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Research Article

In situ Remediation of Saline Soils Using a Consortium of Halophilic Microscopic Fungi

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ABSTRACT

Based on series of experiments conducted in the microbiology laboratory of the faculty of Agricultural Sciences and Biosystems Engineering at the Georgian Technical University, a unique consortium of halophilic microscopic fungi has been created. Using this consortium, compost was prepared under laboratory conditions and remediation of salt-degraded soils (ex situ) was implemented. The present research represents a logical continuation of the laboratory experiment and its gradual translation to field scale. Before testing the created consortium in field conditions, optimization of biotechnological parameters of the composting process was necessary under laboratory conditions. As a result of this experimental stage, optimal concentrations of inoculum and compost were determined (1.25% and 20%, respectively). In the next stage of the experiment, the laboratory-established protocol was adapted for field conditions; additionally, the effectiveness of both individual strains and the multistructural consortium was evaluated. In situ bioremediation of saline soils revealed the existence of synergistic interactions between the components of the consortium; elimination of salts from the soil by the consortium significantly exceeded the effectiveness of individual strains. In the final stage of the research, a comprehensive assessment of the effectiveness of bioremediation technology was conducted according to phenometric parameters of wheat (Triticum aestivum L.) seedlings. The obtained results provide a foundation for further development and practical implementation of innovative technology for bioremediation of saline soils.

Keywords: Bioremediation, composting, halophilic consortium, remediation potential, synergistic effect.

INTRODUCTION

Soil salinization is one of the most acute ecological challenges that poses a serious threat to global food security and the sustainable functioning of agricultural systems [1]. According to the United Nations Food and Agriculture Organization (FAO), the total area of saline soils worldwide exceeds 1 billion hectares and is increasing by an additional 1.5-2 million hectares annually [2, 3].

Excessive concentration of salts in soil causes osmotic, ionic, and oxidative stress, which significantly inhibits plant growth and development, limits nutrient assimilation, and reduces the activity of microbial cycles [4-6]. Traditional methods for the restoration of saline soils often require significant financial and energy resources, which limits the possibilities for their large-scale application [7, 8].

Under these conditions, interest in environmentally safe and low-budget bioremediation technologies is increasing [9, 10]. The use of halophilic microorganisms - bacteria and microscopic fungi - which are naturally adapted to saline environments and possess various mechanisms for biotransformation of salts, is considered particularly promising [11, 12]. Numerous studies confirm that the use of multicomponent consortia of microorganisms in bioremediation technologies is more effective than individual strains [13-15].

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The successful implementation of bioremediation significantly depends on the correct selection of a number of technological parameters, such as: consortium composition, optimal inoculum concentration, proportion of compost material, and application technique [16-18]. Additionally, it is critically important to evaluate the results obtained in field conditions from an agronomic perspective [19, 20].

The presented research aims to perform *in situ* remediation of saline soils using a multicomponent consortium of halophilic microscopic fungi and to analyze the agronomic potential of the restored soil.

MATERIALS AND METHODS

1. Statistical processing of data

The results of phenometric observations were processed statistically using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test to check for differences between means. All calculations were performed using the statistical program Sigma Plot 12.5.

2. Microbial consortium

The experiment uses a consortium of halophilic microscopic fungi, which was created within the framework of previous research [21]. The consortium consists of halophilic microscopic fungi selected through stepwise screenings (*Penicillium* sp. 1-1-2, *Mucor* sp. 2-1-1, *Aspergillus niger* 3-1-3, *Aspergillus flavus* 5-3-3, *Aspergillus* sp. 11-1-1, and *Trichoderma viridae* 12-1-1), which showed resistance to high salt concentrations and possessed high ability to degrade compost substrate.

3. Preparation of inoculum for laboratory tests

3.1 *Cultivation of individual strains*

Cultivation of each microscopic fungal strain was carried out by the stationary method in 1000 ml Erlenmeyer flasks containing 300 ml of liquid nutrient medium with the following composition (g/l): $NaNO_3$ - 3.0; KH_2PO_4 - 1.0; $MgSO_4 \times 7H_2O$ - 0.5; KCl - 0.5; $FeSO_4 \times H_2O$ - 0.02; glucose - 15.0; malt sprouts - 1.0; pH 5.5-5.8. Cultivation was conducted for 10 days at 30°C in a controlled thermostat (TS 608-G/2i).

3.2 *Biomass collection and homogenization*

After incubation, the culture liquid was filtered through a sterile filter. The mycelial biomass (50-60 g) obtained from filtration was transferred to a sterile vessel and homogenized in 500 ml of sterile water using a homogenizer. This represented the initial suspension of the individual strain.

3.3 *Preparation of consortium inoculum*

To prepare the consortium inoculum, biomass from all six cultures was taken in equal amounts (10 g each) and homogenized in 500 ml of sterile water. The resulting initial suspension represented a standardized sample of the consortium inoculum.

