Potential role of mycorrhizae combined with Pinus tabuliformis in repairing soil contaminated by lanthanum and cerium

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Case study

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Abstract

In order to repair light rare earth soil effectively, plant and mycorrhizae technology were applied together. It will provide theoretical basis for ecological restoration of light rare earth contaminated soil. The method of greenhouse pot experiment was used in this study. The concentrations of lanthanum (La) in tested soil samples were 107.15, 329.41, and 2,031.71 mg/kg and cerium (Ce) were 362.11, 741.81, 4,162.03 mg/kg. The ectomycorrhiza (Boletus luridus Schaeff. (BLS), Inocybe lilacina (Boud.) Kauffm. (ILK), Russula foetens (Pers.) Pers. (RFP), Lactarius sanguifluus (Paul.) Fr. (LSF)) was inoculated on Pinus tabuliformis. The inoculation rate and biomass of mycorrhiza, as well as the absorption, transfer and root retention of La and Ce, light rare earth elements (LREE) by plants were determined to provide the theoretical basis for the treatment of La and Ce contaminated soil. The results showed that a symbiotic relationship was established between ectomycorrhiza and Pinus tabuliformis. The mycorrhizal infection rate of Pinus tabuliformis ranged from 0.51–64.81%. The biomass results showed that the dry weight of aboveground organs and roots increased by 1.46, 1.67 and 1.88 times, 1.73, 1.98 and 2.08 times, respectively. With the increase of the concentration of La and Ce in the soil, the increase of one LREE in the host plants inoculated with mycorrhizae was greater than that in the blank control group. Following mycorrhizal inoculation, La and Ce transfer coefficients in P and root retention coefficients increased, which may lead to the decrease of absorption and transfer capacity of hyperaccumulators. This study showed that mycorrhizae can improve the absorption of La and Ce by host plants, demonstrating potential value in the environmental remediation of LREE contaminated soil.

1. Introduction

With the increasing global demand for rare earth products, China is increasing the mining volume of rare earth minerals, resulting in the enrichment of exogenous rare earth in the soil and posing a serious threat to the ecological environment and human health [1]. To date, China's rare earth reserves accounted for about 23% of the world's total reserves. Bayan Obo light rare earth mine in Baotou City, Inner Mongolia, China accounts for about 85% of China's rare earth reserves [2]. Baotou city is known as the world's rare earth capital and has an open-pit mining history of more than 60 years. The content of light rare earth elements (LREE) in the soil of this area is approximately 400 times higher than the background value of Inner Mongolia. Light rare earth elements migrate throughout the soil environment, leading to many environmental issues in the soil, including farmland yield loss, soil desertification, and so on. Soil pollution by rare earth elements has proven to be of great concern [3]. Studies have shown that concentrations of exogenous rare earth in soil higher than 400 mg/kg, the biomass of Oryza sativa L. significantly decreases and the plants die when the concentration is 1,500 mg/kg [4]. Therefore, the environmental damage caused by light rare earth pollution and the mechanism of phytoremediation is a problem requiring an urgent solution.

Mycorrhizae are symbiotic with some fungi and host plant roots in natural soil [5]. Mycorrhizae can expand the absorption area of plant roots and increase the absorption of nitrogen, phosphorus, potassium, and other minerals by host plants [6]. Mycorrhizae coexist with host plant roots and expand throughout the soil, absorbing nutrients from host plants on one hand and helping plant roots to absorb water and nutrients from the soil on the other hand [7]. Based on morphological and anatomical characteristics, mycorrhizae are classified into ectomycorrhizae and endomycorrhizae. Approximately 3% of plants contain ectomycorrhizae, the majority of which are trees. Ectomycorrhizae form a mantle around the root system of the host, protecting the new root and expanding mycelia to the surrounding soil to replace the root hairs of the host plant to absorb water and nutrients. Mycorrhizal fungi play an important role in the remediation of soil polluted ecological environment through the combination of mycorrhizal fungi and host plants. In nature, mycorrhizal fungi have certain selectivity to host plants. This requires that mycorrhizal remediation should follow the principle of "suitable for plants, suitable for fungi" [8].

