Bioremediation of Heavy Metal Contaminated Soils Originated from Iron Ore Mine by Bio-augmentation with Native Cyanobacteria

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ABSTRACT

The soil in the Sangan iron-mining region in the east of Iran is contaminated with a high concentration of heavy metals, especially iron. The release of these pollutants into environment results in the transfer and accumulation of iron through the food chains, hence a reasonable solution is required to restore it. Bioaugmentation is an environmental friendly option to reduce the hazard effects of heavy metal in the contaminated soil. In this study, the consortia of two indigenous cyanobacteria isolated from soil of Sangan iron mining and used to bioremediate soil contaminated with iron, chromium, copper, lead, and nickel. The experiments were carried out by three treatment methods, including control soil, surface soil sprayed with cyanobacteria, and soil mixed with cyanobacteria for six months under laboratory condition. The scanning electron microscope showed the development of a network of filaments of the inoculated cyanobacteria (*Oscillatoria* sp. *and Leptolyngbya* sp.) with soil particles in both treatments. Bio-augmentation of the soil increased initial nitrogen content from 406 mg/kg in control to 664 mg/kg in soil mixed with cyanobacteria and 710 mg/kg in soil sprayed with cyanobacteria. Cyanobacteria inoculation decreased the available concentration of lead and nickel. The non-available heavy metal of soil in sample sprayed with cyanobacteria was in decreasing order: Cr > Fe > Ni > As > Pb > Cu. The maximum metal removal efficiency was 32%. In soil mixed with cyanobacteria increased in the root and hypocotyl lengths of radish and lettuce was observed compared to that in the control soil, indicated in the improvement of soil quality after bioremediation.

INTRODUCTION

Soil contamination with heavy metals is a global problem that poses a severe threat to ecological health, human health, and safe food production. Human activities such as mining, smelting, military training, power generation, electrical industries, burning fossil fuels, industrial waste disposal, pesticide application, and irrigation have introduced a significant amount of heavy metals into the soil [1]. The accumulation of heavy metals in the soils occurred due to the continuous exposure from these activities, which results in heavy metal contamination when the concentrations exceed the natural background levels of the metals [2, 3]. High concentrations of heavy metals, such as lead, chromium, iron, and copper in soils, are reported in many countries, including China, India, Bangladesh, Australia, America, and many European countries [4–9].

Some metals such as Cr, Fe, and Cu are essential for biological systems (e.g., proteins and enzymes). However, high concentrations of these metals create harmful effects on living organisms [4]. Other heavy metals, such as Pb and As, have no known biological functions. These metals are toxic, which can cause chronic and acute health effects on humans such as headache, memory loss, mental confusion, sense of unreality, distorted perception, allergies, vision problems, cardiovascular disease, diabetes, muscle cramping, and death [10, 11]. From contaminated soils, these toxic metals can be transported to humans via multiple routes, including direct contact or indirectly through the contaminated plant and animal foods. Therefore, it is required to restore the contaminated area, especially where extensive mining activities have destroyed the soil health.

A number of physicochemical methods are utilized for remediation of metal-contaminated soils, namely surface capping [12], solidification [13], vitrification, electrokinetics [14], chemical immobilization [15], encapsulation [16], and soil washing [17]. Alternatively, biological methods, namely bioremediation involving plants [18] and microbes [19] or their enzymes, are used for the restoration of the contaminated soils. Among these, exploiting metal resistant microbial

activities has become more prominent because they are environmental friendly, cost-effective, and simple to implement [1, 20]. Studies have shown that microorganisms (fungi, algae, bacteria, etc.) can be used as a tool for the purification of metal contaminated soils through transformation mechanisms (e.g., valence transformation and volatilization), and through biosorption (to the cell surface or intracellularly) [21, 22].

