The object of the study** *M.antarcticus* RB2.

**The subject of** the study *M.antarcticus* RB2.

**Research methods** Metagenomic analysis.

**The scientific novelty** Metagenomic analysis.

**The practical** finding a new and effective remediation method for soil contaminated by heavy metals.

# CHAPTER 1
# LITERATURE REVIEW

## 1.1 Soil heavy metal pollution

With the progress of the times and the development of science and technology, while promoting the progress of human society, some social development problems are gradually emerging, such as: air pollution, soil pollution, water pollution, etc., especially in recent years, with the development of industry, the situation of soil heavy metal pollution is not optimistic, and has become a hot spot in research[1]。 According to statistics, nearly $2\times10^7$ $hm^2$ of cultivated land is polluted by cadmium, lead, arsenic, chromium and other heavy metals in China, accounting for about one-fifth of the total cultivated land area in China. Among them, $1\times10^7$ $hm^2$ of cultivated land was polluted by industrial wastes, and $3.3\times10^6$ $hm^2$ of farmland irrigated by sewage [2].

### 1.1.2 Lead contamination

With the progress of industrial technology and human society, in the past 50 years, about $7.83\times10^5$t of the world has entered the environment, most of which has entered the soil, causing soil lead pollution. At present, the lead that causes soil pollution can be divided into two categories: one is primary lead, which is the lead preserved in the soil parent material, and its content generally does not exceed the local soil background value, but with the mining of rock minerals, the heavy metals in it enter the soil; The second category is exogenous lead, which mainly comes from the atmosphere, mainly including automobile exhaust, atmospheric deposition and lead-containing gas produced by smelters in industrial activities. According to the National Soil Pollution Survey Bulletin released in 2014, 1.5% of the heavy metal lead exceeded the standard, of which 1.2%, 0.2%, 0.1% and 0.1% were lightly, mildly, moderately and severely polluted, respectively It can be seen that the situation

of lead pollution in China's soil is not optimistic. As a heavy metal, lead can enter the human body through a variety of routes [3].

### 1.1.2 Sources of heavy metals in soil

Human factors: Mineral mining is an important source of soil heavy metal pollution, through mineral mining, mineral source heavy metal particles are discharged into the atmosphere, and deposited in the atmosphere, through atmospheric precipitation, dust and other into the soil[4]；The waste discharged from various industrial activities contains a large number of heavy metal elements, which enter the soil after discharge, causing soil pollution; In modern agricultural activities, the use of chemical fertilizers and pesticides may also cause soil heavy metal pollution, the development of modern aquaculture, feed and various additives have also become one of the potential sources of soil heavy metal pollution; Some of people's daily life activities are also one of the causes of soil heavy metals, such as: traffic exhaust, dust from construction sites and urban landfills2。

Natural causes: Natural minerals on the surface of the earth contain many different kinds of heavy metals, which carry heavy metals into the soil during the weathering process 3；Heavy metal dust from volcanic eruptions can also penetrate into the soil along with rainwater.

### 1.1.3 Hazards of soil heavy metal pollution

Heavy metal pollution will have an important impact on the soil, it will destroy the soil microenvironment, so that the microorganisms in the soil will be affected, may lead to the death of poorly tolerant microorganisms, break their original balance, and destroy their community structure. Moreover, soil heavy metal pollution will affect plant growth and have negative effects, and it will take decades or even hundreds of years for polluted soil to be alleviated without human intervention.

Affects soil microbial communities. Microorganisms are the active substances in the soil, which can be seen by the naked eye to reflect the changes of soil quality,

and the soil microbial community can better reflect the stability and ecological mechanism of the soil, and heavy metal pollution will interfere with and destroy the structure and function of soil microbial cells, and inhibit their biological activity4。

When microorganisms are exposed to a large number of heavy metal elements, the lipids and proteins of the cell membrane can bind to them, which may lead to changes in the permeability of the cell membrane, and then affect the physiological activity of the cell. Moreover, the presence of some heavy metals, it will bind to the DNA of microorganisms, thereby interfering with their replication and transcription, leading to genetic mutations; Heavy metal ions can bind not only to their DNA, but also to the active centers of their enzymes, thereby inhibiting the activity of enzymes, which may lead to changes in the structure and function of soil microbial populations in the long run. Heavy metal ions can also cause oxidative damage to cellular proteins, lipids, and DNA of microorganisms, affecting the growth and metabolism of microorganisms 5.

Affect the normal growth and development of plants. Soil is the survival condition of plants. Plants absorb various substances from the soil for their own growth needs. After heavy metals are absorbed by plant roots, heavy metal ions are sent to various parts of the plant through active and passive transportation, thus affecting the physiology, biochemistry and growth of plants. For example, lead can affect the photosynthesis of plants, which cannot be carried out normally, resulting in the normal synthesis of related nutrients, thus affecting the normal growth and development of plants. In addition, lead can also affect the respiration of plants, weaken the intensity of fat metabolism, greatly increase the oxygen consumption of plants, and the sugar produced by photosynthesis cannot be effectively accumulated. This eventually leads to plant death [9]. It will also destroy the membrane system of the plant, affect the normal function of the organelles, and thus make the plant metabolism disorder.

Threats to human life and health. In the process of plant growth, heavy metals in the soil will be absorbed, and plants, as producers of the ecosystem, will be eaten

by consumers in the ecosystem, and humans will be consumers of the ecosystem, so the heavy metals absorbed by plants will be ingested by the human body through the food chain, and because of the enrichment of the food chain, the human body will absorb a large number of heavy metals, which will cause serious damage to the tissues and organs of the human body, resulting in changes in the structure of nucleic acids in the human body, resulting in genetic mutations7. In recent years, the harmful incidents caused by heavy metals have been vividly remembered, such as "cadmium rice" and "excessive lead in children's blood"8. And after research has shown that some heavy metals have a carcinogenic risk, such as Cr, As, Cd, Ni, these heavy metals have been found to be carcinogenic to the human body, Hg is not carcinogenic but also affects human health, when its content is high, it will damage the human nervous system, especially for the intellectual development of young children 9.

### 1.1.4 Lead Pollution Hazards

With the development of productivity and production technology, more and more lead pollutants are produced, which eventually causes all kinds of lead pollution, and the lead in the soil is absorbed by plants, affecting the growth and development of plants, and makes lead accumulate in the plant body and is absorbed by the human body through the food chain. After research, no lead in the soil has been found to be beneficial to living organisms10.Moreover, lead enters the human body not only through the food chain, but also through inhalation through the mouth and nose, contact with the skin, etc. Moreover, the heavy metal lead has been listed as a Group 2B carcinogen by the World Health Organization, and the heavy metal lead can cause lead poisoning in the human body and cause damage to human tissues and organs.

## 1.2 Remediation methods for soil heavy metal pollution

### 1.2.1 Traditional soil remediation techniques

Physical remediation method: refers to the use of physical methods for restoration, mainly including guest soil, soil replacement, deep ploughing and soil turning, electric repair and thermal desorption, electrothermal remediation or soil leaching restoration.

The difference is that the guest soil method adds new soil to the original soil to dilute the concentration of heavy metals, while the soil exchange method adds unpolluted soil after removing the contaminated soil. The deep ploughing method refers to mixing the deep soil with the soil contaminated by heavy metals on the surface, so as to play a similar role in dilution; Electric remediation technology refers to the insertion of the anode and cathode into the soil respectively under the action of an electric field, and by applying a low-power electric current, the heavy metals are moved and enriched in the direction of the corresponding electrode, and then processed intensively by precipitation or removal. This is a method of separating heavy metal ions from the soil and then collecting them in a centralized manner, which can effectively shorten the remediation time and have relatively high economic benefits12.Thermal desorption technology refers to the evaporation of heavy metals by heating them.

