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

Z. Biglari Quchan Atigh 1, A. Heidari 1*, A. Sepehr 1, M. Bahreini 2, K. R. Mahbub 3

1 Department of Environmental Science, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, Iran
2 Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
3 School of Life Sciences, University of Technology Sydney, Ultimo, Australia

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 saline 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. Chamizzo 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 algalculture 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 ×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 °C 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 Alghammi 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 bioaugmentation 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)
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 ellipso sporum HH-205 and Nostoc punctiforme HH-206 inoculation to salty soil and Chamizo et al. [24] for Phormidium ambiguum and Scytomena javanica 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 SMC 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

---

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

| Soil sample | Soil texture | C\(_{\text{org}}\)(g/kg) | N(mg/kg) | EC(µs/m) | pH |
|-------------|--------------|-------------------|---------|---------|----|
| CS          | Sand (%) 47  | Silt (%) 33.2  | Clay (%) 19.8 | 26.93 | 406 | 701 | 8.7 |
| SSC         |              |                   |          |         |     |     |     |
| SMC         |              |                   |          |         |     |     |     |
**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].

**Bioresidation 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  |

![Figure 4](https://via.placeholder.com/150)

*Figure 4. Heavy metal concentration in the soil with different treatments (CS, SMC, and SSC) “a” indicated that Fe concentrations are based on weight percent*

![Figure 5](https://via.placeholder.com/150)

*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*
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 inhibition of root growth and intereval 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 hypocotyl 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.

ACKNOWLEDGMENT

This research project was supported by Ferdowsi University of Mashhad (FUM), Mashhad, Iran (Research Grant No. 44618). The authors would like to appreciate Dr. Habibollah Younesi from Tarbiat Modarres University for his assistance in measuring heavy metals and Mr. Naser Parvian for technical assistant in an environmental laboratory.

REFERENCES

1. Liu, L., Li, W., Song, W. and Guo, M., 2018. Remediation techniques for heavy metal-contaminated soils: Principles and applicability. Science of the Total Environment. 633: 206-219.
2. Barkett, M.O. and Akin, E., 2018. Heavy metal contents of contaminated soils and ecological risk assessment in abandoned copper mine harbor in Yedidalga, Northern Cyprus. Environmental Earth Sciences, 77(10): 1-14.
3. He, Z.L., Yang, X.E. and Stoffella, P.J., 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace elements in Medicine and Biology, 19(2-3): 125-140.
4. Iqbal, N., Hayat, M.T., Zeb, B.S., Abbas, Z. and Ahmed, T., 2019. Phytoremediation of Cd-Contaminated Soil and Water. In: Cadmium Toxicity and Tolerance in Plants. Academic Press, pp. 531-543.
5. Loganathan, P., Hedley, M.J. and Grace, N.D., 2008. Pasture soils contaminated with fertilizer-derived cadmium and fluorine: livestock effects. In: Reviews of Environmental Contamination and Toxicology. Springer, New York, NY., pp. 29-66.
6. Bolan, N., Kunhi Krishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B. and Scheckel, K., 2014. Remediation of heavy metal (loid) s contaminated soils– to mobilize or to immobilize?. Journal of hazardous materials, 266: 141-166.
7. Mahbub, K.R., Krishnan, K., Andrews, S., Venter, H., Naidu, R. and Megharaj, M., 2017. Bio-augmentation and nutrient amendment decrease concentration of mercury in contaminated soil. Science of The Total Environment. 576: 303-309.
8. Bhattacharya, S., Gupta, K., Debnath, S., Ghosh, U.C., Chattopadhyay, D. and Mukhopadhyay, A., 2012. Arsenic bioaccumulation in rice and edible plants and subsequent transmission through food chain in Bengal basin: a review of the perspectives for environmental health. Toxicological & Environmental Chemistry, 94(3): 429-441.
9. Beiyyan, J., Li, J.S., Tsang, D.C., Wang, L., Poon, C.S., Li, X.D. and Fendorf, S., 2017. Fate of arsenic before and after chemical-enhanced washing of an arsenic-containing soil in Hong Kong. Science of the total environment, 599: 679-688.
10. WHO, Arsenic in Drinking-water 2011. Available from: https://www.who.int/news-room/fact-sheets/detail/arsenic
11. Kushwaha, A., Hans, N., Kumar, S. and Rani, R., 2018. A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. Ecotoxicology and environmental safety, 147: 1035-1045.

