Effects of Mycorrhiza, Zeolite and Superabsorbent on Growth and Primary Establishment of *Agropyron desertorum* in Mining Field (Case Study: Mashhad's Shargh Cement Factory, Iran)

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Abstract. The most important and sensitive level in the modification of rangelands surrounding the mine is the initial seedling establishment of plants, which often faces failure due to adverse environmental conditions, poor soil nutrients, disturbance of soil different layers and its instability and unstable soil around the mines. New methods of rangeland improvement using biological and non-biological materials (zeolite, superabsorbent and mycorrhiza) and strategies may help to establish plants in the soil around abandoned mines. This research aims to investigate the possibility of improving the primary establishment and growth of *Agropyron desertorum* Schult (Fisch.Ex Link) in mine areas infected with heavy metals. Initially, seeds were sown in paper pots. After two months, pots were treated with *Glomus intraradices*, zeolite and superabsorbent along with control. Pots were transplanted in the field in semi-arid lands of East Mashhad cement factory using a completely randomized block design with four replicates in 2016. The plants were irrigated only once for the establishment. Data were collected about establishment rate, plant height, the percentage of mycorrhiza root inoculation and shoot, root and total dry weight. The results showed that the application of mycorrhiza, zeolite and superabsorbent increased plant establishment rate (50, 42 and 25%) and root dry weight (62, 72 and 15%) higher than that for control. Application of the mycorrhiza and zeolite treatments increased plant height (44 and 42%), aboveground dry biomass (51 and 61%) and total dry matter biomass (54 and 66%) higher than that for control treatment. The results showed that mycorrhiza and zeolite treatments had the greatest effect on increasing the initial establishment and improving the growth characteristics of *A. desertorum*. It was concluded that using zeolite and *Glomus intraradices* may be effective method for the initial establishment of plants in areas contaminated with heavy metals in the mining area in the semi-arid regions.

Key words: Heavy metals, Inoculation, Seedling establishment
Introduction
Today, it has become clear that mining and extraction operations are one of the major factors in the destruction of vegetation (Sheoran et al., 2010). These areas can contaminate air, water, soil and ground water and affect the environment in case of the non-recovery of vegetation in the mined areas and stabilization of soils contaminated with heavy metals (Laurence, 2011; Bacchetta et al., 2015). Heavy metals contamination in soils is one of the world's major environmental problems, posing significant risks to human health as well as ecosystems (Moameri et al., 2017). The usage of plants for the refining and clearing of the environment and their roles in restraining and controlling the pollution have been discussed as an effective method in order to conduct soil treatments in situ (Norozi Fard et al., 2015). The continuous erosion of abandoned mining lands can affect soil stability, vegetation regeneration and water quality. Toxic heavy metals in the "wastes" of abandoned mines migrate into the environment, causing severe and extensive contamination of rangeland soils, agricultural soils and waterways (Beane et al., 2016; Ma et al., 2016). In recent decades, restoring the vegetation of contaminated soils in industrial, agricultural and urban areas has been a big challenge due to human activities (Li et al., 2015; Xiao et al., 2015). Establishment of vegetation in mining areas often fails due to poor environmental conditions, poor nutrient content, the collapse of different soil layers and soil instability. The soil of these areas usually has high levels of heavy metals, toxic substances and nutrient poverty (Wu et al., 2009). Also, the slippage of unstable soils of the mining lands on the steep slopes causes to move the soil, cut off the root of the plants and ultimately destroy the plants (Adams & Lamoureux, 2005).