Chemical remediation method: chemical remediation technology is to add reagents that can react with heavy metals to the soil polluted by heavy metals, with the help of the adsorption and passivation effects of chemical reagents, or to change the morphology, solubility and mobility of heavy metals in the soil, so as to achieve the purpose of soil remediation13.Chemical remediation techniques include methods such as leaching, chemical modification, and curing/stabilization techniques. Chemical improvement technology is to add corresponding chemical agents to the soil polluted by heavy metals, and reduce the bioavailability of heavy metals through adsorption, redox and precipitation between chemicals and heavy metals.

### 1.2.2 Bioremediation techniques

Bioremediation technology is a hot research topic in recent years, and it plays an important role in the remediation process of soil heavy metal pollution. Bioremediation technology is a method to degrade or transform heavy metal pollutants in soil by using the activity and metabolic capacity of organisms. It has the advantages of environmental friendliness, economic feasibility and sustainable development [18]. Bioremediation technology mainly includes plant, microbial and animal restoration technology.

Animal remediation technology. It refers to the corresponding animal to reduce the content of heavy metals in the soil through its own activities, such as using the excrement of earthworms, rodents and other animals to adsorb heavy metals such as Pb and Cd to achieve the purpose of restoration.

Phytoremediation techniques. It is to use plants that have an enrichment effect on heavy metals to absorb heavy metals in the soil, and through the photosynthesis of plants, the heavy metal particles can be converted and removed. Phytoremediation technologies include plant fixation technology, plant volatilization technology and plant extraction technology. However, phytoremediation technology has requirements for the selection of plants, requiring the use of plants that have an enrichment effect on heavy metals.

Microbial remediation technology. Microbial remediation technology is to reduce the biological toxicity of heavy metals in soil through a series of metabolic activities of microorganisms and the secretions of microorganisms, mainly through the respiration and redox of microorganisms to transform heavy metals, which can change the highly toxic heavy metal ions into low-toxicity metal ions through valence changes, thereby reducing the toxicity of heavy metals. Moreover, microorganisms can reduce the toxicity of heavy metals through metabolism and produce some substances, for example, the organic acids produced by microorganisms in the metabolic process can complex and dissolve heavy metals, and polysaccharides can adsorb heavy metals in the soil. In the actual application process, the resistant strains

added to the soil will produce polysaccharides, glycoproteins and other substances in the soil to form complexes with heavy metals, which can reduce the content of heavy metals in the soil.

### 1.3 Rhizosphere soil microorganisms

Microorganisms are the decomposers in the ecosystem, it is the dominant component of the soil decomposition system, is the most active component of the soil, the interaction of plants-soil-rhizosphere microorganisms together form a multi-functional complex organic whole, and the characteristics of soil microorganisms (soil microbial population, community structure and functional group, microbial biomass, enzyme activity, etc.) can reflect the evolution of soil quality and soil fertility15. Rhizosphere microorganisms refer to the microorganisms closely attached to the rhizosphere soil particles, which are mainly bacteria, among which Gram-negative bacteria are dominant, the most common are *Pseudomonas*, *Bacillus flavus*, *Bacillus calyptogenes*, *Agrobacterium* and so on. Plant rhizosphere soil microorganisms are one of the most important parts of the soil ecosystem, microorganisms can decompose the organic matter entering the soil from the outside world, and convert the organic matter into nutrients, so that plants can absorb and utilize, and play a vital role in the energy flow of the forest ecosystem. These myriad microscopic organisms directly or indirectly affect plant growth and community composition through nutrient cycling, pathogen inhibition, growth promotion, and stress resistance 17.

### 1.4 Effects of heavy metal pollution on bacterial community composition in rhizosphere soil

As more and more heavy metals are discharged into the environment, more and more heavy metals are accumulated into the soil, and the microorganisms in the soil are bound to be affected. It can affect the composition, structure and metabolic function of microbial community.

Studies have shown that when the concentration of heavy metals is high, microorganisms will develop more tolerance or resistance to them, destroying their community structure and causing serious consequences to the microbial ecosystem 18.

Studies have shown that the diversity of microbial communities in soil decreases with the increase of heavy metal content in soil. Moreover, the effect on fungal population richness was more pronounced than that of bacteria 20. Moreover, the effects of soil heavy metal pollution on microorganisms showed a general pattern: severe heavy metal pollution had an inhibitory effect on soil matter, and mild heavy metal pollution had an activating effect on soil microorganisms. Studies have shown that various microorganisms have different tolerances to heavy metal pollution, and the results show that the microbial diversity in the soil polluted by heavy metals for a long time does not decrease, but the population composition of the soil microbial community changes 22.

### 1.5 Marine bacteria and extracellular polysaccharides

Marine bacteria are prokaryotic single-celled organisms that do not contain chlorophyll and phycocyanin living in the ocean. They are widely distributed in the ocean. Marine bacteria play an important role in the Marine ecology. When the Marine ecology is damaged, Marine bacteria adjust the ecosystem quickly through their strong adaptability and extremely fast reproduction rate. In recent years, seawater has been polluted by various human factors such as domestic garbage and industrial wastewater, and Marine bacteria have been found to degrade various pollutants and poisons, so Marine bacteria have been applied to sewage treatment.

Living under special conditions such as high salt, high pressure and high temperature, the quality and content of exopolysaccharides produced by Marine bacteria are different in different environments [26]. Due to the particularity of its function and structure, it has attracted wide attention. Exopolysaccharide is an important part of extracellular polymer, which mainly refers to the sugar coating

composed of microcapsule, capsule, mucous layer and bacterial micelle, which can protect microorganisms from the toxicity of heavy metal ions and also protect microorganisms from the phagocytosis of host cells. Some exopolysaccharides contain a number of negatively charged functional groups in the structure so that it has flocculation ability, can absorb heavy metals in the environment, or REDOX reactions with heavy metal ions, remove or reduce the toxicity or biological availability of heavy metals, and studies have shown that exopolysaccharides can promote plant growth. With the deepening of the research on Marine bacteria, more and more Marine extracellular polysaccharides with special structures and activities have been discovered, and their functions have been studied in many fields, such as anti-tumor, anti-oxidation, immune regulation and flocculation [27].

**Conclusions to chapter 1**

1. Soil contaminated lead can currently be divided into two categories: one is primary lead and the second is exogenous lead. There are two main causes of soil pollution, one is human factors, such as the use of chemical fertilizers and pesticides, industrial emissions, etc., and the other is natural factors, such as the weathering of surface minerals.

2. There are many hazards caused by heavy metals in the soil, it will harm the growth of plants, and it will also affect the corresponding microorganisms, and the pollution of heavy metals in the soil will cause damage to the human body.

3. Soil microbial remediation methods include physical remediation, chemical remediation and biological remediation.

4. Plant rhizosphere soil microorganisms are one of the most important parts of the soil ecosystem and play a vital role in the energy flow of the ecosystem. Soil heavy metal pollution will affect the composition and metabolic function of microbial communities.

5. Marine bacteria, which live under special conditions such as high salt, high pressure and high temperature, are prokaryotic single-celled organisms that do

not contain chlorophyll and phycocyanin, and play an important role in marine ecology.

6. The structure of exopolysaccharides contains a number of negatively charged functional groups, which makes it have flocculation ability, which can adsorb heavy metals in the environment, or react with heavy metal ions such as redox to remove or reduce the toxicity or bioavailability of heavy metals, and exopolysaccharides can promote plant growth.

