

Cyanobacteria and bio-crusts communities restore degraded soil: Examples of Iran

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Abstract

Soil is the major natural resource on which human life depends for the production of food, feed, fiber, renewable energy and raw materials, as well as the protection of the planet's natural ecosystems and climate system. Increasing soil degradation and subsequent ecosystem degradation is a current concern in agriculture and the environment. Soil degradation poses many challenges to feed the world's growing population. This food insecurity will also reduce the income of the agricultural sector, which will lead to a decrease in economic income. Soil degradation will lead to environmental threats, the extinction of animals and plants, the reduction of biodiversity, water loss, and disruption of water and carbon cycles. The use of new technologies such as biological crusts has provided great hope for the recovery and restoration of degraded ecosystems. Inoculation of biological crusts, especially cyanobacteria, has had positive results on improving soil conditions and restoration degraded soils. In this article, has been introduced the biocrusts communities considering cyanobacteria species to restore degraded soils. We reported the results of cyanobacteria applications in soil conservation in arid environments of Iran.

Cyanobacteria and biocrusts communities restore degraded soil: Examples of Iran Atoosa Gholamhosseinian^a and Adel Sepehr^{a,b}

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adelsepehr@um.ac.ir **Abstract** Soil is the major natural resource on which human life depends for the production of food, feed, fiber, renewable energy and raw materials, as well as the protection of the planet's natural ecosystems and climate system. Increasing soil degradation and subsequent ecosystem degradation is a current concern in the hyper-arid areas. Soil degradation has created many challenges for residents of arid and semi-arid regions of Iran. The use of new technologies such as biological crusts has provided great hope for the recovery and restoration of degraded ecosystems. Inoculation of biological crusts, especially cyanobacteria, has had positive results in improving soil conditions and restoring degraded soils. In this article, has been introduced the bio-crusts communities considering cyanobacteria species to restore degraded soils. We reported the results of cyanobacteria applications in soil conservation in arid environments of Iran. **Key words:** biological soil crusts, cyanobacteria, inoculation, soil degradation, soil restoration **1. Introduction** Indiscriminate human interference in the exploitation of nature and the environment will have numerous consequences. The growing need of the population for food has put a lot of pressure on the ecosystem, especially in arid and desert areas. Soil resources especially in arid and semi-arid regions are rapidly degraded and polluted (Oldeman et al., 1990). Today, soil degradation in arid and semi-arid regions is recognized as a serious and widespread problem. Soil degradation is defined as the loss and reduction of soil yields, which in recent decades has become a serious problem in the world and a threat to the ability to produce agricultural products and human life (Chisholm and Dumsday, 1987). Increasing soil degrada-

tion and subsequent ecosystem degradation is a current concern in agriculture and the environment (Scherr, 1999; UNEP, 1997). Soil degradation poses many challenges to feed the world's growing population. In arid areas, soil degradation is referred to as desertification (Blaikie and Brookfield, 2015). 75% of Iran's area is covered by arid and semi-arid areas. Human and geographical factors have led to an increasing trend of soil degradation in Iran. Soil degradation processes in Iran have created many challenges. Therefore, the use of effective methods in soil conservation and restoration is essential. The use of biological crusts is expanding as restoration of degraded soils as well as protection against erosion and degradation. Moss, lichen and cyanobacteria are classified as biological soil crusts (BSCs). Mosses have organs similar to stems, leaves and rhizoids, have a relatively high photosynthetic capacity and are similar to plants (Giuliani et al., 2020). Cyanobacteria and algae, which usually act as pioneers in desert soil, are low-growing plants with low photosynthesis (Chaplot, 2021). Lichens are a collection of algal cells that are covered in epiphytic mycelium and can withstand very dry environments (Goiris et al., 2012). These organisms are characterized by different physiological characteristics and require different conditions for rapid growth, resulting in the complexity of artificial repair for each type of BSCs (Scherr, 1999). From the beginning of the twentieth century, the use of compounds extracted from cyanobacteria and green algae has been considered by farmers (Taddese, 2001). At that time, most attention was paid to the role of cyanobacterial products in increasing the quality of products, increasing production, reducing plant stress, and so on (Kovda, 1983). Over time and increasing pressure on the soil, soil erosion and destruction also increased, and this issue led to special attention to the role of these crusts in soil conservation and restoration (Buringh, 1981). In this study, we have tried to investigate the role and importance of biological crusts in soil regeneration in arid and semi-arid regions of Iran and examples of the use of these shells in Iran are given. Areas in which a combination of factors such as wind and water erosion, soil salinization, loss of fertility and soil quality, declining groundwater levels, migration, poverty, etc. have led to obvious signs of desertification.