Cyanobacteria are a group of microorganisms that inhabit marine, freshwater, and terrestrial environments and are considered as a most ancient group of biocrusts [23, 24]. These organisms participate in many ecosystem functions such as nutrient fixation, dust entrapment, enhancement of soil structure, stability, fertility to establish the plants and the animals [25, 26]. Recently, several studies have explored the inoculation of cyanobacteria in the degraded soil to improve its stability and fertility [27–30]. For example, Nisha et al [31] investigated the effect of the inoculation of two indigenous heterocystous cyanobacteria (*Nostoc ellipsosporum* HH-205 and *Nostoc punctiforme* HH-206) on salty soil. They found that the indigenous cyanobacterial species enhanced the productivity of soil and they are useful option for remediation [32] examined the impacts of cyanobacterial inoculation on dryland soil restoration. They concluded that this organism as a bio-tool could rapidly improve soil fertility and structure. Chamizo et al [24] investigated the ability of a non-N-fixing cyanobacterium (*Phormidium ambiguum*), and an N-fixing cyanobacterium (*Scytonema javanicum*) for the modification of stability, hydraulic and fertility properties of the soil by inoculation method. They proved that the development in soil fertility and stability assist the viability of using cyanobacteria to rehabilitate degraded arid soils. So far, researchers have focused on the cyanobacteria inoculation to remediate saline soil remediation. However, to the best of our knowledge, there is a scientific gap about the potential use of cyanobacteria as a remediation technique for heavy metal-contaminated soils. Various genera of cyanobacteria were isolated from metal-polluted sites such as mine tailings and proved to have metal tolerance ability. Several metal-tolerance mechanisms such as extracellular binding or precipitation, impermeability or exclusion, and internal detoxification were adopted by these species [33].

This study aimed to investigate the bio-augmentation of contaminated soil by heavy metal using cyanobacteria inoculation. The soil is collected from a site adjacent to the Sangan iron-mining region, which is contaminated with a high concentration of Fe, and low concentration of other heavy metals such as Cr, As, Cu, Ni, Pb, etc. [34]. Then its indigenous strains of cyanobacteria are isolated, cultured, and then introduced to polluted soil to remediate it. From this primary goal, three specific objectives were derived: (1) determination of soil organic carbon and nitrogen content before and after bio-augmentation treatment; (2) Evaluation of the removal efficiency of heavy metals from contaminated soil by cyanobacteria inoculation; and (3) investigation on bioremediation efficacy by planting.

MATERIAL AND METHOD
Soil sampling and characterization
The soil was collected from an active mine named Sangan iron ore mine (SIOM) in the east of Iran in June 2017. SIOM is one of the largest mineral areas in Iran and located near the Khaf County in Khorasan Razavi Province at 60° 22′ 28″ E and 34° 27′ 22″ N (Figure 1). About nine soil samples (2 kg) were taken from 0-15 cm deep at a different location near SIOM and were transferred to the laboratory. For analysis, soil samples were air-dried in shed, avoiding direct sunlight, sieved to 2 mm, and stored in polyethylene bags for further testing. The physicochemical properties (e.g., soil pH, organic carbon, nitrogen, EC, percent of silt-sand-clay) of samples were determined.

Isolation of cyanobacteria
Indigenous strains of cyanobacteria from the contaminated soil collected from the mining site were isolated, and cultured by the standard technique reported by Kaushik [35] using BG 11 as growth medium at 28 °C under light: dark cycles (12:12 h) and irradiance of 30 μ mol m^{-2} s^{-1}. Cyanobacteria were purified using agar culture method [35]. The morphological characteristics of the isolated native cyanobacteria were determined by observing under the light microscope (magnifications 400–1000 x (Olympus CH-2)) and Scanning Electron Microscope (SEM) (Leo-Germany 1450VP) in conjunction with the taxonomic references [36–39].