# CHAPTER 2
# OBJECT, PURPOSE AND METHODS OF THE STUDY

The use of microorganisms to repair soil contaminated by heavy metals has become a research hotspot in recent years. Through the study of Marine microorganisms in recent years, many Marine microorganisms with high exopolysaccharides have been found and applied to the treatment of water pollutants. Based on this, some scientists have shifted their attention to the remediation of soil heavy metal pollution. However, few studies have been done on this subject. The secretion of exinsic polysaccharide (EPS) by Marine microorganisms is a common phenomenon, and the properties of EPS, a highly effective bioadsorbent material, and M. antarcticus RB2, a microbial membrane isolated from the body surface of abalone seedlings, have the ability to degrade a variety of organic pollutants. It has a good prospect in the treatment of low temperature pollution. The bacterium M.antarcticus RB2 can produce a large amount of high yield exopolyaccharides, and exopolyaccharides, as an important component of extracellular polymer EPS, are highly efficient bioadsorbent materials.

Moreover, there are few researches on the composition of rhizosphere soil microbial community after PGPR inoculation. Therefore, the purpose of this study was to analyze the bacterial community composition of rhizosphere soil under different lead stress conditions of M. antarcticus RB2 strain inoculated with a high yield of exopolysaccharide, in order to explore the mechanism of RB2's remediation of lead-contaminated soil, as well as the changes of microbial community after inoculation with strain RB2 compared with that of the untreated group. It is hoped that this study can find a more effective and green remediation method for the remediation of heavy metal pollution in soil by revealing the repairing effect of the original flora in soil under the condition of lead pollution.

**Conclusions to chapter 2**

The tested strain M. antarcticus RB2, a microbial membrane isolated from the body surface of abalone seedlings, can produce large quantities of high-yielding exinsic polysaccharides, which can degrade a variety of organic pollutants, and has good prospects for low temperature pollution treatment. Therefore, this study hopes to find a more effective and green remediation method for soil heavy metal pollution through the study of strain RB2.

# CHAPTER 3
# EXPERIMENTAL PART

## 3.1 Experimental Materials

The test strain was obtained by *Micrococcus antarcticus* RB2 from the microbial membrane on the surface of abalone seedlings，keep in the laboratory. The tested soil was taken from the experimental base of the Maize Research Institute of Shandong Academy of Agricultural Sciences, China, located at 117°22′ east longitude and 36°44′ north latitude. The soil is osmanthus loess. According to the USDA's texture classification system, this soil is classified as Chromic Cambisol. The initial pH value of soil was 7.8, and the organic matter, total nitrogen, total phosphorus, and total potassium were 13.72, 1.45, 0.64, and 2.41 g/ kg, respectively. The test plant was Chinese cabbage, and the seeds of Chinese cabbage were purchased from the Shandong Academy of Sciences.

## 3.2 Experimental Methods

### 3.2.1 Culture of strain RB2

Preparation of seed liquid medium: yeast paste 1g, peptone 5 g, sea salt 35g, distilled water 1000mL, pH 7.6 ~ 7.8.

Preparation of bacterial suspension: The activated strain *M. antarcticus* RB2 was inoculated in sterile seed liquid medium and incubated at 25°C for 8 h on a shaker at 180 r/min. After 8 h, the culture solution was centrifuged (5000 rpm, 4 min) to remove the supernatant and resuspended with sterile deionized water.

### 3.2.2 Treatment of the tested soil

Collection and processing of soil samples: Soil at a depth of 0-15cm from unpolluted farmland of Shandong Academy of Agricultural Sciences was collected, gravel, gravel and other debris were removed, and PbCl2 solution was configured (3 concentration gradients were set: 0, 25 and 50mg/kg, respectively), mix the prepared

PbCl2 solution well with the collected soil, each (set up three replicates of the experiment per gradient). Each concentration gradient weighed 4.8 kg of the mixture and placed it in pots with a diameter of 28 cm and a height of 35 cm, and then equilibrated for 45 days, during which time the soil moisture content was kept at about 70% of the water holding capacity of the field, so deionized water was added, and the soil of each pot was loosened once a week during this period.

### 3.2.3 Potted plant experiments

Chinese cabbage sowing: The soil was well balanced in pots and placed in a greenhouse with a temperature of 25±3°C, a relative humidity of 70%, and an average photoperiod of 12 h/d, with 6 parallels (i.e. 6 pots) for each $Pb^{2+}$ concentration. The Chinese cabbage seeds collected from the Academy of Agricultural Sciences were selected by flotation method, and the seeds with uniform size and full particles were selected, and then they were soaked in 5% sodium hypochlorite solution for disinfection for 10 min, and after disinfection, they were fully washed with sterile water for three times, and finally the seeds were put on sterilized and clean filter paper to fully absorb the water on the surface of the seeds. Then select the whole and undamaged seeds to sow into the pots, 30 seeds in each pot, maintain greenhouse conditions, water regularly, and wait for the seeds to germinate and dilute.

Dilute seedlings: After the seeds germinate, wear sterile gloves, dilute the planting density to 15 plants per pot, keep the seedlings with relatively robust growth and good growth, make them evenly distributed in the pot, and record the numbers respectively. The diluted Chinese cabbage was kept in a greenhouse with a temperature of 25±3°C, a relative humidity of 70% and an average photcycle of 12 h/d for 45 days. Deionized water was added daily, and the soil moisture was kept at 70% field humidity.

Root irrigation treatment: When the Chinese cabbage grows to the third leaf stage, the root irrigation treatment is carried out. Root irrigation treatment: When the

Chinese cabbage grows to the third leaf stage, the root irrigation treatment is carried out.After root irrigation, deionized water was added daily to maintain soil moisture at 70% field humidity for 45 days, during which greenhouse conditions were maintained (temperature of 25±3 °C, relative humidity of 70%, and average photoperiod of 12 h/d).

### 3.3 Methods of sample collection and determination

#### 3.3.1 Collection and processing of samples

Collection and preservation of rhizosphere soil: Carefully remove the plants with rhizosphere soil from the pot and gently shake off the soil sticking to the root system;Collect the soil (used as rhizosphere soil) by gently brushing off the soil tightly bound to the root system with a soft bristle brush, and store the collected soil at -80 °C for subsequent extraction of total DNA.

#### 3.3.2 High-throughput analysis of soil microorganisms in the rhizosphere of Chinese cabbage

Bacterial genomic DNA from soil samples was extracted with a fast DNA spin kit, The quantity and quality of extracted DNA were determined with spectrophotometer (NanoDrop 100，ThermoScientific, USA) and gelelectrophoresis, respectively . The extracted DNA was amplified with primers 338 F（5′-ACTCCTACGGGAGGAGCA -3′）and 806 r（5′-GGACTACHVGGT-WTCTAAT -3′，It targets the V4 region of bacterial 16S rRNA. Sample-specific 7-bp barcode fused to primers. The PCR reaction conditions were: Predenaturation at 98 ℃ for 3 min; Denatured at 98 ℃ for 30 s; Annealing at 50 ℃ for 30 s; Extend for 30 s at 72 ℃; 27 cycles; Keep warm at 72 °C for 5 min and store at 4 °C. At the end of the reaction, a 2% agarose gel was prepared for electrophoresis to detect the PCR product. Soil bacterial community structure in the rhizosphere of Chinese cabbage was analyzed using Illumina-Miseq high-throughput sequencing technology, and sequences were clustered into actionable taxonomies (OTUs) using the QIIME

software package to set 97% similarity. All downstream data analysis was performed in QIIME and R packages (v.3.2.0). Richness estimates, diversity index, and Simpson index to assess species evenness. Perform non-metric multidimensional scaling (NMDS) on a distance matrix and use coordinates to plot 2D graphics. The abundance of taxa at the phylum, class, order, family and genus levels was statistically compared between samples or groups through meta-states, and species abundance tables and multi-sample species distribution maps at different taxonomic levels (phylum and class) were generated, and redundancy analysis (RDA) was used to study the relationship between bacterial communities in water bodies and environmental factors in rhizosphere soils.