2. Soil restoration

Ecosystem restoration has become an important goal of environmental protection and international policy (Zhao et al., 2013). Ecosystem restoration means that plants, microorganisms, soil, and even higher levels of nutrition, such as animals, are targeted for restoration (Giraldo-Silva et al., 2019). Soil restoration in degraded arid and semi-arid soils faces many challenges due to long periods of drought and irregular rainfall (Berdugo et al., 2020). Most arid and semi-arid regions are exposed to large-scale human disturbances, including overgrazing, severe fire processes, the use of chemical fertilizers to raise productivity, and mining (Bateman and Mooz Noz-Rojas, 2019). In recent years, several new strategies have been adopted to overcome these challenges and revitalize these ecosystems, including the use of amendments (Luna et al., 2018) or inoculation of soil microorganisms (Moreira-Grez et al., 2019). In recent years, research has shown promising potential for the effects of these organisms on restoring soil fertility and preventing water and wind erosion (Chamizo et al., 2017, 2018; Rossi et al., 2017). Cyanobacteria are prokaryotes that, thanks to their characteristics such as high UV tolerance, soil salinity, drought, high temperature, etc., can be established in many damaged and critical environments (Adessi et al., 2018). Cyanobacteria as the initial crust and one of the most important microorganisms in biological crusts play an important role in ecological, hydrological and biogeochemical processes (Rodríguez-Caballero et al., 2018; Gholamhosseinian et al., 2020). Cyanobacteria are the best choice for soil regeneration due to their high resistance in poor environments, against intense UV radiation and exopolysaccharide production, as well as rapid growth and multiplication (Rajeev et al., 2013; Lan et al., 2015).

2.1 The importance of biological crusts

2.1.1 Improving the physical and chemical properties and soil ecosystem

The presence of unique features in biological crusts has made it possible for them to live in harsh environments (Markou et al., 2014). For example, algae live in different environments such as fresh water, salt water of the sea and oceans, snow, rocks and on the surface of plants and animals (Maestre et al., 2011). The algae *Oscillatoria Brevis*, *Scytonema elongates*, *Heterormogonium*, can withstand temperatures up to 70 ° C, which is why they are found in abundance in tropical and desert soils. Many algae are able to withstand sunlight and only slow down when they are exposed to strong light conditions (Zhang et al., 2016). Many green algae are found in a wide range of pH but have the highest growth and yield at neutral or alkaline pH (Bilgrami and Saha, 2004; Heidarpour et al., 2019). After death and decomposition of these microorganisms, organic matter is added to the soil and after a few years, it enriches the soil and makes it suitable for cultivation. Cyanobacterial play an important role in soil physicochemical

properties (Thajuddin and Subramanian, 2005; bashtian et al., 2019; sepehr et al., 2019; Gholamhosseinian et al., 2020) (Fig. 1). [Fig. 1] In recent years, inoculation of cyanobacteria into the soil due to their vital role in the ecological cycle (Brown et al., 2019; Gholamhosseinian et al., 2020), such as The redistribution of precipitated rainwater; dust capture and use of airborne, interaction with vascular plants; and enhancing the soil physical structure and stability(Fig. 2), reducing the wind and water erosion, increasing nutrients accessibility, increasing the roughness of the surface, so enhancing dust trapping capacity (Fearnough et al., 1998; Belnap and Lange, 2003; Warren et al., 2019; Weber et al., 2016); improving the essential nutrient concentration in plants (Belnap and Harper, 1995), enhancing nitrogen and carbon fixation rate (Evans and Lange, 2001; Yang et al., 2014; Madsen et al., 2016), so, increasing the mineralization and bioavailability rate of nitrogen (Delgado-Baquerizo et al., 2013; Hu et al., 2015); thus, biocrusts nutrient enrichment has important role in soil fertility and nutrient bioavailability. Therefore, has become one of the most promising biotechnology strategies to restore soil performance in degraded arid and semi-arid areas have become (Lal, 1995; Lutz et al., 1996; Eswaran, 1999; Muñoz -Rojas et al., 2018a). Xie et al., (2007) found that by inoculating cyanobacteria into the soil and conducting a 3-year study, these shells with 70% coverage in the soil and penetration to a depth of 0.5 mm could have long-term positive effects on soil stability. In Figure 3, you can see the difference in the structure of soil particles in the presence and absence of crusts (Semi-arid regions in eastern Iran). Increased roughness, albedo and trapping of airborne particles are other well-known features of these crusts (Chamizo et al., 2016; Gholamhosseinian et al., 2020). Adessi et al, (2018) in an experiment showed that by removing the exopolysaccharides of cyanobacteria from the soil, the water holding capacity was reduced by up to 80%. [Fig.2] [Fig. 3]