Inoculum preparation and bioremediation experiment
For bio-augmentation experiments, the algaculture was poured inside the 50 mL polypropylene tubes and centrifuged (Dinamica, Velocity 14, France) at 4000 g for 30 min. Then the obtained biomass fragmented in a sterile tube with a sterile spatula. The dry weight of the biomass was measured by an oven drying method at 80 °C for 24 h. An amount of 50 mg (dry weight) of cyanobacteria biomass was added to Petri dishes (2 cm height $\times$ 10 cm diameter) containing 80 g contaminated soil in two ways: (1) by spraying on the surface soil (2) and by mixing. The samples were placed in an incubator with 28±2 ℃ and 30 μ mol m^{-2} s^{-1}. Samples were kept wet by spraying 25 mL of distilled water every three days for six months. Three treatment types, including control soil (CS), soil sprayed with cyanobacteria (SSC), soil mixed with cyanobacteria (SMC) were designed for bioremediation experiments.

Analytical methods
Organic carbon and nitrogen content
The weight loss-on-ignition (WLOI) method demonstrated by Wang et al. [40] was used to measure total organic carbon (Corg) of soil. In summary, 3 g of soil was placed in a crucible and dried at 105 °C for 12 hours to specify the moisture content of the samples. After that, the sample was placed in a furnace (AFE1200L, ATRA, Iran) and burned for 12 h at 500 °C. Then, it was cooled to room temperature in a desiccator and weighed. All treatments were carried out in triplicate. Total Kjeldahl nitrogen (TKN) was measured using the Kjeldahl method [31].

Cyanobacterial biomass
Cyanobacterial biomass was determined by chlorophyll-a concentration as the method reported by Park et al. [41]. Two grams of dry soil was added to 5 ml of ethanol and placed in a 50 ml Falcon tube. After putting in a water bath at 80 °C for 5 min, it was cooled at room temperature and centrifuged at 2500 g for 5 min. The absorbance of the supernatant solution

was measured with a UV-visible spectrometer (DR 2000, Hach, USA) at 665 nm. The chlorophyll concentration was calculated by the equation previously stated by Ritchie [42].

Total metal determination

$CaCl_2$ extraction was used to determine the heavy metal (Fe, As, Cr, Cu, Pb, and Ni) availability or exchangeable fraction of the elements; because it considered as a dependable indicator of metal availability in polluted soil. Briefly, 3 g of soil was placed in 50 mL glass flask, and 30 mL of 0.01 M $CaCl_2$ solution was added. Then, the suspension stirred for 24 h at room temperature. After that, it was centrifuged at 3500 rpm for 20 min and filtered using a 0.45 μm pore size filter. The heavy metal concentration infiltrate measured using ICP-OES (ICP-OES, SPECTRO ARCOS-76004555). Total heavy metal concentrations of soil samples were measured by acid digestion according to the method described by Alghanmi et al. [43], and Turan et al. [44]. First, one gram of soil sample was mixed with a 3: 1 ratio of 70% HNO_3 37% HCl and stirred at room temperature for 12 h. Then, the obtained mixture was refluxed at 130 °C for three h. The final solution was filtered and diluted to 50 ml with 0.5 M HNO_3 for analysis. Finally, the concentrations of heavy metal ions were determined.

Bioremediation efficacy testing

The success of bioremediation was investigated by planting lettuce (*Lactuca sativa*) and radish (*Raphanus sativus* L.) seeds in the experimental soils before and after bioremediation, as suggested by Mahbub et al. [7] and Aparicio et al. [45]. Seeds from each plant were sterilized with ethanol, 97%, and then were planted in each of the three soil treatments (CS, SSC, and SMC). After 12 days, germination percent of seeds were recorded, and the length of root and hypocotyl was measured on a millimeter scale. Besides, vigor factor ((mean root length + mean shoot length) × (percent germination/10)) was calculated [45–47].

Statistical analysis

Any statistically significant differences of observed parameters (organic carbon, nitrogen, metal recovery, root length, and hypocotyl length) between various treatments were determined using one-way ANOVA at 95 % level of significance. The comparison between the means in all variables except organic carbon was performed with Tukey's post hoc test. The later was conducted with the Duncan test. Analyses were carried out using IBM SPSS 22.