### 3.4 Analysis of the structural diversity of rhizosphere soil microbial community

#### 3.4.1 Effect of RB2 inoculation on the relative abundance of dominant bacteria

As shown in Figure 1A, the relative abundance of *Acidobacter* phylum decreased first and then increased with the increase of $Pb^{2+}$ concentration after inoculation with *M. antarcticus* RB2 strain. When the concentration of $Pb^{2+}$ was 25 $mg \cdot kg^{-1,}$ the relative abundance of *Acidobacteria* of inoculated strains was lower than that of the uninoculated control group, However, when the concentration of $Pb^{2+}$ was 50 $mg \cdot kg^{-1}$, the abundance of *Acidobacteria* in the inoculated strain was significantly higher than that in the non-inoculated control group (Fig. 3.1).

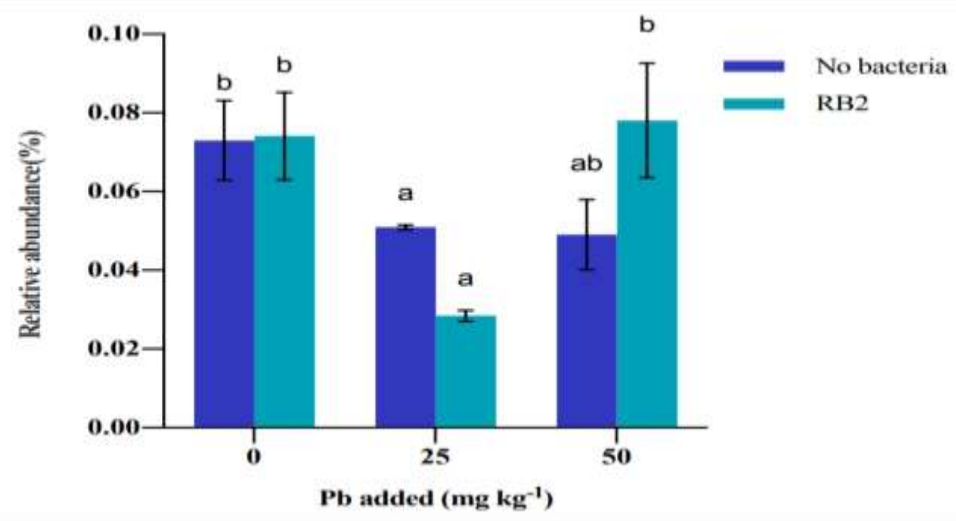


Figure 3.1– Effect of strain *M.antarcticus* RB2 on the relative abundance of *Acidobacteria* in rhizosphere soil. The data were the mean of 3 replicates ±SD (n=4), with different letters representing significant differences between treatments $p \leq 0.05$

The relative abundance of *Proteobacteria* increased first and then decreased with the increase of $Pb^{2+}$ concentration after inoculation with the strain *M. antarcticus* RB2, while the relative abundance of *Proteobacteria* of the uninoculated strain *M. antarcticus* RB2 decreased with the increase of lead contamination concentration. However, the concentration of $Pb^{2+}$ remained unchanged after 25 $mg \cdot kg^{-1,}$ and the relative abundance of *Proteobacteria* inoculated with strain *M. antarcticus* RB2 in uncontaminated lead soil was lower than that in the uninoculated group,However, when the concentrations of $Pb^{2+}$ were 25 $mg \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$, the relative abundance of *Proteobacteria* inoculated was significantly higher than that of the non-inoculated control group (Fig. 3.2).

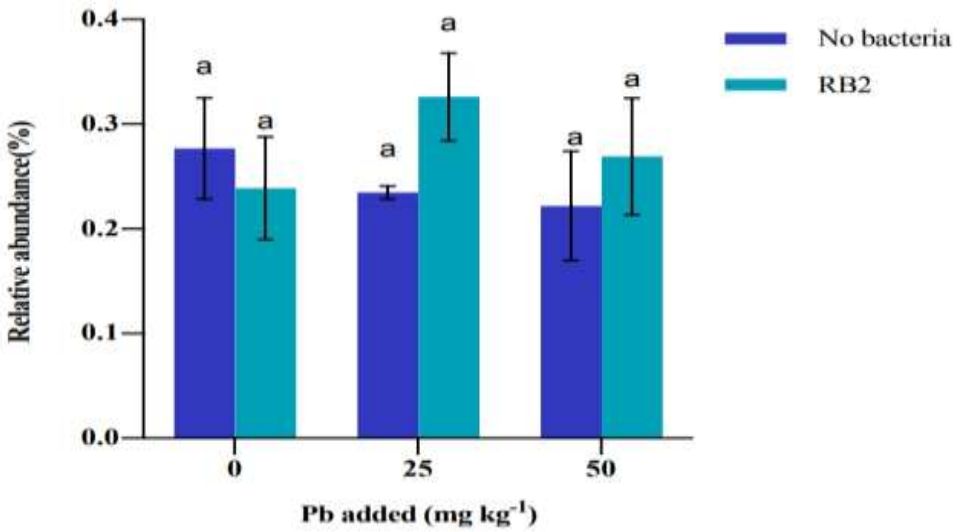


Figure 3.2 –Effect of strain *M.antarcticus* RB2 on the relative abundance of *Proteobacteria* in rhizosphere soil. The data were the mean of 3 replicates ±SD (n=4), with different letters representing significant differences between treatments $p \leq 0.05$

The relative abundance of *Cyanobacterial* phylum inoculated with the strain *M. antarcticus* RB2 decreased with the increase of $Pb^{2+}$ concentration, However, the relative abundance of *Cyanobacteria* of *M.antarcticus* RB2 did not change much, but the relative abundance of *Cyanobacteria* of *M.antarcticus* RB2 was significantly higher than that of the control group (Fig. 3.3).

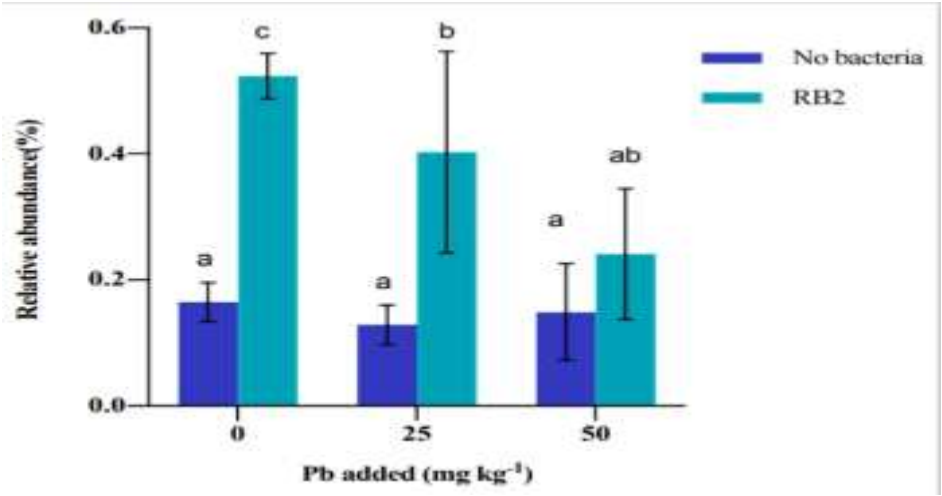


Figure 3.3 – Effect of strain *M.antarcticus* RB2 on the relative abundance of *Cyanobacteria* in rhizosphere soil. The data were the mean of 3 replicates ±SD (n=4), with different letters representing significant differences between treatments $p \leq 0.05$

The relative abundance of *Proteobacteria* and *Acidobacterium* did not change significantly with the increase of $Pb^{2+}$ concentration in rhizosphere soil without inoculation with the inoculation of strain *M. antarcticus* RB2,However, the relative abundance of *Cyanobacteria* in contaminated soil was higher than that in uncontaminated soil. In 25mg·kg$^{-1}$$Pb^{2+}$ contaminated soil, the relative abundance of *Acidobacteria* in the inoculated RB2 group was lower than that in the uninoculated control group, the relative abundance of *Proteobacteria* and *Cyanobacteria* were higher than those in the non-inoculated control group. When the concentration of $Pb^{2+}$ was 50 mg·kg$^{-1}$, the relative abundance of *Acidobacteria*, *Proteobacteria* and *Cyanobacteria* was significantly increased compared with the non-inoculated control group (Fig. 3.4).