3.4 *Determination of the optimal amount of compost*

To determine the optimal amount of compost, soil samples (150 g) were collected from the research location and placed in individual 0.5 L containers. Within the experiment, various concentrations of compost material inoculated with the microbial consortium were added to the soil samples: 5%, 10%, 15%, and 20% (w/w) (for detailed methodology of compost preparation, see section 4.2 below). The same soil without compost addition was used as a control variant. The effectiveness of different compost amounts was evaluated by quantitative analysis of initial and final values of soluble salt concentration in the soil.

3.5 *Determination of the optimal amount of inoculum*

To determine the optimal amount of inoculum, different quantities of consortium biomass - 50, 25, 12.5, 6.2 g (corresponding to 10%, 5%, 2.5%, 1.25%) were prepared according to the described methodology (section 3.3). Using these quantities, composts were prepared and applied to remediation soils at 20% (for compost preparation

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methodology, see section 4.2). The effectiveness of different inoculum concentrations was evaluated by quantitative analysis of initial and final values of soluble salt concentration in the soil.

4. Preparation of inoculum for field experiments

The protocol described above was scaled proportionally for field experiments. During scaling, the identical ratio of biomass to water was maintained, which ensured obtaining a suspension of standardized concentration.

4.1 *Remediation location, preparation of experimental plots*

For the field experiment, a location with moderate salinity was selected in Lagodekhi municipality. Seven identical plots were allocated for the experiment, each with an area of 4 m^2. All experimental sites were cleared of weeds and tilled to a depth of 0-20 cm to ensure optimal conditions for microbial colonization.

4.2 *Biocompost preparation for laboratory and field experiments*

For laboratory and field experiments, biocompost was prepared using an identical method with proportional scaling. Dry wheat bran was used for composting (500 g in laboratory conditions, 100 kg in field conditions), which was moistened with water at a 1:1 weight ratio. The hydrated substrate was supplemented with the appropriate amount of the inoculum of microbial consortium (1,25g inoculum per 100g of substrate). Homogenization of materials was carried out by mechanical mixing. The resulting substrate was covered with polyethylene layer for 1-2 hours to ensure even distribution of moisture and adaptation of microorganisms. In laboratory conditions, compost fermentation proceeded for 5-7 days, while in field conditions - for 21 days, with regular aeration and moisture control.

4.3 *Determination of easily soluble salt content in soil*

The content of soluble salts in the soil was determined by the method recommended by Gartley (2011). A soil sample (20 g) sieved through a 1 mm diameter mesh was suspended in 40 ml of distilled water (1:2 ratio). After allowing the suspension to settle (3 minutes), the electrical conductivity (EC) of the solution was measured using an electrical conductometer (HI8733), expressed in decisiemens per meter (dS/m) [22]

RESULTS AND DISCUSSION

As a result of sequential research conducted in the microbiology laboratory of the faculty of Agricultural Sciences and Biosystems Engineering at the Georgian Technical University, a unique consortium of halophilic microscopic fungi was produced. Using this consortium, compost was prepared under laboratory conditions and remediation of salt-degraded soils (*ex situ*) was successfully implemented. The obtained results are documented in a scientific article [21].

Before applying the laboratory-developed technology in field conditions, it was deemed advisable to optimize two important parameters: determining the optimal amounts of compost to be added to the soil and the optimal amount of consortium inoculum. This research was focused on the economy and profitability of the technology; the goal was to achieve maximum efficiency of bioremediation with minimal amounts of compost and inoculum.

Determination of the compost optimal amount

In the first stage of the research, under laboratory conditions different amounts of compost were applied to samples, taken from moderately saline soils (3.7 dS/m) in the Lagodekhi municipality: 5%, 10%, 15%, and 20%. The compost was prepared using the inoculum of the halophilic microscopic fungi consortium, the concentration of which was 2% in each variant. The content of soluble salts in the soils was measured before and after remediation (on the 21st day of composting).

The obtained results demonstrate that with the increase in the amount of compost, the content of soluble salts in the soil significantly decreased. Bioremediation was most effective in the soil where 20% compost was applied. Based on this, the use of 20% compost was considered appropriate for further research (Fig. 1).