Mycorrhizae can improve the ability of host plants to absorb water and nutrients from the soil and enhance their resistance to stress [9]. Greenhouse pot experiment was used in this study. The soil was collected from the surface soil in Bayan Obo Mining District. In this study, we investigated the effect of ectomycorrhizoa (BLS), (ILK), (RFP), (LSF) the mycorrhizal infection rate, biomass (ground organs dry weight and root dry weight) and the absorption, transfer, and retention coefficients of La and Ce LREE of Pinus tabuliformis as well as that intraradices, to provide a theoretical basis and technical support for the environmental remediation and treatment of LREE contaminated soil.

2 Materials And Methods

2.1 Soil collection and treatment
More than 90% of the LREE in the Bayan Obo Mining District exist in the form of monazite [3]. The LREE in the soil mainly exist in the form of residue and few in the form of weak acid extracts [10]. Their soil content is high but biological activity is low, making dissociation a challenge. In addition, when exogenous LREE enter the soil, the majority remain in the surface layer, and their concentration in the soil, therefore, presents the characteristics of gradient decline [11]. Therefore, surface soil from 0 to 40 cm was collected in this study from the Bayan Obo Mining District, Baotou City, Inner Mongolia (N 41°49′28″, E 109°58′42″) in July 2019. Five samples of 1.6 kg of soil were collected at each sampling point with a ring knife. Following uniform mixing, each sample was divided into 4 equal parts (each of the parts was 2 kg). One part was randomly selected and reserved in a self-sealing bag [12]. The collected soil was screened with a 2-mm soil sieve and sterilized for 2 hours in an autoclave at 121°C [13]. The basic physicochemical properties of soil are shown in Table 1.

| Substance | pH | Organic matter | Nitrogen | Available nitrogen | Phosphorus | Available phosphorus | Potassium | Available potassium | Bulk density | Water content |
|-----------|----|----------------|----------|--------------------|------------|---------------------|-----------|--------------------|--------------|---------------|
| Soil      | 8.52 | 1.69 | 0.22 | 69.84 | 0.33 | 13.19 | 13.44 | 42.85 | 1.78 | 5.76 |

2.2 Plants and mycorrhizae

Seeds of *P. tabuliformis* and *L. bicolor* used in this experiment were obtained from the seed management station of Baotou Municipal Bureau of Agriculture and Pastoral Administration, Inner Mongolia. Before sowing, seeds were surface sterilized and then placed in a constant temperature incubator (20°C) for germination treatment. Seeds were sown when they were white. *P. tabuliformis* was inoculated with ectomycorrhiza IL collected in the field [14]. The inoculation agents included rhizosphere sand-soil mixture, spores, hyphae, and infected plant root segments. The inoculants were obtained by propagation in the experiment. The inoculants included sand soil mixture, spores, hyphae and infected root segments.

2.3 Experimental design and treatment

The greenhouse pot method was used in this study to set up three types of soil contaminated with lanthanum (La) or cerium (Ce) LREE with varying concentrations. *P. tabuliformis* and *L. bicolor* were either inoculated with ectomycorrhizae, endomycorrhizae or nothing (blank control group). Three replicates were done for each treatment, totaling 54 pots that were randomly arranged [15]. Two kg of sterilized soil and 50 g of inoculation agent were added to each experimental pot, while 2 kg of sterilized soil and 50 g of fungicide were added to each pot in the control group. Each pot was seeded with 5 healthy and plump germinated *P. tabuliformis* or *L. bicolor*. Thinning was carried out one week after emergence, where three plants with similar growth potential were left in each pot [16]. The experiment was carried out in the experimental greenhouse of landscape plant restoration in the dry and cold mine of Inner Mongolia University of Science and Technology, with natural lighting, temperature ranging from 21 to 35°C, and relative humidity ranging from 32 to 60%. The plants were watered regularly, and the data were collected 90 days after seedling emergence.

2.4 Sample collection

The above-ground organs and roots of the harvested plants were cut off, cleaned with tap water, rinsed with distilled water three times, dried at 70°C and weighed. The mycorrhizal infection rate was determined by collecting 1 g of fresh roots from randomly selected experimental plants and preserved in 50% ethanol. The root frequency standard method was used to calculate the mycorrhizal infection rate [17]. The prepared test samples were stained with 0.05% trypan blue lactate glycerol solution (lactic acid:glycerol, 1:1) and sectioned[18].