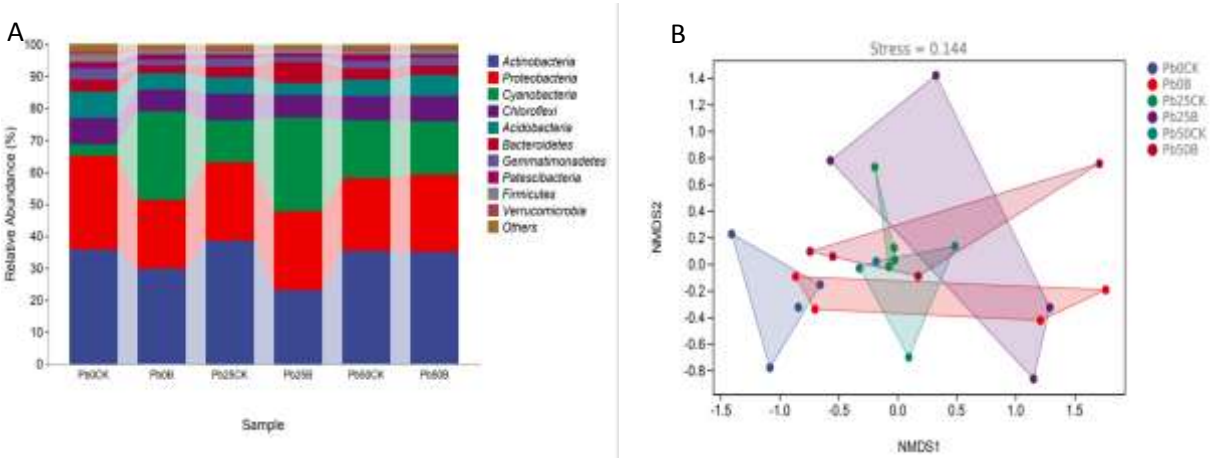


Figure3.4 – Effects of strain RB2 on the relative abundance of soil communities under different treatments at gate level (A). The non-metric multidimensional Scale (NMDS) shows weighted Uni Frac distances based on all communities. Each colored dot represents a sample (B).

### 3.4.2 Community composition analysis

A total of 45 phyla were identified in all rhizosphere soil samples, and the microbial composition of all samples was basically the same at the gate level.Phyla with higher relative abundance include: *Actinobacter*, *Proteobacteri*, *Cyanobacteria*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*. *Among Actinomycetes* (22.8%~38.4%) were the dominant phyla, followed by *Proteobacteria* (21.3%~29.1%),

*Cyanobacteria* (3.5%~29.1%), *Chloroflexi* (6.9%~8.3%), and *Acidobacteria* (3.9%~8.2%), *Bacteroidetes* (2.3%~6.3%). Through the analysis, it is found that the application of $Pb^{2+}$ and RB2 affects the diversity of rhizosphere soil flora.

### 3.4.3 Heatmap analysis

Figure 3.5 shows the top 30 dominant phyla in the rhizosphere soil of Chinese cabbage at different $Pb^{2+}$concentrations, such as *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, and *Bacteroidetes*. When the uninoculated control group was stressed with $Pb^{2+}$, the relative abundance of *Actinobacteria* showed a trend of first increasing and then decreasing with the increase of $Pb^{2+}$ concentration. The relative abundance of *Proteobacteria* and *Chloroflexi* decreased with the increase of $Pb^{2+}$ concentration. The relative abundance of *Cyanobacteria* increased with the increase of $Pb^{2+}$ concentration, which was quite the opposite of *Proteobacteria*. The relative abundance of *Enterobacteria* and *Latescibacteria* decreased significantly. As shown in Figure 3.5, in 25mg·$kg^{-1}$ $Pb^{2+}$ contaminated soil, the relative abundance of *Chloroflexi* and *Firmicutes* in the inoculated RB2 group was slightly lower than that in the uninoculated control group, but not significant. The relative abundance of *Epsilonbacteraeota*, *Tenericutes* and *Actinobacteria* was lower than that of the unvaccinated control group. The relative abundance of *Cyanobacteria*, *Bacteroidetes* and *Latescibacteri* was higher than that of unvaccinated control group. In the soil inoculated with strain RB2, when the concentration of $Pb^{2+}$ was 50mg·$kg^{-1}$, compared with the uninoculated control group, The relative abundance of *Chloroflexi*, *Proteobacteria* and *Firmicutes* increased, while the relative abundance of *Nitrospirae* and *Enterobacteriae* increased. The relative abundance of *Entotheonellaeota* and *WS2* increased significantly (Fig. 3.5).

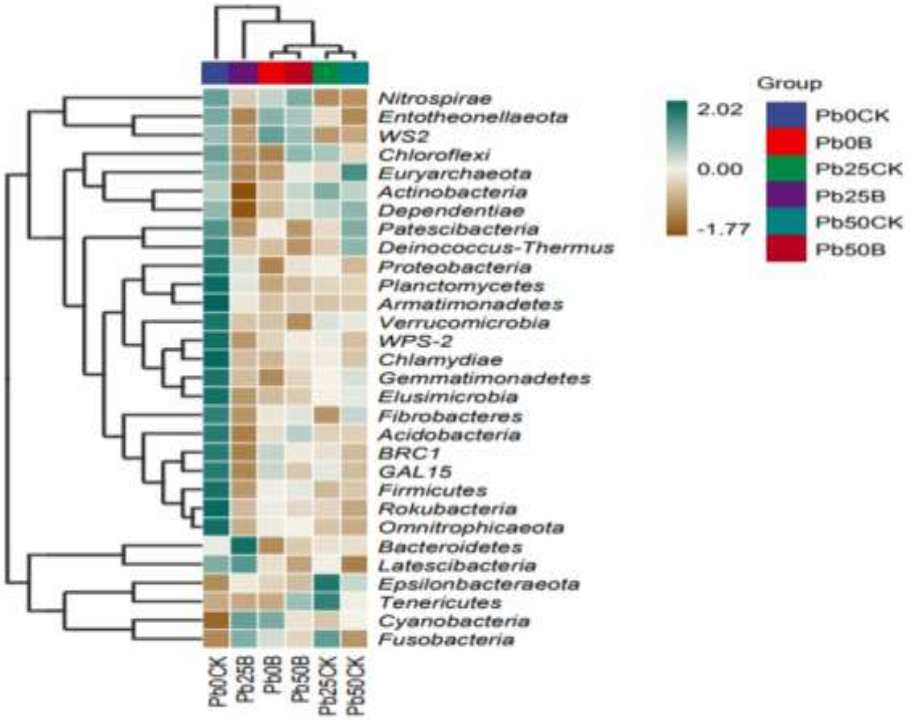


Figure 3.5 – For the first 30 phyla in the rhizosphere soil under different $Pb^{2+}$ concentrations, the relative values of each phyla or genus are depicted by color intensity according to the legend in the upper right corner