RESULTS AND DISCUSSION

Morphology of cyanobacteria

The morphology of cyanobacteria isolated from the soil samples collected from SIOM and cultured in BG-11 was characterized based on their microscopic features. Two cyanobacteria species were recovered from the soil, which was identified as *Oscillatoria* sp. and *Leptolyngbya* sp. (Figure 2). Both species are stranded, lacking akinete and heterocysts. *Oscillatoria* sp. was darker than the *Leptolyngbya* sp. and thinner at the end of the strand. Sepehr et al. [36] have previously reported the presence of these two species in arid soils in northeastern Iran. Besides, the SEM images of cyanobacteria after 6 months inoculation into soil

samples revealed that cyanobacteria developed a network of filaments among soil particles (Figure 1c and d). They also presented extracellular polymeric substances (EPS), which coated on mineral materials and soil fragments.

Changes in soil parameters

By inoculating cyanobacteria cells into the soil, its parameters before and after treatments were changed (Table 1). The soil texture was a loamy (19.8 % clay, 32.2 silt, 47% sand). The pH value of the control soil sample was 8.7, which after bio-augmentation with cyanobacteria during 6 months it decreased to 8.36 for SMC and 8.21 for SSC. Similar trend was reported by Singh [48] which concluded that cyanobacteria growth reduced soil pH. Besides, the electrical conductivity of the soil samples reduced significantly (more than 30 %) after cyanobacterial inoculation. This finding could be confirmed by other researchers [31, 49]. They explored the bioremediation of salt soil using cyanobacteria and proved that cyanobacteria application remarkably declined soil EC. This phenomenon ascribed the adaption of cyanobacteria with salinity and high pH by retaining inorganic ion level [50] and the contribution of ion transport processes [51] and different metabolic adjustments [52]. Besides, they secrete extracellular polysaccharide, which chelates cations and decreases its bioavailability due to having a negative charge like uronic acid [31].

Enhanced organic carbon content of the soil

By inoculating cyanobacteria cells into the soil, organic carbon increased from 26.9 g kg^{-1} in CS to 28.4 g kg^{-1} in SMC and 32.6 g kg^{-1} in SSC (Table 1). Although 1.5 to 5.6 percent increment of organic carbon was observed after inoculation, this difference was not statistically significant ($p > 0.05$). The development in the organic carbon content of the soil was the result of the carbon fixation by photosynthetic cyanobacteria. Recently, few studies have shown that the cyanobacteria inoculation of soil could improve the soil organic carbon. Park et al. [41] demonstrated that the organic carbon content of sandy soil increased from 2.8 g kg^{-1} to 3.1 g kg^{-1} after 12 months, in response to addition of cyanobacteria, but this

Figure 1. Map of the sampling site (Sangan Iron Ore Mine)

TABLE 1. Summary of physicochemical properties of the soil before and after treatments

Soil sample	Soil texture			C_{org}(g/kg)	N(mg/kg)	EC(µs/m)	pH
	Sand (%)	Silt (%)	Clay (%)				
CS	47	33.2	19.8	26.93	406	701	8.7
SSC				28.4	664	516	8.36
SMC				32.61	710	549	8.21

enhancement was not statistically significant. Also, Nisha et al. [31] reported that by introducing *Nostoc* sp. in saline soils for 240 days enhanced the organic carbon content up to 32-36%. Besides, Muñoz-Rojas et al. [32] stated that the cyanobacteria inoculation of mine waste substrate over 90 days resulted in a significant increase in the amount of organic carbon from 0.6 g kg^{-1} to 1.9 g kg^{-1}.

Improvement in nitrogen content of the soil
The influence of cyanobacteria inoculation on the nitrogen content of contaminated soil was measured (Table 1). The nitrogen quantity of the control sample was 406 mg kg^{-1}, which increased to 664 mg kg^{-1} in SMC and 710 mg kg^{-1} in SSC. Overall, cyanobacteria inoculation significantly led to more than 1.5 fold improvement in total Kjeldahl nitrogen (TKN). Similar results were reported by Nisha et al. [31] for *Nostoc ellipsosporum* HH-205 and *Nostoc punctiforme* HH-206 inoculation to salty soil and Chamizo et al. [24] for *Phormidium ambiguum* and *Scytonema javanicum* inoculation on different textured soils. Although nitrogen fixation is the main characteristic of heterocystous cyanobacteria, most cyanobacteria can use atmospheric nitrogen as a source of nitrogen using heterocysts, which called nitrogen fixation. Many non-heterocystous cyanobacteria such as *Oscillatoria* sp. and *Leptolyngbia biryani* can fix nitrogen by nitrogenases under anaerobic conditions [53, 54]. Nitrogenases of non-heterocystous cyanobacteria are oxygen-sensitive and activated in an anoxic condition [55].