The collected soil samples were naturally air-dried, then dried at 105°C for 4 hours, and sieved through a 100-mesh sieve to remove impurities. Then, 0.20 g of both the reserved soil samples and the standard soil samples were weighed and placed in a tetrafluoroethylene digestion tank for digestion [19]. The content of La and Ce in soil samples was determined by Inductively Coupled Plasma Mass Spectrometer (ICP-MS, NexION 300Q, PerkinElmer) [20].

After the above-ground organs and roots of the plants were cleaned, they were dried for 4 hours at 105°C, ground, and screened for use through a 100-mesh sieve. Then, 0.25g of both types of soil were weighed and digested with Multiwave 3000 microwave digestion apparatus. The contents of La and Ce LREE were determined by ICP-MS [21].

2.5 Data processing
The infection rate was calculated using the following formula [18]: Mycorrhizal infection rate = (number of infected root segments / total number of observed root segments) x100%

The formula of the biological transfer coefficient (BTC) [22] was as follows:

Where $T_u$ is the content of above-ground organs of plants; $T_r$ is the root content of plants. BTC reflects the ability of plants to transfer La and Ce LREE from roots to above-ground organs. When BTC is less than 1, the transfer ability is weak. When BTC is greater than 1, the transfer ability is strong and when active absorption occurs [23–25].

The root retention coefficient (RC) [26] was calculated as follows:

Excel 2017 and SPSS 19.0 were used for data calculation and variance analysis to test the significance of the difference between samples or treatments. The difference was considered to be significant or very significant if the P-value was less than 0.05 or less than 0.01, respectively.

3 Results

3.1 Mycorrhizal infection rate and plant biomass

At various concentrations of La and Ce polluted soil, the infection rate and biomass of $P. tabuliformis$ were measured by inoculating ectomycorrhiza. There was no mycorrhizal infection in the control group. The measured data are shown in Table 2.

According to La and Ce concentrations in the soil (Table 2), the tested soil was divided into three concentration zones: high concentration (2,031.74, 4,162.03 mg/kg), medium concentration (329.41, 741.81 mg/kg), and low concentration (107.15, 362.11 mg/kg). Plants successfully established a symbiotic relationship with the tested mycorrhiza. The infection rate of ectomycorrhiza in $P. tabuliformis$ was lower than that of endomycorrhizae in $L. bicolor$. There was no mycorrhizal infection in the control group. The mycorrhizal infection rate of $P. tabuliformis$ ranged from 1.18 to 34.16%, while the rate in $L. bicolor$ ranged from 0.51 to 64.81% (among which BLS was the highest). The mycorrhizal infection rate of the plants decreased with the increase of LREE concentration. The biomass of above-ground organs and roots of $P. tabuliformis$ and $L. bicolor$ decreased significantly with the increase of LREE concentration. The biomass of the plants both increased after inoculation. The dry weight of above-ground organs of $P. tabuliformis$ in different soil samples increased 1.59, 1.19 and 1.03 times, respectively. The dry weight of roots increased 1.86, 1.30 and 1.30 times, respectively. The biomass of $L. bicolor$ inoculated with BLS mycorrhiza increased the most, with the dry weight of above-ground organs increasing 1.46, 1.67 and 1.88 times, respectively, and the dry weight of roots increasing 1.73, 1.98 and 2.08 times, respectively.
Table 2
Effects of inoculation with Mycorrhizal Colonization and Biomass of Pinus