At the dependent level, it can be seen from Figure 3.6 that when the rhizosphere soil containing $Pb^{2+}$ was not inoculated with strain RB2, compared with the soil without $Pb^{2+}$, The relative abundance of *MND1*, *KD4-96*, *Gitt-GS-136*, *Bacillus*, *Sphingomonas*, *Ramlibacter*, *Subgroup_6* and *67-14* decreased significantly. The relative abundance of *Streptomyces* and *Nocardioides* in soil decreased, but not significantly. The relative abundance of *Microcoleus_PCC-7113*, *Microcoleus_Es-Yyy1400*, and *SBR1031* increased. When the concentration of lead pollution in rhizosphere soil was 25 $mg \cdot kg^{-1}$, The relative abundance of *Pseudomonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Lysobacter* in the inoculated strain treatment group was significantly higher than that in the uninoculated control group. The relative abundance of *Kribbella*, *Lechevalieria*, *Mycobacterium*, *Herpetosiphon*, *IMCC26256* and *Aeromicrobium* was

significantly lower than that of unvaccinated control group. The relative abundance of *SBR1031* and *A4b* in rhizosphere soil was significantly higher than that in uninoculated control group when the concentration of Pb contamination in rhizosphere soil was 50 $mg \cdot kg^{-1}$. The relative abundance of *Subgroup_6*, *Bacillus*, *Sphingomonas* and *Lysobacter* were slightly higher than those of the unvaccinated control group. The relative abundance of *Saccharimonadales* and *Haliangium* was significantly lower than that of unvaccinated control group (Fig. 3.6).

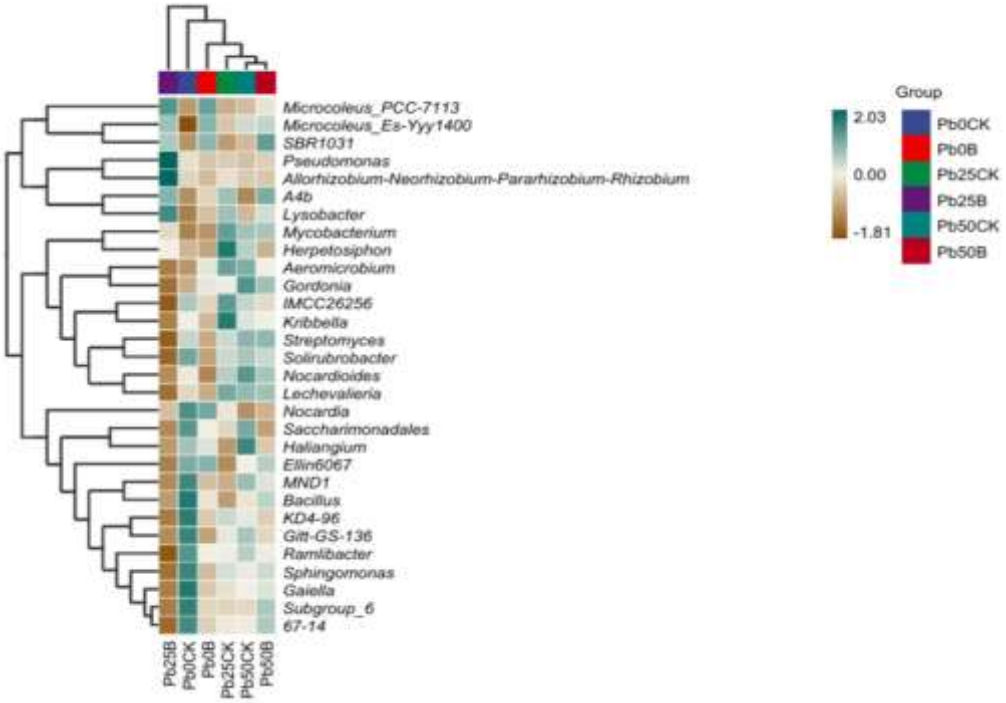


Figure3.6 – Heat maps of the first 30 genera (D) in the rhizosphere soil of calcareous Chinese cabbage at different concentrations of $Pb^{2+}$. According to the legend in the upper right corner, the relative value of each phylum or genus is depicted by color intensity

**Conclusions to chapter 3**

1. The strain used in this study was Micrococcus antarctica isolated from the microbial membrane layer on the surface of abalone seedlings Micrococcus antarcticus RB2.
2. Culture of strain RB2.

3. Treatment of the tested soil.

4. Bonsai experiments.

5. Sample collection and processing.

6. High-throughput analysis of soil microorganisms in the rhizosphere of Chinese cabbage.

7. A total of 45 phyla were identified from rhizosphere soil samples, and the microbial composition of all samples was basically the same at the phylum level. The analysis showed that the application of Pb2+ affected the diversity of rhizosphere soil microbiota.

8. The relative abundance of the top 30 dominant phyla and strains RB2 in the rhizosphere soil of Chinese cabbage under different Pb2+ concentrations was different between inoculated and uninoculated groups.

9. The relative abundance of dominant bacteria in the inoculated strain RB2 and the untreated group was different under different lead pollution concentrations.

# CONCLUSIONS

1. RB2 can increase the relative abundance of relevant microflora with the ability to remove heavy metals. Microorganisms can degrade or transform heavy metals in soil through their own actions, such as adsorption and redox by microorganisms. The adsorption of microorganisms is to use the extracellular polymers and metal-binding proteins produced by microorganisms to adsorb or bind metal ions and reduce the toxic effect of metal ions on plants. The strain RB2 used in this study is a strain that can produce exopolysaccharides, which can adsorb positively charged heavy metal ions due to their structural specificity, and exopolysaccharides are a low-toxicity, biodegradable, and sustainable substance.

In this study, the application of strain RB2 increased the relative abundance of bacteria with heavy metal scavenging ability, such as *Proteobacteria*, *Cyanobacteria*, and *Firmicutes*. *Proteobacteria* and *Cyanobacteria* belong to Gram-negative bacteria, and Gram-negative bacteria can absorb heavy metal cations due to their more lipopolysaccharides in the outer layer of their cell wall and strong negative charge. Some bacteria in *Firmicutes* have a degrading effect on heavy metals. In this study, the relative abundance of *Proteobacteria*, *Cyanobacteria* and *Firmicutes* in the inoculated strain RB2 treatment group was significantly higher than that in the uninoculated strain treatment group when the concentration of lead pollution in the rhizosphere soil of Chinese cabbage was 50 $mg \cdot kg^{-1}$. In this study, the application of strain RB2 increased the relative abundance of bacteria with the ability to scavenge heavy metals, such as *Bacillus* and *Pseudomonadaceae*, which could change the valence state of heavy metals into poorly mobile, valence ions with low toxicity and water solubility**Ошибка! Источник ссылки не найден.**; *Mycobacterium* has an adsorption effect on heavy metals. In this study, the relative abundance of the above microbiota in the rhizosphere soil inoculated with strain RB2 under lead contamination conditions was significantly higher than that in the rhizosphere soil of uninoculated Chinese cabbage. Therefore, the strain RB2 could change the structure

of soil biota and increase the relative abundance of microflora with heavy metal scavenging ability in the rhizosphere soil of Chinese cabbage under the stress of lead pollution. The above experimental results showed that the relative abundance of bacteria with heavy metal scavenging ability was higher than that of the non-inoculated treatment group due to the presence of RB2, so the strain RB2 could achieve the purpose of soil remediation by changing the rhizosphere soil community structure of Chinese cabbage under the stress of lead pollution.