2.1.2 vegetation establishment, seed germination

These microorganisms can lead to optimal plant growth (Condon and Pyke, 2018; Havrilla et al., 2019); These shells may act as armor-like barriers to seed, but if seeds can be successfully created, the presence of biological shells can increase seedling growth rate (Ferrenberg et al. 2018; Slate et al., 2019). Research has shown the positive effect of cyanobacteria on germination and grain growth of wheat and rice (Muñoz-Rojas et al., 2018). In addition, the use of cyanobacteria on the seeds can be a useful tool for inoculation into the soil after germination (O'Callaghan, 2016). Soaking the seeds in cyanobacterial suspension in addition to rapid seed germination and better seedling growth provides favorable conditions for inoculation of cyanobacteria into the soil (Gupta and Lata, 1964; Mahmood et al., 2016). The ability of cyanobacteria as a biological initiator to regenerate soil in an arid region of Western Australia was investigated, and the results showed that cyanobacteria due to their high protein content have positive effects on native plant growth and improved soil substrate characteristics (Flemming and Wingender, 2010). Cyanobacteria can be used as biological controllers against fungi and bacteria harmful to plants (Sing et al., 2016) and also capable of producing plant growth regulating hormones (PGR) (Venkataraman, 1981) and gibberellin (Shen-Rui and Shen, 1997) and organic acids (Hellebust, 1974). Studies have shown that the use of cyanobacterial extract increases plant growth (Offer et al., 1992) and controls fungal damping disease (Caire et al., 1976). The use of cyanobacteria is recommended to increase seed germination as a biological fertilizer and to increase the growth of many plants (Strick et al., 1997). Due to these characteristics, their high dispersion and establishment in any environment, cyanobacteria can be used as a potential tool for soil repair in arid areas (Rossi et al., 2017).

2.1.3 Application of EPS production in improving soil properties and soil stability

One of the reasons for the importance of cyanobacteria in the restoration of degraded soils is the synthesis of Extracellular polymeric substances (EPS), which are called the most vital chemicals produced by these microorganisms. These polysaccharides play an important and vital role in improving soil physicochemical parameters (Weber et al., 2016). These polysaccharides form a protective layer around the cells called the capsule (De Philippis and Vincenzini, 1998); that protects the cell under drought stress (Costa et al., 2018). Studies have shown that another important role of the EPS matrix is the aggregation capacity of soil particles, which improves soil structure and soil fertility (Boonchai et al., 2014). These polymers can improve soil quality, health, fertility and structure (Hwang et al., 2004; Escribano et al., 2009). EPS is produced to protect the cell under environmental stresses such as salinity, pH changes, drought, temperature changes, etc. (Wingender et al., 1999). It also protects the cyanobacteria from the negative effects of heavy metals and antimicrobial chemicals by creating a layer around them, as a result, it allows cyanobacteria to survive in very harsh conditions where other microorganisms are unable to survive. EPSs are important for improving

soil quality because they trap nutrients and make the environment suitable for chemical reactions. As EPS increases the ability of soil particles to accumulate, it can increase soil nutrients.

3. Examples of research experiences and use of biological crusts (considering cyanobacteria) in Iran

The location of Iran in the arid region and the increasing desertification process in this country has multiplied the importance of soil protection and soil restoration. Decreased soil quality and health, increased dust storms, reduced crops, increased migration, poverty, unemployment, etc. are all consequences of increasing soil degradation and desertification. In this section, we have tried to give examples about the use of biological crusts, especially cyanobacteria in arid and desert areas of Iran, as an effective solution to solve the mentioned problems.