Heavy metal removal of the contaminated soil
The influence of cyanobacteria inoculation on heavy metal availability (exchangeable fraction of elements) in soil was determined and shown in Figure 3. Compared to the control soil sample, the available concentration of Pb and Ni was decreased. However, there was no distinct effect on Fe, Cr, and As availability with cyanobacteria bioaugmentation. Figure 4 presented the Cu, Pb, As, Fe, Cr, and Ni concentrations in the contaminated soil before and after treatment. It revealed the heavy metal concentrations in soil, which was not bioavailable or easily exchangeable. In other words, it indicates the quantity of metal bounded to carbonate, Fe oxide, organic, etc. Heavy metal concentrations in SSC sample, compared to control were in decreasing order: Cr > Fe > Ni > As > Pb > Cu while in SMC sample it was Cr > As > Ni > Fe > Pb > Cu. Previous studies on the aqueous environment have shown that cyanobacteria are effective in the removal of metal ions from the environment [56–58]. This is due to the release of some extracellular polymeric

substances by cyanobacteria, which can chelate free metal ions or sequestering them in their extracellular surfaces [59].

Maximum heavy metal removal in both treatment methods was obtained for Cr. It reached 26.9 and 32 % for SSC and SMC, respectively. This result confirmed that other researches proved that cyanobacteria could tolerate and interact with chromium ions by adsorption or absorption mechanisms [60, 61]. Faisal et al. [62] have previously reported two cyanobacterial strains, *Oscillatoria* sp. and
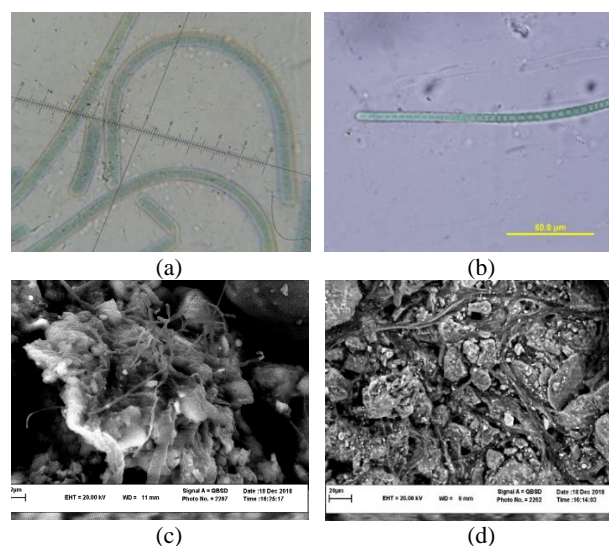
Figure 2. Morphological characterization of cyanobacteria (a) *Oscillatoria* sp., (b) *Leptolyngbya* sp., and (c and d) SEM micrographs of cyanobacteria inoculated to the soil
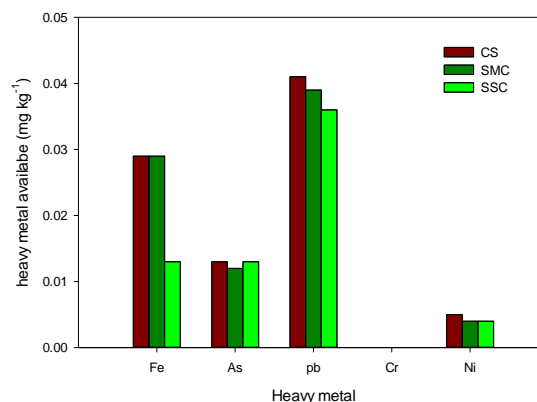
Figure 3. The concentration of available heavy metal of soil with cyanobacteria bioaugmentation (The Fe concentration was based on percent)