Crested wheatgrass (Agropyron desertorum) is a perennial bunchgrass and one of the important species of the Poaceae family (Mueller & Richards, 1986). This plant is resistant to dry and cold conditions. It grows well, except in heavy clay or sandy soils and is somewhat resistant to soil alkalinity. This species is prevalent due to the ease of planting and growth in unfavorable conditions (Bassiri et al., 1988). The success of establishment of vegetation in these lands depends on the combination of important elements in the contaminated bed and the choice of appropriate amendments and inoculants (Neagoe et al., 2005; Azimi et al., 2014). Zeolites are minerals of which aluminum silicates are the main component. Zeolites have a large surface and liquid absorption properties that are widely used in the removal of heavy metal ions through ion exchange processes, adsorption, surface deposition and dissolution (Mozgawa et al., 2005; Doula et al., 2012). Other amendments such as super-absorbents or hydrogels, a hydrophilic polymer network store water several times more than their weight. These materials contract to provide water and fertilizers to roots (Behbahani et al., 2005). Hydrogel maintains elements such as nitrates, phosphates, potassium, iron, zinc, and various types of vitamins and prevents from wasting them (Khodadadi Dehkordi, 2016). In a research in the coalmine of China, they succeeded in planting soybeans and revitalizing the mining area using soybean and corn plants with superabsorbent at the time of cultivation.

Application of mycorrhiza treatments increased plant establishment higher than that for control (Huang et al., 2015). Other amendments include mycorrhiza bio-fertilizers which help the initial establishment of plants in harsh environmental conditions (Azimi, 2013). Research showed that arbuscular mycorrhiza fungi increased the growth and establishment of an indigenous grass (Panicum virgatum L.) in areas around
mines with less phosphorus (Jonson, 1998). In a study, it was determined that the mycorrhiza fungi coexistence with the *Atriplex nummularia* Lindl restored the vegetation and areas contaminated with oil materials and saline soils (Keiffer et al., 2002). Currently, many studies have been limited to laboratory and greenhouse studies and only a small number of studies have been carried out in real-world conditions and actual results are likely to be different from laboratory or greenhouse studies because in reality, different factors affect them simultaneously. Factors such as temperature, precipitation, humidity, plant pathogenic factors and herbivores, unequal distribution of pollutants, soil type, acidity and soil structure affect the phytoremediation. Initial establishment and plant production should be investigated in real terms in order to assess the feasibility of this technology for commercialization. Therefore, this study was carried out to investigate the application of biological and non-biological approaches to increase production and the possibility of initial establishment of *A. desertorum* in soils contaminated by heavy metals around the abandoned mines of Shargh Mashhad Cement Factory.

### Materials and Methods

In this experiment, the effect of mycorrhiza (*Glomus intraradices*), superabsorbent (A200) and zeolite (Clinoptilolite) were assessed on the establishment and production of *Agropyron desertorum* in the semi-arid area (mining lands of Shargh Mashhad cement factory) in latitude 36° 28’ 29.96” and longitude 59° 44’ 46.99”. The average rainfall of the study area is 255 mm. Precipitation occurs in the cold season with ununiformed distribution. The area is located at an altitude of 1120 to 1130 m above sea level. Before plants cultivation, soil samples were taken from depths of 0 to 30 cm. Then, soil properties, heavy metals, soil acidity, electrical conductivity and soil texture were measured (Table 1).

### Table 1. Some chemical and physical properties of soil, cement factory mining area of East Mashhad

<table>
<thead>
<tr>
<th>EC (dS.m⁻¹)</th>
<th>pH</th>
<th>Soil texture</th>
<th>Cd</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
<th>Cu</th>
<th>Cr</th>
<th>Mn</th>
<th>Ar</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6</td>
<td>8.16</td>
<td>Silty loam</td>
<td>1.1</td>
<td>18.46</td>
<td>58</td>
<td>88.23</td>
<td>45</td>
<td>45.7</td>
<td>287.5</td>
<td>6.7</td>
<td>12</td>
</tr>
<tr>
<td>Allowable level of Heavy metals in soil (mg.kg⁻¹)</td>
<td>0.0-0.7</td>
<td>1-10</td>
<td>2.50</td>
<td>3-50</td>
<td>1-20</td>
<td>2-50</td>
<td>15-150</td>
<td>0.4-5.5</td>
<td>0.5-5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* (Raisi, 2013; Vodyanitskii, 2016)