Sepehr et al., (2019) With a study in a semi-arid region located in eastern Iran examined the importance of EPS in soil stability. The results of this study showed that the genera *Microcoleus sp.*, *scillatoria sp.*, *Lep- tolyngbya sp* and *Phormidium sp* are among the dominant species in arid regions of Iran and among these two species *Oscillatoria splendida* and *Oscillatoria tenuis* had the highest tolerance to soil salinity. The EPS secreted by these cyanobacteria increases the stability of the aggregates and reduces the dispersion at the soil surface.

Hassanzadeh Bashtian et al., (2019) by examining the relationship between soil parameters and the development of biological crusts showed that crusts have an effective role in increasing organic carbon, nitrogen, calcium carbonate and reducing salinity and sodium in soil. Gholamhosseinian et al (2020) also reached this conclusion by studying the biological crusts in the semi-arid region of Iran; The presence of crusts in the soil helps to improve the chemical and physical conditions of the soil and also increases the diversity of soil minerals by affecting the geochemical processes of the soil and the deposition of dust. Due to the known roles of cyanobacteria, soil inoculation with cyanobacteria has been proposed as a sustainable technology to improve soil quality and control and regenerate degraded soils in arid and semi-arid regions (Rossi et al. 2017). Cyanobacteria are able to retain more water in the soil and store moisture by secreting exopolysaccharides, in lichens and mosses, this role is played by filaments (Fig. 4).

[Fig. 4] The study of biological communities in these saline soils south of the Caspian Sea showed that these biological crusts have positive effects on increasing soil quality. These effects include increasing nitrogen, phosphorus, copper and iron and decreasing pH, sodium, sodium adsorption ratio and percentage of exchangeable sodium compared to crustless soils (Jafari et al., 2004; Kakeh et al., 2018; Bashtian et al., 2019). Also, the presence of these biological crusts leads to increased infiltration and soil moisture (Kakeh et al., 2018). Deposition of non-rainfall water in arid and semi-arid regions is of great importance. Maintaining moisture is very important for the survival of vegetation and maintaining water balance in these areas. In the desert areas of Iran, the lack of vegetation leads to a decrease in moisture and water loss through evaporation and transpiration, as well as runoff. Therefore, the role of biological shells to solve this problem is of particular importance.

270 samples containing cyanobacteria were tested under artificial rainmaker compared to the control sample. Samples with cyanobacterial crust had higher permeability and less runoff production and also reduced soil salinity (Kakeh et al., 2020). In addition to soil surface, these crusts also affect soil properties, so they play an important role in conservation, restoration and rehabilitation soils in arid and semi-arid regions. Dust storms are a common phenomenon in desert areas. In recent years, the intensity and frequency of these storms have increased sharply in Iran (Cao et al., 2015). The problems of sandstorms in Iran cause a lot of damage to agricultural products every year and create problems such as migration and poverty (Khusfi et al., 2020). Inoculation of cyanobacteria can be an effective solution to this problem. In northwestern Iran, two species of *Nostoc sp.* and *Oscillatoria sp.* were obtained from the dry bed of the river. After inoculating cyanobacteria, sandy soils were tested in a wind tunnel at a speed of 72 km / h. The presence of cyanobacterial filaments among the sand particles caused the particles to coalesce and reduced wind erosion by 96.6% (Kheirfam and Asadzadeh, 2020). A study was conducted to evaluate the success of cyanobacterial inoculation in reducing runoff in a large-scale degraded land with natural rainfall in northwestern Iran. The results showed that inoculation of two species of *Nostoc sp.* and *Oscillatoria sp* in soil reduced 57-32% of runoff (Fig 5). Also, roughness and stability index did not show positive results (Sadeghi et al., 2020). A study in northern Iran showed that inoculation of cyanobacteria into degraded soils after 60 days showed a significant difference in chemical parameters such as carbon and nitrogen (Kheirfam et al., 2017). Biological crusts and sediments were collected from the loess plateau of Northern Iran. Cyanobacterial diversity and grain stabilization were studied by light and scanning electron microscopy. The results reported in this study