| Plants | Mycorrhizal name | Inoculation( mg/kg) | Mycorrhizal infection rate(%) | Shoot dry weight(g) | Root dry weight(g) |
|--------|-----------------|---------------------|-----------------------------|---------------------|-------------------|
|        |                 | La                  | Ce                          |                     |                   |
| Pinus  | BLS             | 2031.71             | 4162.03                     | 4.36 ± 0.39         | 3.98 ± 0.49b      | 2.26 ± 0.16b      |
|        |                 | 329.41              | 741.81                      | 35.75 ± 2.79        | 8.12 ± 0.22b      | 4.87 ± 0.65a      |
|        |                 | 107.15              | 362.11                      | 64.81 ± 6.33        | 12.57 ± 0.46a     | 6.93 ± 0.77b      |
| ILK    |                 | 2031.71             | 4162.03                     | 2.57 ± 0.02         | 3.59 ± 0.58b      | 1.37 ± 0.64b      |
|        |                 | 329.41              | 741.81                      | 28.83 ± 1.83        | 5.56 ± 0.89b      | 3.34 ± 0.25a      |
|        |                 | 107.15              | 362.11                      | 42.23 ± 3.93        | 9.11 ± 0.55a      | 3.42 ± 0.37a      |
| RFP    |                 | 2031.71             | 4162.03                     | 0.51 ± 0.01         | 3.12 ± 0.12a      | 1.32 ± 0.41b      |
|        |                 | 329.41              | 741.81                      | 25.24 ± 1.09        | 5.51 ± 0.35b      | 3.27 ± 0.28a      |
|        |                 | 107.15              | 362.11                      | 39.03 ± 3.37        | 7.88 ± 0.97a      | 3.86 ± 0.32b      |
| LSF    |                 | 2031.71             | 4162.03                     | 1.18 ± 0.02         | 1.16 ± 0.27b      | 0.26 ± 0.01b      |
|        |                 | 329.41              | 741.81                      | 13.53 ± 1.63        | 2.43 ± 0.61a      | 0.73 ± 0.03a      |
|        |                 | 107.15              | 362.11                      | 34.16 ± 3.18        | 3.20 ± 0.24b      | 1.22 ± 0.05a      |
| C K    |                 | 2031.71             | 4162.03                     | 0.00 ± 0.00         | 0.73 ± 0.33a      | 0.14 ± 0.01a      |
|        |                 | 329.41              | 741.81                      | 0.00 ± 0.00         | 2.04 ± 0.17b      | 0.56 ± 0.01a      |
|        |                 | 107.15              | 362.11                      | 0.00 ± 0.00         | 3.11 ± 0.74a      | 0.94 ± 0.02a      |

Note: the data in the table are the average value ± standard error of three repetitions. Different letters in the same column indicate significant difference, a indicates significant difference (P < 0.05), B indicates significant difference (P < 0.01)

3.2 La and Ce content in Pinus tabuliformis

The tested soil was divided into a high concentration zone, medium concentration zone, and low concentration zone (Table 3). With the increase in the content of LREE (La and Ce) in the soil, the content of LREE in P. tabuliformis also increased, while the content in above-ground organs was lower than that in the root system. After IL mycorrhizal inoculation, the La element transfer coefficient of P. tabuliformis decreased by 0.00, -0.17, and − 0.15 and the root retention coefficient increased by 0.00, 0.17, and 0.10. The Ce element transfer coefficient decreased by -0.02, -0.07, and − 0.15 and the root retention coefficient increased by 0.02, 0.07, and 0.15.
The contaminated soil was divided into high concentration area, medium concentration substrate and low concentration substrate, and then the tolerant earthworm was added to the test. Pinus tabulaeformis was inoculated with ectomycorrhizal BLS, ILK, RFP and LSF respectively, and the blank control group was set up. The results showed that the contents of pollutants absorbed by Pinus tabulaeformis were as follows: high concentration zone > medium concentration zone > low concentration zone; the contents of pollutants in organs of Pinus tabulaeformis were root > stem > leaf, and the contents of pollutants absorbed by Pinus tabulaeformis inoculated with mycorrhiza were BLS > ILK > RFP > LSF.

### 3.3 La and Ce content in Lespedeza bicolor

With the increase of La and Ce content in the soil, the content of LREE in *L. bicolor* also increased, while the content in above-ground organs was greater than in the roots (Table 5). The content of LREE increased following inoculation with RI, FM and GV. LREE content was highest in *L. bicolor* inoculated with RI. The La element transfer coefficient of *L. bicolor* inoculated with RI decreased by -0.02, -0.11, and -0.04 and the root retention coefficient increased by 0.02, 0.12, and 0.04. The Ce element transfer coefficient increased by 0.06, 0.07, and 0.04, while the root retention coefficient decreased by 0.06, 0.11, and -0.04.