### Research Method

Seeds of the *Agropyron desertorum* were sown in 160 trays in greenhouse of the Department of Agricultural, Ferdowsi University of Mashhad in mid-January 2016. After one month, the seedling was transferred to pots (dimensions 7 × 9 cm) containing about 160 g of soil. The soil of the pots was the same as the mining area of the Mashhad Cement Factory. During the transfer of pots to the mining area (March 19th), in separate treatments, *Glomus intraradices* mycorrhiza were added to soil from 1 to 10 (as a layer to 2 kg of soil) (Maestre et al., 2002; Azimi et al., 2014; Azimi et al., 2016; Sharma & Sharma, 2013) and zeolite treatments at rate of 2% (40g.kg⁻¹) (Bagherifam et al., 2014; Yari et al., 2013) and 0.4% superabsorbent (8 g.kg⁻¹ soil) (Al Humaid & Moftah, 2007; Abedi Koupai & Mesforoush, 2009; Banedj Schafie, 2015) was added into the pits containing 2kg soil and then cultivated. Mycorrhiza soil was obtained from Fanavar Turan Company. According to the company's catalog, there were at least 50 live spores per gram of mycorrhiza soil. The inoculum also included soil, spore, plant roots, and mycorrhiza fungal hyphae. Zeolite was prepared from Alvand Sangsar Agricultural Development Company of Semnan and Polymer was used from the Polymer Research Institute of Iran.
Data collection
The plants of pots were transplanted in the field based on a completely randomized block design with four replications in March 2016 and data were collected at the end of the experiment in July in the same year. Irrigation was done only once at planting time. Data were collected prior to plants going to yellowish leaves and falling. At the end of the experiment, for each treatment, two seedlings in each plot were randomly selected and establishment rate, plant height, and dry weight of leaves, roots, and stems of plants were measured. Then, the samples were dried in an oven at 70°C for 48 h and reweighted for dry matter yield. The root colonization percent was assessed in two experiments. In the greenhouse experiment, the coexistence of plant with mycorrhiza in the soil of mining area was assessed. For this propose, the plants of four pots were inoculated with mycorrhiza and grown in the greenhouse until March 2016. Then, the percentage of colonization was examined in the roots of the plants. In the field, experiment seedlings were inoculated with mycorrhiza during plant growth in the field and at the end of July, the percentage of roots colonization of was measured.

To determine the root colonization by mycorrhiza, a part of the fresh root of the plants (about 0.2 g) was randomly selected, washed and cut from 1 cm and then, transferred into the glasses containing 10% KOH and stored at room temperature for 60 h. In the next step, the roots were placed in 1/10 molar HCl solution to neutralize the alkaline medium for two minutes. The roots were stained with Phillips & Hayman (1970) method. Then, the method of Giovannetti et al. (1980) was used to determine the degree of symbiosis of mycorrhiza fungus with roots.

The experimental design was based on a completely randomized block design with four replications including mycorrhiza, zeolite and super absorbent and control treatments in the mining lands of Mashhad cement factory. Data were analyzed for variance using SPSS18 software and the means comparison was done using Duncan method. The non-normal data were converted.

Results
The effect of treatments on seedling establishment and growth of A. desertorum based on the analysis of variance, it was determined that zeolite, mycorrhiza and superabsorbent treatments had a significant effect on plant height, dry matter of shoot, root and whole plant and establishment rate of the A. desertorum in the field (Tables 3 and 4).

For Root dry weight, the effect of zeolite, mycorrhiza and superabsorbent was significant (p≤0.05) (Table 2). Treatments of mycorrhiza, zeolite and superabsorbent increased dry root weight (59, 72 and 15%, respectively) more than that for control (Table 3).

For shoot dry weight, the effects of zeolite and mycorrhiza were significant (p≤0.01) (Table 2). Means comparison of treatments showed that the inoculated plants with mycorrhiza and zeolite had the highest dry weight of the shoot as 51 and 61% more than that for control, respectively (Table 3).

For plant, dry weight result showed that zeolite and mycorrhiza treatments had a significant effect on total plant dry weight (p≤0.01) (Table 2). Regarding the means comparison between treatments, the result showed that mycorrhiza and zeolite treatments increased total dry weight (54 and 66%) more than that for control (Table 3).