confirm the integral role of cyanobacteria in the formation of loess. EPS layers and cyanobacterial filaments enclosing fine and coarse soil particles, respectively. This is the first step in loess formation (Dulić et al., 2017). The formation of loess prevents the dispersion of clay particles. Many pests cause a lot of damage to Iranian farms every year. The use of chemical pesticides also has its disadvantages and leads to chemical contamination in the soil and endangers the health of the soil. The use of cyanobacteria as a killer of bacteria and pathogenic fungi is a completely harmless process and is also useful for improving the quality and health of the soil. In order to find the potential of cyanobacteria for the production of antibacterial and antifungal compounds in rice fields in northern Iran, 150 strains of cyanobacteria from *Hapalosiphon* sp, *Fischerella* sp, *Stigonema* sp, *Nostoc* sp were isolated and studied. The results of this experiment showed that the genera *Hapalosiphon* sp, *Fischerella* sp, *Stigonema* sp have the greatest effect on antifungal and bacterial activities as well as reducing lead in the environment (Ghasemi et al., 2003). The combination of Tjipanazoles obtained from *Tolypothrix tjipanensis* also showed significant fungicidal effects against wheat blight, rice blast and leaf rust (Borowitzka, 1995). Another study of 76 species of cyanobacteria found that 17 species, generally from the four families Nostocaceae, Stigonemataceae, scillatoriaceae and Chroococcaceae, had antibacterial effects (soltani et al., 2005). The use of chemical fertilizers to increase yields may be effective at first, but over time it leads to pollution and degradation of soil and the environment. In recent decades, the use of cyanobacteria to increase seed germination rate, seedling establishment and increase crop growth has been widely used. Shriatmadari et al., (2013) in order to investigate the effect of blue-green algae on crop growth, the dominant *Anabaena* sp. and *Nostoc* sp. were identified and isolated from paddy soils. After 40 days of inoculating the algae extract into the potting soil, the parameters of root length, fresh and dry weight of the plant, number of leaves and plant height were measured. Liquid chromatography confirmed the production of plant growth stimulants such as phytohormones and the results showed a significant difference compared to the control. [Fig. 5]

4. Conclusion The interaction between biocrusts and soil is very important and good awareness from that help to better manage soil specially in arid and semi-arid areas. New techniques for soil restoration in arid area are of particular importance. Successes in inoculating cyanobacteria in the soil could provide a new perspective on ecosystem restoration in Iran. Numerous studies for inoculation of cyanobacteria as an artificial induction strategy in the fight against desertification and rehabilitation of degraded soils (Evans et al., 1999). Given the properties of biological crusts, especially cyanobacteria, the idea of adding them to the raw material to increase soil stability and reduce losses due to water and wind erosion seems to be useful. Research into BSC inoculation, especially the use of cyanobacteria, has made great strides so far. cyanobacteria have successfully been inoculated in soils under laboratory conditions (Issa et al., 2007; Muñoz-Rojas et al., 2018b) and in local field settings (Chen et al., 2006; Wang et al., 2017). For countries located in very hyper-arid, such as Iran Like Iran, using these biological crusts for soil restoration can be considered a very useful and efficient technology.

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Fig. 1 Fig. 2Fig. 3Fig. 4Fig. 5Caption of Figures:**Figure 1.** The schematic shows that many ecosystem processes are affected by the presence of biological crusts**Figure 2.** In Figures A, B, and D, you can see that the presence of crusts has led to the accumulation and strength of the soil. The pictures c and e also show the interconnection of soil particles in the presence of lichens**Figure 3.** Above images show an arid region in eastern Iran including a surface without biological crust cover (a) and at the right side a surface with biological crusts (b). Images of c and d show the electron microscope (SEM) of these surfaces which involves coarse particles in image c with little coherence and presence of polysaccharide and cyanobacterial filaments surround the conducting particles in image d.**Figure 4.** (a) cyanobacterial colonies on the soil surface before rain and (b) cyanobacteria during rainfall by water absorption have formed a protective layer on the soil surface and (c) microscopic image of soil particles surrounded by cyanobacteria and (d) Formation of fine soil structures by cyanobacterial filaments. (Kheirfam et al. 2020)**Figure 5.** Electron microscope images a) Cyanobacterial filaments extracted from soil b) - Soil particles among polysaccharide filaments c) Polysaccharide filaments of cyanobacteria (red circle) hold soil particles together d) Close view of a polysaccharide filaments. The red arrow indicates fine soil particles