![Table 3](image)

Table 3

| Element | Plant organs | Pinus |  |  |  |  |
|---------|--------------|-------|---|---|---|---|
|         |              | BLS   | ILK | RFP | LSF | CK |
| La      | Shoot (mg/kg)| 57.34±5.33 | 53.53±5.22 | 51.13±4.87 | 50.95±4.13 | 20.63±2.13 |
|         | Root (mg/kg) | 62.30±6.24 | 57.90±4.91 | 55.34±3.35 | 6.90±1.24 | 2.94±0.50 |
| Ce      | Shoot (mg/kg)| 59.47±5.34 | 53.55±2.45 | 53.97±5.24 | 9.42±1.24 | 9.42±1.24 |
|         | Root (mg/kg) | 106.77±11.87 | 91.14±7.19 | 84.26±6.82 | 17.47±1.51 | 8.06±1.18 |
The tested soil was divided into three concentration zones (Fig. 1). The plant species were inoculated with mycorrhizae while the control group was not. The results showed that LREE content absorbed by the plants was as follows: high concentration zone > medium concentration zone > low concentration zone. The LREE content in plants was as follows: mycorrhizae-inoculated plants > control group. LREE content in *L. bicolor* inoculated with four mycorrhizae was as follows: BLS > ILK > RFP > LSF.

### 4. Discussion

Mycorrhizal infection rate is an index to measure the affinity between mycorrhizae and host plants [27]. The infection rate is affected by various factors, such as soil nutrients, types and concentrations of pollutants, and the combination of strains and host plants [28]. In this study, we found that in the light rare earth elements La and Ce polluted soil, the plants developed symbiotic relationships with mycorrhizae, indicating that the mycorrhiza could survive and reproduce under the certain concentration of LREE contamination in the soil [26]. The infection rate of ectomycorrhiza in *P. tabuliformis* ranged from BLS 4.36–64.81%, ILK 2.57–42.23%, RFP 0.51–39.03%, and LSF 1.18–34.16%. The mycorrhizal infection rates in this study were slightly lower in comparison to previously published studies. Studies have shown that the infection rate of endomycorrhiza in *Zea mays* in Ce contaminated soil ranges from 5.29 to 78.79% [13]. Similarly, the infection rate of mycorrhiza in Glycine max L. in Ce contaminated soil is 67.46% [29]. The primary reason for this variation lies in the high concentration of LREE in the soil tested in this study. In addition, this study found that both the biomass of above-ground organs and the roots of the plants decreased significantly with the increase of LREE concentration. When the concentration of element La increased by about 19 times and the concentration of element Ce increased by about 11 times, the dry weight of above-ground organs in *P. tabuliformis* increased by approximately 36.25%, the dry weight of roots increased by approximately 21.31%, the dry weight of above-ground organs in *L. bicolor* increased by 31.66–47.21% and the dry weight of roots increased by 32.61–40.06%. Our study showed a lower biomass increase compared to previously published data. The dry weight of above-ground organs and roots of *Lolium perenne* and *Cynodon dactylon* inoculated with mycorrhiza *Glomus aggregatum* and *Glomus constrictum* increased by 203% vs. 482% and 119% vs. 291%, respectively [29]. Inoculation of mycorrhiza *Glomus versiforme* increased the dry weight of organs and roots of *Elymus dahericus* by 83% and 103% [30]. The fresh weight of *Brassica campestris* decreased by about 76% when the Ce element concentration increased from 5 mg/L to 100 mg/L [31]. Increased Ce content in soil resulted in decreased biomass of host plant *Raphanus sativus* L. [32]. In this study and other studies, the "Hormesis" effect of mycorrhizal infection rate increasing first and decreasing later appeared with the increase of