For plant height, results showed that zeolite and mycorrhiza treatments had a significant effect on total plant dry weight (p≤0.01) (Table 2). Regarding the means comparison between treatments, the result showed that mycorrhiza and zeolite treatments increased total dry weight (54 and 66%) more than that for control (Table 3).

For plant height, results showed that the effects of zeolite and mycorrhiza were significant (p≤0.05) (Table2). Application of the zeolite and mycorrhizae treatments increased the plant height to 44 and 42% as compared to control (Table 3).
In general, for all of traits, mycorrhiza with average values of 0.6 g/p, 0.8 g/p, 1.4 g/p, 17.7 cm and 06 % had higher root, shoot, total dry weight, plant height, and Establishment, respectively. For zeolite, the average values of 0.7 g/p, 0.9 g/p, 1.6 g/p, 17.4 cm and 87 % had higher root, shoot, total dry weight, plant height, and Establishment, respectively (Table 3).

**Table 2.** The result of analysis of variance and the effect of zeolite, mycorrhiza and superabsorbent treatment on seedling establishment and growth of *A. desertorum*

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>0.04</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>0.17*</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.01</td>
</tr>
<tr>
<td>CV</td>
<td>-</td>
<td>48.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Root dry weight</th>
<th>Shoot dry weight</th>
<th>Total dry weight</th>
<th>Height plant</th>
<th>Establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>10.0 ± 0.1</td>
<td>46 ± 6.4</td>
</tr>
<tr>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>17.4 ± 1.1</td>
<td>87 ± 6.4</td>
</tr>
<tr>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>11.8 ± 1.1</td>
<td>71 ± 6.4</td>
</tr>
<tr>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>17.7 ± 1.1</td>
<td>96 ± 6.4</td>
</tr>
</tbody>
</table>

**Table 3.** Means comparison of the effect of zeolite, mycorrhiza and superabsorbent treatments on seedling establishment and growth of *A. desertorum.*

In each column and for each factor, averages that have common letters have no significant difference in 0.05% level according to Duncan's multiple range test (Average ± Standard error)

**Root colonization using mycorrhiza (greenhouse vs. field)**

The root colonization of *A. desertorum* using *Glomus intraradices* was significant in the greenhouse and after transfer and cultivation in the field (p≤0.01) (Table 4). The result showed colonization of *A. desertorum* root with *G. intraradices* mycorrhiza in the field (66%) and greenhouse (80%) indicating 14% higher values of root colonization in glasshouse than the field (Fig. 1). The success of the arbuscular mycorrhiza can be explained by fungal colonization (arbuscular, vesicles, and hyphae) in the plant roots (Fig. 2).

**Table 4.** Analysis of variance of root colonization percent of *A. desertorum* with mycorrhiza at the site of cultivation (field and greenhouse)

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>1.39</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>18.52</td>
</tr>
<tr>
<td>CV</td>
<td>-</td>
<td>11.31</td>
</tr>
</tbody>
</table>