Synechocystis sp. reduced Cr (VI) to Cr (III). Fe ion was the second most reduced in the SSC sample. The Fe removal efficiency (15 %) of the SSC sample was significantly higher than in the SMC sample. Cyanobacteria require large quantities of the essential micronutrient iron to maintain their photosynthetic apparatus. The iron requirements of cyanobacteria are exceptionally high than other photosynthetic microorganisms [63]. The chlorophyll-a content of the SSC sample was three times that in the SMS sample, which is the reason for the higher Fe ion removal of the SSC sample. Their results indicated that a 19 % removal yield was achieved for Fe ion. Besides, in the SMC sample, As ion was achieved the second place of removal efficiency (17.11 %). This finding indicated that although arsenic is not a necessary element, cyanobacteria can bioaccumulate it. Also, bioinformatics studies have proved that most sequenced cyanobacterial genomes have arsenic resistance genes [64, 65]. Shaheen et al. [66] reported that the soil cyanobacteria had different tolerance limits for As and it depended on metal concentration. Some species like *Nostoc sp.* and *Phormidium sp.* would be able to survive at 10,000 ppm of As. Liu et al [67] investigated the bioremediation of As from contaminated soil by genetically engineered bacteria and reported the removal efficiency of 2.2 -4.5 % for 30 days. Moreover, SMC and SSC samples indicated the Ni removal of 11.1 and 11.9 % from contaminated soil, respectively. The reason is that nickel is an essential element for the growth of cyanobacteria and has an essential role in cellular physiology [68]. The quantity of Cu removal from contaminated soil was low, 2.7 % for SSC, and 0.19 % for SMC. It is referred to as copper is also a crucial micro-nutrient that is needed for the production of several cuproenzymes [68].

Bioremediation efficiency

Previous studies confirmed that lettuce and radish could be used as a biomarker for monitoring bioremediation [7, 45, 69, 70]. The roots and hypocotyls lengths and the vigor index (VI) were measured for both species, and are shown in Table 2 and Figure 5. The root length of lettuce in bioremediation soils (SSC and SMC) was developed to 3.58 cm, which was significantly longer ($p < 0.05$) than its size, 2.3 cm, in control

TABLE 2. Vigour index (VI) of lettuce and radish seedlings in the soil with different treatments

Soil Treatment	VI	
	Lettuce	Radish
CS	22.3	64.75
SSC	32.4	94.74
SMC	29.8	128.4

(a)

(b)

(c)

(d)

Figure 5. Mean root (a and b) and hypocotyl (c and d) lengths of lettuces and radish in the soil with different treatments (CS, SMC, and SSC). a and b show the probability level of p < 0.05 and p > 0.05, respectively
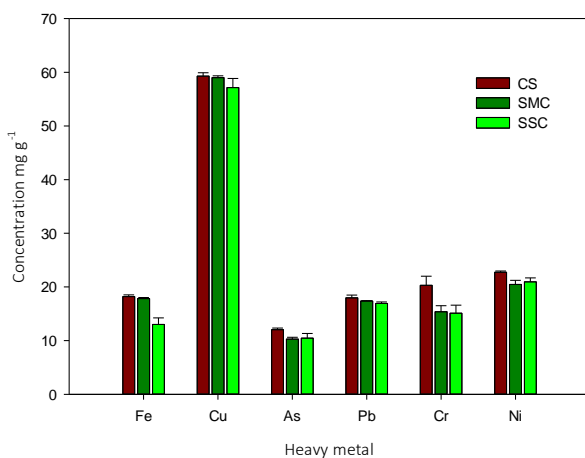