2. RB2 increases the relative abundance of flora associated with the ability to promote plant growth. *Firmicutes* participates in the environmental carbon cycle and organic matter decomposition process, which can promote plant growth. *Proteobacteria* play an important role in the nitrogen cycle and can transform inorganic phosphates for plant utilization**Ошибка! Источник ссылки не найден.** Therefore, in this study, when the concentration of lead pollution in the rhizosphere soil of Chinese cabbage was 50 $mg \cdot kg^{-1}$, the relative abundance of *Proteobacteria* and *Firmicutes* in the inoculated strain RB2 treatment group was significantly higher than that in the uninoculated strain treatment group. *Bacteroides* has a strong ability to degrade cellulose, promote carbon cycle and promote plant growth. In this study, the relative abundance of *Bacteroides* in the inoculated strain RB2 treatment group was significantly higher than that in the uninoculated strain treatment group when the concentration of lead contamination in the rhizosphere soil of Chinese cabbage was 25 $mg \cdot kg^{-1}$. Pseudomonas in the phylum *Proteobacteria* will produce a variety of antibacterial substances in the metabolic process, such as antibiotics, volatile bacteriostats, etc., which have an antagonistic effect on plant pathogenic bacteria, which can well inhibit the growth of pathogenic bacteria, and the rhamnose and other substances produced by *Pseudomonas* can also play an insecticidal role. It can also produce plant growth hormone that promotes plant growth. In this study, when the concentration of lead pollution in the rhizosphere soil of Chinese cabbage was 25 $mg \cdot kg^{-1}$, the relative abundance of *Pseudomonas* in the inoculated strain RB2 treatment group was significantly higher than that in the uninoculated control.

Bacillus spp., which belongs to the phylum *Firmicutes*, has the function of nitrogen fixation to promote plant growth, and produces auxin that promotes plant growth during its metabolism. and in this study, when the concentration of lead pollution in the rhizosphere soil of Chinese cabbage was 50 $mg \cdot kg^{-1}$, The relative abundance of *Bacillus* in the inoculated strain RB2 treatment group was significantly higher than that in the non-inoculated treatment group. *Lysobacterium*, which belongs to the phylum *Proteobacteria*, can also secrete a variety of antibiotics and extracellular hydrolases to inhibit the growth and reproduction of plant pathogens. Bacteroidetoides sphingosinomonas can produce hormones and metabolites that stimulate plant root development, such as indoleacetic acid, gluconic acid, and glucosamine. when the concentration of lead contamination in the rhizosphere soil of Chinese cabbage is 50 $mg \cdot kg^{-1}$. The relative abundance of *Sphingomonas* and *Lysobacter* in the RB2 inoculation group was higher than that in the non-inoculation group. According to the above results, inoculation with strain RB2 could increase the relative abundance of beneficial microflora in the rhizosphere soil of Chinese cabbage under lead pollution stress, which was more conducive to plant growth.

According to the analysis of the above studies, inoculated strain RB2 had a significant effect on the diversity of soil community in the rhizosphere of Chinese cabbage. RB2 did not change the dominant bacterial species in the region, but only the relative abundance of the microflora in the region. Under lead stress, inoculation with RB2 increased the relative abundance of bacteria with heavy metal ion scavenging ability and plant growth in the rhizosphere soil of Chinese cabbage, and almost all of the dominant bacteria were tolerant to heavy metal ions, scavenging heavy metal ions or beneficial to plant growth.

# LIST OF REFERENCES

1. Chen Nengchang, Zheng Yuji, He Xiaofeng, Li Xiaofei & Zhang Xiaoxia. (2017). Analysis of the Communiqué of the National Soil Pollution Survey. *Journal of Agro-Environmental Science* (09), 1689-1692.

2. Chen Nengchang, Zheng Yuji, He Xiaofeng, Li Xiaofei & Zhang Xiaoxia. (2017). Analysis of the Communiqué of the National Soil Pollution Survey. *Journal of Agro-Environmental Science* (09), 1689-1692.

3. Hua Lanying, Xia Ye, Zou Bendong, Zhang Jin & Zhao Hongshuai. (2024). Pollution sources, hidden dangers and remediation technologies of soil heavy metals. *Environmental Ecology* (02), 90-94.

4. Fang Jin, Zheng Juhao & Qian Jiali. (2024). Briefly describe the research progress of soil heavy metal pollution source analysis and remediation technology. *Leather Making and Environmental Technology* (03), 100-102. doi:10.20025/j.cnki.CN10-1679.2024-03-34.

5. Yu Qiongyang, Li Wanyi, Zhang Ning, Zhao Ling, Wang Yuemei, Song Jiayin... & Shen Renfang. Research progress on heavy metal pollution in farmland soil and safe utilization technology. *Soil* 1-13.

6. Zhao Yuxin. (2024). Review of the effects of heavy metal pollution on the ecological characteristics of soil microorganisms. *Comprehensive Utilization of Resources in China* (03), 89-91.

7. Chen Lixia, Xu Dongmei, Sun Huanru & Li Tong. (2024). Research progress on the application of microbial technology in the remediation of soil heavy metal pollution. *Cleaning the World* (03), 70-73.

8. Lin Meili. (2022). Current status of soil heavy metal pollution and research progress on detection and analysis technology. *Chemical Design Bulletin* (07), 145-147.

9. Yang Xue, Wang Huifang, Li Yuancheng & Lv Dongpeng. (2022). A brief discussion on the status quo of soil heavy metal pollution and its ecological restoration. *Environmental Protection and Circular Economy* (08), 55-57.

10. Li Mingyuan & Lv Yanqiong. (2023). Analysis and application of remediation technologies for soil heavy metal pollution. *Journal of Contemporary Chemical Research* (09), 79-81. doi:10.20087/j.cnki.1672-8114.2023.09.025.

11. Ji Yaokun. (2023). Analysis of microbial remediation techniques for heavy metal pollution in farmland soil. *Industrial Microbiology* (04), 125-127 .

12. Wang Yan, Lin Hai, Fu Datian, Mo Wenwen & Han Rui. (2023). Research progress on the characteristics and clinical diagnosis and treatment of lead poisoning in children. *Journal of Clinical Military Medicine* (08), 874-877. doi:10.16680/j.1671-3826.2023.08.28.

13. Wang Hanying. (2023). Analysis of microbial diversity in lead-contaminated Soil and Study on Characteristics of Pb Mineralized bacteria Master's Degree (Dissertation, Guizhou Minzu University). Master's https://link.cnki.net/doi/10.27807/d.cnki.cgzmz.2023.000422 doi: 10.27807 /, dc nki. CGZMZ. 2023.000422.Yang Xue, Wang Huifang, Li Yuancheng & Lv Dongpeng. (2022). A brief discussion on the status quo of soil heavy metal pollution and its ecological restoration. *Environmental Protection and Circular Economy* (08), 55-57.

14. Li Ding, Zhou Ziqiang & Hong Yang. (2022). Research and analysis of soil heavy metal pollution and remediation methods. *Agricultural Disaster Research* (04), 147-149.

15. Ren Yu, Cao Wengeng, Xiao Shunyu, Li Xiangzhi, Pan Deng & Wang Shuai. (2024). Research progress on the distribution, hazards and treatment technologies of heavy metals in soil. *Geology in China* (01), 118-142.

16. Fang Jin, Zheng Juhao & Qian Jiali. (2024). Briefly describe the research progress of soil heavy metal pollution source analysis and remediation technology. *Leather Making and Environmental Technology* (03), 100-102. doi:10.20025/j.cnki.CN10-1679.2024-03-34.

17. Li Jinge, Cui Dong, Yan Jiangchao, Lv Liqin, Liu Xuan, Wu Yuxin & Jiang Zhicheng. (2023). Research progress on rhizosphere soil microorganisms of Lycium barbarum. *Journal of Yili Normal University (Natural Science Edition)* (03), 38-45.

18. Zhao Lili & Zhang Xinmin. (2022). Research status and frontier hotspots in the field of soil microbiology in China. *Journal of Library and Information Science of Agriculture* (02), 75-87. doi:10.13998/j.cnki.issn1002-1248.21-0054.