12. Zhou, Z., Huang, T., Yuan, B. and Liao, X., 2016. Remediation of nitrogen-contaminated sediment using bioreactive, thin-layer capping with biozeolite. Soil and Sediment Contamination: An International Journal, 25(1): 89-100.

13. Dassekpo, J.B.M., Ning, J. and Zha, X., 2018. Potential solidification/stabilization of clay-waste using green geopolymer remediation technologies. Process Safety and Environmental Protection, 117: 684-693.

14. Alshawabkeh, A.N., 2009. Electrokinetic soil remediation: challenges and opportunities. Separation Science and Technology, 44(10): 2171-2187.

15. Tajudin, S.A.A., Azmi, M.A.M. and Nabila, A.T.A., 2016. Stabilization/solidification remediation method for contaminated soil: a review. In IOP Conference Series: Materials Science and Engineering (Vol. 136, No. 1). IOP Publishing. doi: 10.1088/1757-899X/136/1/012043

16. Bertagnolli, C., Grishin, A., Vincent, T. and Guibal, E., 2016. Recovering heavy metal ions from complex solutions using polyethyleneimine derivatives encapsulated in alginate matrix. Industrial & Engineering Chemistry Research, 55(8): 2461-2470.

17. Guo, X., Wei, Z., Wu, Q., Li, C., Qian, T. and Zheng, W., 2016. Effect of soil washing with only chelators or combining with ferric chloride on soil heavy metal removal and phytoavailability: field experiments. Chemosphere, 147: 412-419.

18. Sasaki, L. and Muthuraman, G., 2016. Removal of heavy metals from wastewater using Trichobis terrestris herbal plants powder. Iranian (Iranica) Journal of Energy and Environment, 7(1): 39-47.

19. Deepali, A., 2011. Bioremediation of Chromium (VI) from textile industry’s effluent and contaminated soil using Pseudomonas putida. Iranian (Iranica) Journal of Energy & Environment, 2(1): 24-31.

20. Li, X., Peng, W., Jia, Y., Lu, L. and Fan, W., 2016. Bioremediation of lead contaminated soil with Rhodobacter sphaeroideus. Chemosphere, 156: 228-235.

21. Marques, A.P., Rangel, A.O. and Castro, P.M., 2011. Remediation of heavy metal contaminated soils: an overview of site remediation techniques. Critical Reviews in Environmental Science and Technology, 41(10): 879-914.

22. Wu, G., Kang, H., Zhang, X., Shao, H., Chu, L. and Ruan, C., 2010. A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. Journal of Hazardous Materials, 174(1-3): 1-8.

23. Issa, O.M., Défarge, C., Le Bissonnais, Y., Marin, B., Duval, O., Bruand, A., d’Acqui, L.P., Nordenberg, S. and Annnerman, M., 2007. Effects of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical plant. Soil and plant, 290(1-2): 209-219.

24. Chamizo, S., Mugnai, G., Rossi, F., Certini, G. and De Philippis, R., 2018. Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. Frontiers in Environmental Science, 6. https://doi.org/10.3389/fevens.2018.00049

25. Mager, D.M. and Thomas, A.D., 2011. Extracellular polysaccharides from cyanobacterial soil crusts: a review of their role in dryland soil processes. Journal of Arid Environments, 75(2): 91-97.

26. Zhao, Y., Zhu, Q., Li, P., Zhao, L., Wang, L., Zheng, X. and Ma, H., 2014. Effects of artificially cultivated biological soil crusts on soil nutrients and biological activities in the Loess Plateau. Journal of Arid Land, 6(6): 742-752.