Figure 4. Heavy metal concentration in the soil with different treatments (CS, SMC, and SSC) "α" indicated that Fe concentrations are based on weight percent
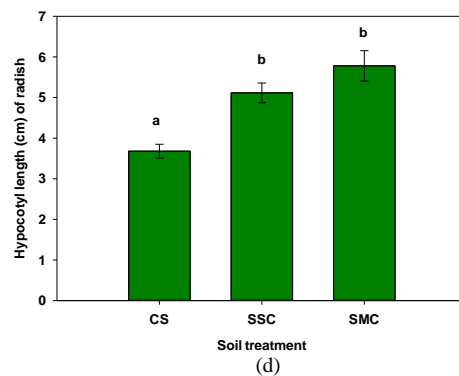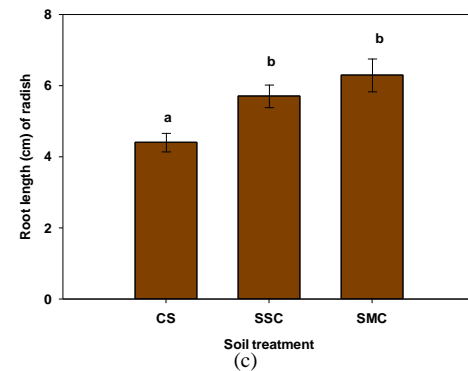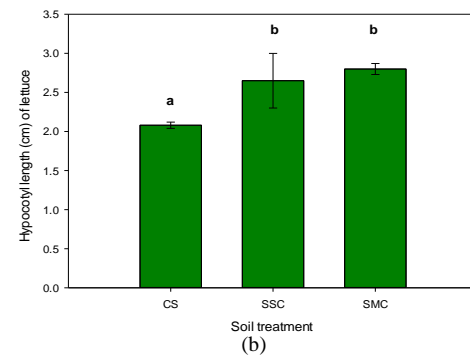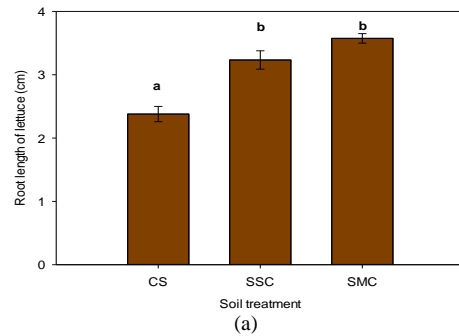
soil (CS). The root length of radish in SSC and SMC samples significantly increased ($p < 0.05$) compared to the CS sample. Also, the hypocotyl length of lettuce increased statistically ($p < 0.05$) from 1.93 cm in control soils (CS) to 2.8 cm in bioremediation soils (SSC and SMC). Hypocotyl length of radish in SSC and SMC samples improved significantly ($p < 0.05$) than the CS sample. Besides, there were no significant differences between SSC and SMC samples for root and hypocotyl lengths of both species. Besides, as shown in Table 2, the vigor index of radish and lettuce in bioremediation soils was significantly higher than the control soil.

In general, cyanobacteria inoculation into polluted soil resulted in an increase in root and hypocotyl lengths and vigor index due to high nutrient content and less heavy metal bioavailability. As mentioned above, cyanobacteria growth in the soil, nitrogen, and carbon (nutrients) contents were improved due to the photosynthesis process and heavy metal bioavailability was reduced due to complexation/chelation reaction. High availability of heavy metals such as Cr, As, Fe, etc. can lead to biochemical and physiological changes such as inhabitation of root growth and interveinal chlorosis with chlorophyll reduction [71, 72]. Similar results were reported by Aparicio et al. [45] and Mahbub et al. [7] for the bioremediation efficiency test of heavy metal contaminated soil by lettuce.