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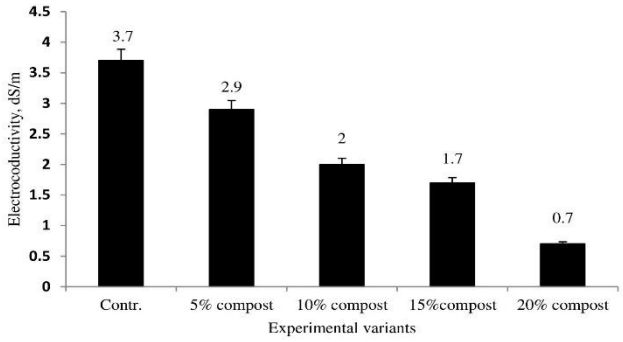

Fig. 1 Influence of the compost amount on the efficiency of bioremediation

Determination of the optimal concentration of inoculum

In the next stage of the research, the effect of different concentrations of consortium inoculum on the efficiency of bioremediation was studied. For this purpose, compost was prepared using different concentrations of consortium inoculum (0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75%, 2%, and 2.25%); after which the compost was applied to remediation soils at 20%.

The results clearly show that under low inoculum concentrations (0.5% and 0.75%), remediation practically did not occur (0% and 5.4%, respectively). A significant reduction in soluble salts in the soil begins when using 1% inoculum (50% reduction in salinity). However, the maximum effect is observed when using inoculum concentrations of 1.25%-2% (reduction in salinity by 79.7% - 81.1%); while with 2.25% inoculum, the effect slightly decreased (80.5%), indicating that further increase in inoculum amount does not increase the efficiency of bioremediation and is economically unjustified. Based on these results, the use of 1.25% consortium inoculum was considered appropriate for further research (Fig. 2).

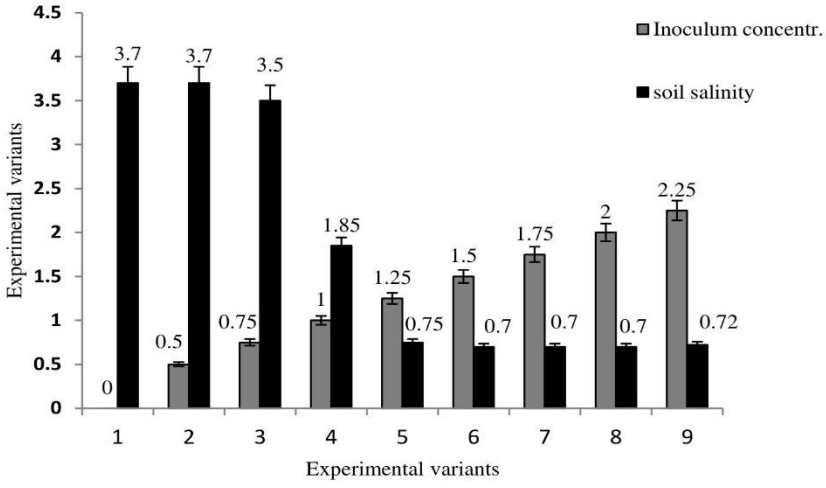

Fig. 2 Influence of the inoculum concentration on the efficiency of bioremediation

Evaluation of individual strain effectiveness

After determining the optimal composting parameters, the remediation technology was tested in open soil. Remediation was carried out on moderately saline soils in the Lagodekhi municipality (3.7 dS/m), with a composting duration of 1.5 months. During the testing of the bioremediation technology in field conditions, the role of individual strains of the consortium in the process of saline soil remediation was studied.

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Research Article

Analysis of the obtained results shows that each member of the consortium individually possesses the ability to remediate saline soils, although their effectiveness differs significantly. The strain *Trichoderma viridae* 12-1-1 demonstrated the lowest remediation potential, reducing soil salt content by only 18.9%. *Penicillium* sp. 1-1-2 and *Aspergillus flavus* 5-3-3 were distinguished by maximum efficiency, reducing salt content by 48.6% and 51.4%, respectively. However, the effectiveness of individual strains significantly lags behind the effectiveness of the consortium. Using the fungal consortium, salt content in soil was reduced by 70.3%, which significantly exceeds the effectiveness of any individual strain (Fig. 3). These results indicate a synergistic effect of the microorganisms in the consortium. Presumably, consortium members complement each other, ensuring more efficient absorption of salts and soil remediation process.

In the final stage of the research, a comprehensive assessment of bioremediation technology effectiveness was carried out based on the number and height of wheat (*Triticum aestivum* L.) seedlings. The experiment compared growth and development indicators of plants cultivated on three different soil types: soil, remediated with halophilic consortium; non-remediated, saline soil (negative control); and fertile, non-saline soil (positive control). This approach allows for the assessment of remediation effectiveness using a biological indicator and the agronomic potential of remediated soil in terms of suitability for plant growth and development.