**Fig. 1.** Means comparison of colonization percent for *A. desertorum* at field and greenhouse
Discussion
The results showed that zeolites and mycorrhiza had a significant impact on some morphological characteristics and plant growth of A. desertorum. In this study, mycorrhiza increased plant height, root and shoot dry weight. The fungus absorbs water and nutrients through the net of hyphae outside root and gives them to the plant, and the plant provides the carbohydrates needed for the fungus (Azimi et al., 2014). In addition, mycorrhiza fungi with the production of plant hormones can intensify plant growth or root growth, thus increasing the absorption of nutrients and keeping the plants in avoiding drought (Barea et al., 2005). A research conducted on the coexistence of mycorrhiza G. intraradices of Sorghum bicolor L. in lead-contaminated soils showed higher values of shoot and root dry weight than that for control, but it had no significant effect on the plant height (Amanifar et al., 2010). In the study on Trifolium subterraneum L, inoculated plant with the Glomus family mycorrhiza enhanced the plant roots growth and conversion of insoluble nutrients into accessible state, increased the absorption of nutrient elements of plants and ultimately increased plant biomass (Li-ping et al., 2009). Another study suggested that application of zeolite controls soil pH and N retention, absorption of Pb, Cd, Zn and other heavy metals by cation exchange provided plant nutrients requirement and microorganisms (Fenn et al., 2006; Fenglian et al., 2011). In a study, Shaheen et al. (2015) succeeded in corn establishment via inoculation of plants with 2.5% of zeolites in contaminated soils around the Greek copper mine using mycorrhiza and zeolite, superabsorbent treatments that increased the root dry weight and establishment rate as compared to the control. Hutterman et al. (1999) using superabsorbent found that the seedlings grow, the survival rate of Pinus halepensis were increased under drought conditions in clay loam soils, and they suggested application of 0.4% of superabsorbent. They also showed that water evaporation from the soil surface decreased from 90% to 50%, and the seedlings survival rate increased from 49 to 82 days (Hutterman et al., 1999). Zangooei Nasab et al. (2013) in Birjand, Iran studied Holoxylon sp. by the addition of hydrogel to soil and found higher root dry weight and establishment rate. They suggested that soil polymerization with 0.3% of polymer had the best result (Zangooei Nasab et al., 2013). The effect of A200 superabsorbent polymer on plant growth indices of an ornamental shrub in green space showed that mixing 4 or 6 g of polymer with 1 kg of soil reduces the water needed for the plant by at least one third due to the increase of the usable water (Abedi Koupai & Asadkazemi, 2006). In the similar study, the effect of A200 superabsorbent on the reduction of drought stress of olive trees (Olea
europaea) using superabsorbent in soil at a ratio of 0.3% had a higher dry weight and plant height in drought stress (Talaii & Asadzadeh, 2005). For plant establishment, the mean values of plants inoculated with mycorrhiza and zeolite were higher than that of superabsorbent and control in the field. Similar to our study, application of natural zeolites was effective in removing heavy metals from mines water. Their result showed that zeolite induced better growth of Dieffenbachia amoena and improved growth indices including leaf, stem and root dry weight, leaf number, diameter and plant height, and also improved nutrient uptake by the plant (Mohammadi Torkashvand et al., 2013) This is probably due to increased P, N and K uptake in zeolite-grown plants (Ahmed et al., 2010). Maloupa et al. (1999) concluded that despite the zeolite, the concentration of K and Mg may grow the plant tissue and the plants’ growth in such a bed was better (Maloupa et al., 1999). Mycorrhiza colonization in soils contaminated with heavy elements increases the effective root level for nutrients absorption. The fungal hyphae can penetrate to the large depth of soil and absorb a large number of nutrients including heavy elements. In highly contaminated soils, arbuscular mycorrhiza fungus (VAM) can protect plants against the harmful effects of these metals and improve their response to water loss at cellular, anatomical and morphological levels (Bano et al., 2013). Mycorrhiza like a filter prevents the introduction of certain metals (heavy metals such as Ar and Cd, etc.) into plant cells, thereby reducing plant damage by toxic metals and increasing the probability of the establishment of plants in the field (Sharma & Sharma, 2013). In a study, Kohler et al. (2015) observed that mycorrhiza induced establishment of Anthyllis cytisoides seedlings in semi-arid areas with mining activity infected with heavy metals. They showed that using mycorrhiza with organic fertilizers increased the Anthyllis cytisoides aerial biomass. Symbiotic fungi of Glomus versiforme and G. intraradices contributed to the establishment and optimal growth and absorption of nutrients in maize, soybean, clover, ground clover, tomato and eucalyptus in soils contaminated with Cd, Pb, Zn, Ar and petroleum (Sadravi & Gharacheh, 2013). These fungi inactivate pollutants by fixing them in their hyphal net out of the root. Therefore, symbiotic pollutants can contribute greatly to the biological restoration of contaminated lands (Sadravi & Gharacheh, 2013). The application of mycorrhiza fungi increased the plant’s resistance to environmental stresses, exacerbate photosynthesis, increase the growth and development of plants and increase the yield and absorption of water and nutrients (Porras et al., 2009; Subramanian et al., 2006). The effective symbiosis between plant roots and AMF is very different in terms of nutrient uptake, depending on the species of fungi and genotype of the plant (Marschner, 1995). AMF are affected by soil bed characteristics such as fertilization and physical and chemical properties. However, all types of AMF can carry at least P and N (Khan, 2005; Powell et al., 2007).