CONCLUSIONS

The utilization of metal resistant microbial for bioremediation of contaminated soil is a promising method due to being environmentally friendly, cost-effective, and, simple to implement. The present work described the utilization of indigenous cyanobacteria (*Oscillatoria* sp. and *Leptolyngbya* sp.) to remove heavy metal (Fe, Cr, As, Cu, Pb, and Ni) from the soil. By cyanobacteria inoculation into heavy metal contaminated soil, following finding was obtained: a network of filaments was developed around soil particles, pH and EC of treated soil was reduced, the organic carbon and nitrogen content of treated soils were increased, the bioavailable concentrations of Pb and Ni in treated samples were decreased, in contrast other metals such as Fe, Cr and As did not change. The vegetation of the bioremediation soils with radish and lettuce confirmed that the soil quality increased after the treatment. The roots and hypocotyls lengths, and the vigor index of both species were enhanced in soil inoculated with cyanobacteria compared to the control sample due to high nutrient content and less heavy metal bioavailability. Although cyanobacteria application is promising in soil stability and fertility improvement, this is the first application in heavy metal contaminated soil. To improve the soil nutrients such as nitrogen and carbon, and also reduce its heavy metal bioavailability, it is required to soil inoculated with cyanobacteria mat two or three times on the period of experiments. Finally, cyanobacterial inoculation of soil is in the early stage of development. Future works can be extended to plots in the field and with repeated mat inoculation on the long term basis leads to help the remediation and restoration of heavy metal contaminated soil.

NOMENCLATURE

Arsenic	As
Copper	Cu
Iron	Fe
Lead	Pb
Chromium	Cr
Nickel	Ni
Control soil	CS
Surface soil sprayed with cyanobacteria	SSC
Soil mixed with cyanobacteria	SMC
Scanning electron microscope	SEM
Sangan iron ore mine	SIOM
Total Kjeldahl nitrogen	TKN

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چکیده

خاک نواحی اطراف مجتمع معدن سنگ آهن سنگان در شرق ایران آلوده به غلظت بالایی از فلزات سنگین به ویژه آهن است. انتشار این آلاینده‌ها در محیط زیست منجر به انتقال و تجمع آن‌ها در زنجیره‌های غذایی می‌شود، از این رو یک راه حل مناسب برای احیا آن لازم است. تقویت زیستی یک راه‌حل سازگار با محیط زیست برای کاهش اثرات خطرناک فلزات سنگین در خاک آلوده است. در این مطالعه، بیومس زنده دو گونه سیانوباکتری بومی از خاک معدن سنگان جداسازی شده و بعد از کشت در آزمایشگاه، دوباره به خاک آلوده به فلزات سنگین (آهن، کروم، مس، سرب، آرسنیک و نیکل) تلقیح شده است. آزمایش‌های تلقیح با دو روش(اسپری شده بر روی سطح خاک و مخلوط شدن کامل با خاک سطحی) برای مدت زمان ۶ ماه در شرایط آزمایشگاهی انجام شد. تصاویر میکروسکوپ الکترونی روبشی نشان داد که رشته‌های سیانوباکتری‌های گونه‌های مورد مطالعه (اوسیلاتوریا و لپتولینگبیا) تلقیح شده، در اطراف ذرات خاک شبکه‌ای گستردند. همچنین تلقیح منجر به افزایش مقدار نیتروژن اولیه از ۴۰۶ میلی‌گرم در کیلوگرم در نمونه کنترل به ۶۶۴ میلی‌گرم در کیلوگرم در خاک مخلوط توسط سیانوباکتری‌ها و ۷۱۰ میلی‌گرم در کیلوگرم در خاک اسپری شده با سیانوباکتری‌ها شد. همچنین تلقیح، غلظت در دسترس فلزات سرب و نیکل در خاک را کاهش داد. بعلاوه، غلظت غیرقابل دسترس فلزات سنگین در نمونه خاک اسپری شده با سیانوباکتری‌ها با ترتیب Cu >Pb >As> Ni >Fe >Cr کاهش یافت. حداکثر راندمان حذف برای فلز کروم با مقدار ۳۲٪ بود. نتایج آزمایشات احیا آشکار کرد که طول ریشه و هیپوکوتیل گیاه تربچه و کاهو رشد یافته در خاک تلقیح شده نسبت به نمونه کنترل، افزایش یافت که نشان‌دهنده بهبود کیفیت خاک پس از تلقیح بیولوژیکی است.