| Name | La   | BCT | RC  | Ce   | BCT | RC  |
|------|------|-----|-----|------|-----|-----|
| BLS  | 2031.71 | 1.17 | -0.17 | 4162.03 | 1.15 | -0.15 |
|      | 329.41 | 1.10 | -0.10 | 741.81 | 1.19 | -0.19 |
|      | 107.15 | 1.05 | -0.05 | 362.11 | 1.02 | -0.02 |
| ILK  | 2031.71 | 1.17 | -0.17 | 4162.03 | 1.08 | -0.08 |
|      | 329.41 | 1.18 | -0.18 | 741.81 | 1.23 | -0.23 |
|      | 107.15 | 1.08 | -0.08 | 362.11 | 1.09 | -0.09 |
| RFP  | 2031.71 | 1.19 | -0.19 | 4162.03 | 1.11 | -0.11 |
|      | 329.41 | 1.21 | -0.21 | 741.81 | 1.08 | -0.08 |
|      | 107.15 | 1.03 | -0.03 | 362.11 | 1.04 | -0.04 |
| LSF  | 329.41 | 0.73 | 0.27 | 741.81 | 0.74 | 0.26 |
|      | 107.15 | 0.73 | 0.27 | 362.11 | 0.72 | 0.28 |
| CK   | 2031.71 | 1.19 | -0.19 | 4162.03 | 1.09 | -0.09 |
|      | 329.41 | 1.22 | -0.22 | 741.81 | 1.08 | -0.08 |
|      | 107.15 | 1.09 | -0.09 | 362.11 | 0.98 | 0.02 |

Note: the data in the table is the average value ± standard error of three repetitions.
LREE concentration [33]. Therefore, it is feasible to repair the symbiosis between mycorrhiza and host plants in the soil contaminated with a certain concentration of LREE.

The results of this study showed that after the inoculation of mycorrhiza in P. tabuliformis and L. bicolor, the content of La and Ce LREE in the plants increased with the content of LREE in the soil. Other studies have found that the La element significantly increased in host plants after mycorrhiza inoculation in soils contaminated with La element [34]. When the soil contains a low concentration of La element, mycorrhiza inoculation can increase the content of La in Astragalus sinicus [35]. The above results show that mycorrhizal inoculation can increase the host plant's LREE absorption at certain LREE concentrations. The absorption mechanism may be that mycorrhizae increase the channels through which host plants absorb nutrients such as phosphorus, and at the same time increase the absorption of light rare earth elements. Therefore, the mechanism and interaction of mycorrhizal absorption of LREE should be further investigated.

In this study, we found that P. tabuliformis reduced the transfer coefficients and increased the root retention coefficients following mycorrhizal inoculation. Some studies have found that in soils contaminated with elements La and Pb (lead) at different concentrations, mycorrhizal inoculation significantly reduced the Pb content in above-ground organs of maize and increased the Pb content in roots, effectively inhibiting the transfer of Pb pollutants from roots to above-ground organs [36]. Other studies have shown that endomycorrhizal inoculation in host plants can catalyze the synthesis of chelated synthase (PCs), chelate heavy metal ions in the cytoplasm, transfer heavy metal ions to plant vacuoles, and effectively improve host plant tolerance [37]. Under the chelation of mycorrhizal hyphae and glomamycin, heavy metal ions are fixed to produce filtration effect, reducing the heavy metal ions entering the host plants, thus reducing the transfer ability [38]. Mycorrhizae influence Pb enrichment in plants, and a large amount of Pb is fixed in the root by mycelia and vesicles, which reduce the transfer rate from root to above-ground organs [39], consistent with the results obtained in this study. Therefore, mycorrhizae have a negative effect on the host plant's transfer of LREE in La and Ce polluted soil, leading to a decrease in the transfer ability of hyperaccumulators. This may be related to the interaction between mycorrhizae, host plant, LREE, and heavy metal ions. It is therefore necessary to study the mechanism of the absorption, transfer, and retention of mycorrhizae-inoculated hosts in LREE contaminated soil.

5. Conclusions

(1) In the soils polluted by three different concentrations of La and Ce LREE, symbiotic relationship between ectomycorrhizae and P. tabuliformis, endomycorrhizae and L. bicolor was successfully established. The mycorrhizal infection rate and plant biomass increased first and then decreased with the increase of LREE concentration.

(2) The decrease of transfer coefficient and the increase of root retention coefficient indicated that P. tabuliformis belonged to passive absorption, and there was no significant change in transfer efficiency, as it could only be used as an auxiliary plant in light rare earth ecological restoration.

(3) The results show that mycorrhizae and host plants have potential application value for the environmental remediation of La and Ce contaminated soil. Further research should focus on the infection rate of the most appropriate concentration of LREE, mycorrhizae, and host plant selection.

Declarations

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**Figures**

![Figure 1](image_url)

**Figure 1**

Content in Plant Inoculated with Mycorrhiza
Figure 2

Content of La and Ce Light Rare Earth in Plant Inoculated with Mycorrhiza