Among different treatments, the percentage of establishment in species inoculated with mycorrhiza and zeolite was higher than superabsorbent treatment and control in the field because most mycorrhiza fungi increase host plant resistance (Jones et al., 1988). The effects of these toxic metals can be reduced using suitable fungi as an inoculum in heavy metals contaminated lands (Bano et al., 2013). The intrinsic mycorrhiza relationship is also involved in bioaccumulation and emulsification of toxic heavy metals in the soil. Requena et al. (2001) found that G. intraradices mycorrhiza had the greatest impact on the improvement of plant establishment in
In this research, zeolite and mycorrhiza treatments had a positive effect on the increase in yield and establishment of the *A. desertorum* seedling in the mining lands of a cement factory in Mashhad, Iran. Therefore, given the existence of millions of tons of zeolite in Iran, they can be purchased at very cheap prices and used in agriculture and natural resources. This issue is especially important in the fields of natural resources in arid and semi-arid regions where rainfall and soil moisture content are low and plant establishment depends on rainfall and can be considered by researchers and executives. Mycorrhiza *G. intraradices* can be proposed as a biological fertilizer for...
enhancement of yield, the initial establishment of Agropyron desertorum and restoration of the vegetation of lands contaminated with heavy metals in the mining area of the cement factory of Mashhad.

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تاثیر میکوریزا، زئولیت و سوپرجاذب بر رشد و استقرار اولیه گیاهان در اراضی معدن کاوی شده (مطالعه موردی: کارخانه سیمان شرق مشهد)

Agropyron desertorum (Fisch. ex Link) Schult.

چکیده
مهم‌ترین و حساس‌ترین مرحله در اصلاح اراضی مرتعی اطراف معادن استقرار اولیه نشاء گیاهان است، که اغلب به دلایل نامناسب محیط، فقر عناصر غذایی خاک، فرورفتن لایه‌های مختلف خاک و ناهماهنگی خاک در اطراف معادن با شکست مواجه می‌شود. روشهای جدید اصلاح مرتع مبتنی بر استفاده از رهیافت‌های زیستی و غیرزیستی (زئولیت، سوپرجاذب و میکوریزا) ممکن است به استقرار گیاهان در اراضی معدن مراکه مهک کمک کند. این تحقیق با هدف بررسی این مکانیک بهبود استقرار اولیه و رشد گیاه مرتعی Agropyron desertorum (Fisch. ex Link) Schult. در اراضی معدن کاوش عرصه نیمه خشک کارخانه سیمان شرق مشهد انجام شد. در این تحقیق درصد استقرار، ارتفاع بوته، وزن خشک اندام هوایی و وزن خشک ریشه گیاهان از تعداد چهار تیمار میکوریزا، زئولیت و سوپرجاذب به صورت تصادفی در قالب طرح بلوک کامل تصادفی با پنج تکرار در سال 95-1394 گیاهان به ترتیب 62، 72 و 15 درصد بیشتری به سوپرجاذب و میکوریزا بودند. نتایج نشان داد میکوریزا و زئولیت بیشترین بهبود رشد و تقویت گیاهان را فراهم کردند و بهبود استقرار اولیه گیاهان را نیز بهبود یافتند. این نتایج نشان می‌دهد که استفاده از میکوریزا و زئولیت در اراضی معدن کاوش بهبود ساختار و رشد گیاهان را بهبود می‌یابد و استقرار اولیه و رشد گیاهان را افزایش می‌دهد.

کلمات کلیدی: فلزات سنگین، تلقیح، استقرار گیاهان

担忧 opinion, advice, or suggestion from the above content.