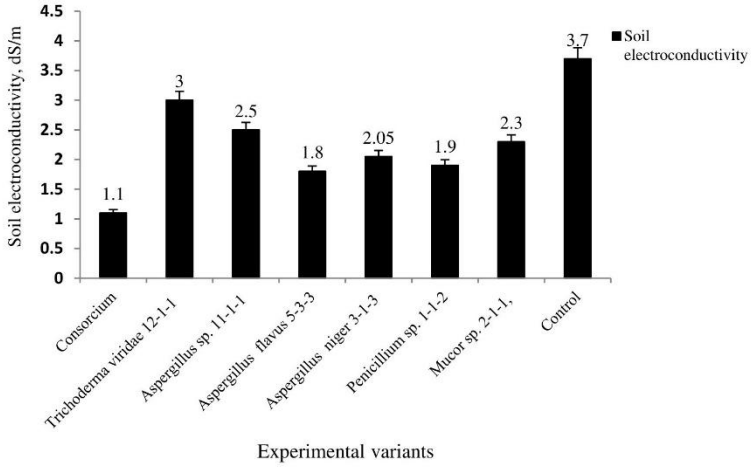

Fig. 3 Effect of the consortium and its particular strains on saline soil remediation

Monitoring was carried out in two stages - on the 10th day after sprouting (early development phase) and on the 30th day (tillering phase). Seed germination rate (%) and seedling height (cm) were selected as assessment criteria.

The results obtained from the conducted experiments demonstrated that bioremediation performed with the halophilic microscopic fungi consortium significantly improved the agronomic characteristics of saline soil (Fig. 4, 5).

It was experimentally determined that the germination rate in remediated soil is statistically significantly higher ($p<0.05$) than the corresponding indicator of non-remediated saline soil and only slightly lower than the indicator of non-saline soil (Fig. 4, 5). By the 10th day, seedling height in remediated soil exceeded the height of plants grown in non-remediated soil by 73.6% and was only 8.9% lower than the height of plants grown in non-saline soil (Fig. 4).

By the 30th day, a similar trend to the 10th day was observed - the height of plants grown in remediated soil significantly exceeded ($p<0.05$) the height of plants grown in non-remediated soil and did not statistically differ from the height of plants grown in non-saline soil ($p>0.05$).

CONCLUSIONS

1. Biotechnological parameters of composting have been optimized for the bioremediation of saline soils. The optimal concentrations of compost (20%) and the inoculum of microbial consortium (1.25%) have been experimentally determined ($p<0.05$).

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2. The use of the consortium reduced the salt content in the soil by 70.3%, which statistically significantly exceeds the effect of individual strains ($p<0.05$).
3. The effectiveness of the consortium is due to the synergistic action of the microorganisms it contains, which ensures comprehensive elimination of salts.
4. The germination rate and seedling height of wheat on remediated soil statistically significantly ($p<0.05$) exceed the same parameters obtained on saline soil, indicating the high agronomic efficiency of the used technology
5. On the 30th day after sprouting, the height of plants grown on remediated soil did not differ from the height of plants grown on non-saline soil ($p>0.05$), which confirms the effectiveness of bioremediation in terms of restoring the agronomic properties of the soil.

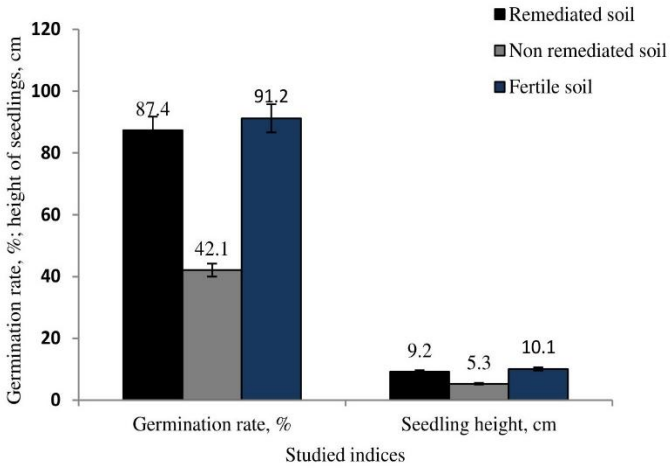

Fig. 4 The germination rate of wheat seeds and seedling height on the 10th day after sprouting

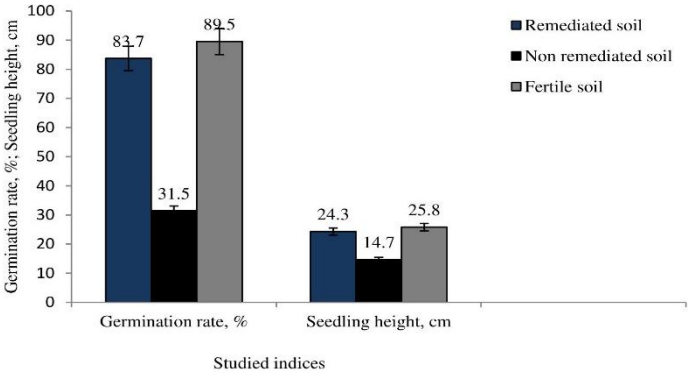

Fig. 5 The germination rate of wheat seeds and seedling height on the 30th day of sprouting

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