19. Wu Pengfei. (2023). Research on rhizosphere Soil Microbial Characteristics and Spatial Distribution Pattern of Pinus sylphus M. (Dissertation, Inner Mongolia Agricultural University). master doi:10.27229/d.cnki.gnmnu.2023.001549.

20. Li Zimeng, Li Xiaoqian, Zhang Wenhui, Ji Yining, Sun Siyu, Wang Sujing... & Zheng Na. (2021). Research progress on the biological effects of heavy metal composite pollution. *Environmental Chemistry* (11), 3331-3343.

21. Xing Yonghui. (2023). *Ecological barrier effect of rhizosphere bacteria on soil heavy metals* Ph.D. (Dissertation, Huazhong Agricultural University). Ph.D https://link.cnki.net/doi/10.27158/d.cnki.ghznu.2023.000190 doi:10.27158/d.cnki.ghznu.2023.000190.

22. Zhang Xueqing, Zhang Qin, CHENG Yuanyuan & Zeng Rong. (2016). Effects of heavy metal pollution in copper ore on soil microbial community diversity and enzyme activity. *Ecology and Environmental Sciences* (03), 517-522. doi:10.16258/j.cnki.1674-5906.2016.03.022.

23. Diao Zhan. (2016). *Effects of exogenous heavy metals on nutrients and microbial activities in different types of soils* M.S. (Dissertation, Northwest A&F University). Master's Degree .

24. Zhang Xiaohua, Wen Changtao, Wang Keqin, Song Yali, YANG Xin & CHEN Bingshen. Effects of fertilization measures on the structure and function of bacterial communities in tobacco planting soil in central Yunnan *Tobacco Tech* 1-15.

25. Wang Yuhan, Chen Lian, Zhang Peizhen, Wang Zhenjiang, Lin Sen, Tang Cuiming... & Wang Yuan. (2024). Response and remediation of root exudates and rhizosphere microorganisms to soil heavy metal pollution (review). *Jiangsu*

*Agricultural Sciences* (05), 19-27. doi:10.15889/j.issn.1002-1302.2024.05.003.

26. Xiang Yanhua, Qin Meiling, Li Yihai & He Xiumiao. (2023). Isolation and biological activity analysis of exopolysaccharide-producing marine animal epiphytic bacteria *Southwest China Journal of Agricultural* Sciences (09), 2051-2058. doi:10.16213/j.cnki.scjas.2023.9.026.

27. Fuwei Wang, Xiaobo Wang, Jincai Li, Aihua Ye, Yan Wang, Wei Che & Lin Zhu. (2015). Effects of fertilization and straw returning on bacterial communities in black soil of Sand ginger *Chinese Journal of Eco-Agriculture* (10), 1302-1311. doi:10.13930/j.cnki.cjea.150267.

28. JI Yaokun. (2023). Analysis of microbial remediation techniques for heavy metal contamination in farmland soil *Industrial Microbiology* (04), 125-127.

29. Qian Tingting. (2021). Structural Characterization and activity of Extracellular polysaccharide from flocculating Active bacteria in Philippine clam adhesion sludge Master's Degree thesis, Zhejiang Ocean University. Master's https://link.cnki.net/doi/10.27747/d.cnki.gzjhy.2021.000061 doi: 10.27747 /, dc nki. Gzjhy. 2021.000061.

30. Abiodun Olusola Omotayo & Oluwadara Pelumi Omotayo. (2024). Potentials of microbe-plant assisted bioremediation in reclaiming heavy metal polluted soil environments for sustainable agriculture. *Environmental and Sustainability Indicators* 100396-.

31. Li Huijie & Liu Yu. (2024). Research on microbial ecological remediation technology of heavy metal pollution in mining area *Environmental Science and Management* (02), 158-161+188.

32. Jia Jianli, Gao Xiaolong, Zhang Ben, Zhao Shenwei, Han Yuxin, Yuan Haokun & Wang Yequan. (2024). Microbial remediation effect and mechanism of heavy metal-PAHs contaminated soil *Clean Coal Technology* (01), 205-216. doi:10.13226/j.issn.1006-6772.KD23112701.

33. HE Xuelian, WEI Chaoqian, LAI Kaidi & DUAN Mingyu. (2023). Research Progress on Heavy Metal Pollution and Remediation Technology in Cultivated Soil

*Resource Conservation and Environmental Protection* (12), 141-148. doi:10.16317/j.cnki.12-1377/x.2023.12.023.

34. Ma Ying, Jiang An, Shi Xiaojun, Li Zhenlun & Chen Xinping. (2024). Synthesis of microbial exopolysaccharides and their mechanism and application in heavy metal remediation *Acta Microbiologica Sinica* (03), 701-719. doi:10.13343/j.cnki.wsxb.20230612.

35. Zeng Huiwen, Wang Yashi, Li Qianru & Lan Wenbo. (2023). Study on the status of heavy metal pollution in soil remediated by microorganisms *Guangdong Chemical Industry* Co., Ltd. (10), 156-157+203.

36. Junjie Liu, Yueyu Sui, Zhenhua Yu, Yu Shi, Haiyan Chu, Jian Jin... & Guanghua Wang. (2014). High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China. *Soil Biology and Biochemistry* 113-122.

37. Yu-shu Tang, Lei Wang, Jian-wei Jia, Xiao-hua Fu, Yi-quan Le, Xiu-zhi Chen & Ying Sun. (2011). Response of soil microbial community in Jiuduansha wetland to different successional stages and its implications for soil microbial respiration and carbon turnover. *Soil Biology and Biochemistry* (3), 638-646.

38. Yanmei Chen, Qiaobei Ding, Yuanqing Chao, Xiange Wei, Shizhong Wang & Rongliang Qiu. (2018). Structural development and assembly patterns of the root-associated microbiomes during phytoremediation. *Science of the Total Environment* 1591-1601.

39. Tang Lingling. (2022). Determination of IAA Content in Metabolites of Three Growth-Promoting Microorganisms and Its Effect on Tobacco Growth Promoting Master (Dissertation, Henan University of Science and Technology). Master's https://link.cnki.net/doi/10.27115/d.cnki.glygc.2022.000365 doi:10.27115/d.cnki.glygc.2022.000365.

40. Sandrin T R, Chech A M & Maier R M. (2000). A rhamnolipid biosurfactant reduces cadmium toxicity during naphthalene biodegradation.. *Applied and environmental microbiology* (10), 4585-8.

41. Hu Fang, Wang Fang, Han Xiaozeng, Xu Min, Fu Yuhao, Yan Jun... & Jiang Xin. (2022). Succession of soil microbial communities in typical black soil areas under different land use patterns *Journal of Soil Science* (05), 1238-1247.

42. Luo Guosheng, Shi Zunji & Wang Gejiao. (2012). Lysobacter arseniciresistens sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil.. *International journal of systematic and evolutionary microbiology* (Pt 7), 1659-1665.

43. Lu Zi Gang, Zhu Lixin, Ji Hongbing & Wang Kang Research progress on the remediation of soil heavy metal pollution by sphingomonas. *Journal of Zhejiang Agricultural Sciences* 1-10.

44. Zhen Jing, Li Guanjie, Du Zhimin, Wang Jiwen, Yang Wenling, Gong Tao & Zhao Junjie. (2020). Degradation and community structure analysis of polycyclic aromatic hydrocarbons in contaminated soil by the fermentation broth of Strain Trametes hirsuta zlh237 *Journal of Southern Agricultural Sciences* (01), 72-79.

45. Pan Yanshuo, Zhu Qinghe, Li Cong, Wang Youjing, Wu Kun, Li Xuanzhen... & Wu Yucheng. (2018). Effects of cellulose, straw and wood chips on soil nitrification and microorganisms in farmland *Journal of Henan Agricultural University* (05), 785-792. doi:10.16445/j.cnki.1000-2340.2018.05.020.