27. Kheirfam, H., Sadeghi, S.H. and Darki, B.Z., 2020. Soil conservation in an abandoned agricultural rain-fed land through inoculation of cyanobacteria. Catena, 187. https://doi.org/10.1016/j.catena.2019.104341

28. Roncero-Ramos, B., Román, J.R., Gómez-Serrano, C., Cantón, Y. and Acín, E.G., 2019. Production of a biocrust-cyanobacteria strain (Nostoc commune) for large-scale restoration of dryland soils. Journal of Applied Phycology, 31(4): 2217-2230.

29. Chamizo, S., Adessi, A., Certini, G. and De Philippis, R., 2020. Cyanobacteria inoculation as a potential tool for stabilization of burned soils. Restoration Ecology. https://doi.org/10.1111/rec.13092

30. Kheirfam, H., 2020. Increasing soil potential for carbon sequestration using microbes from biological soil crusts. Journal of Arid Environments, 172. https://doi.org/10.1016/j.jaridenv.2019.104022

31. Nisha, R., Kiran, B., Kaushik, A. and Kaushik, C.P., 2018. Bioremediation of salt affected soils using cyanobacteria in terms of physical structure, nutrient status and microbial activity. International Journal of Environmental Science and Technology, 15(3): 571-580.

32. Muñoz-Rojas, M., Román, J.R., Roncero-Ramos, B., Erickson, T.E., Merritt, D.J., Aguila-Carrondo, P. and Cantón, Y., 2018. Cyanobacteria inoculation enhances carbon sequestration in soil substrates used in dryland restoration. Science of the Total Environnement, 636: 1149-1154.

33. Tiwari, S., Parihar, P., Patel, A., Singh, R. and Prasad, S.M., 2019. Metals in Cyanobacteria: Physiological and Molecular Regulation. In: Cyanobacteria. Academic Press. pp. 261-276.

34. Dabiri, R., Bakhshti Mazdeh, M. and Mollai, H., 2017. Heavy metal pollution and identification of their sources in soil over Sangan iron mining region, NE Iran. Journal of Mining and Environment, 8(2): 277-289.

35. Kaushik, B.D., 1987. Laboratory methods for blue-green algae. Associated Publishing Company.

36. Sepehr, A., Hassanzadeh, M. and Rodriguez-Caballero, E., 2019. The protective role of cyanobacteria on soil stability in two Aridisols in northeastern Iran. Geoderma Regional, 16. https://doi.org/10.1016/j.geoder.2018.e00201

37. Skinner, S. and Entwistle, T.J., 2001. Non-marine algae of Australia: 1. Survey of colonial gelatinous blue-green macroalgae (Cyanobacteria). Telopea, 9(3): 573-599.

38. Anagnostidis, K. and Komarek, J., 2005. Cyanoprokariota. Teil 2: Oscillatoriales. Band 19/2 Spektrum Akademischer Verlag Heidelberg.

39. Sant’Anna, C.L., Azevedo, M.D.P., Fiore, M.F., Lorenzi, A.S., Kaltsovský, J. and Komárek, J., 2011. Subgeneric diversity of Brasilionema (cyanobacteria, Syctonemataceae). Brazilian Journal of Botany, 34(1): 51-62.

40. Wang, Q., Li, Y. and Wang, Y., 2011. Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources. Environmental Monitoring and Assessment, 174(1-4): 241-257.

41. Park, C.H., Li, X.R., Zhao, Y., Ia, R.L. and Hur, J.S., 2017. Rapid development of cyanobacterial crust in the field for combating desertification. PLoS One, 12(6). https://doi.org/10.1371/journal.pone.0179903

42. Ritchie, R.J., 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynthesis Research, 89(1): 27-41.

43. Alghammri, S.I., Al Sulami, A.F., El-Zayat, T.A., Alhoghi, B.G. and Salam, M.A., 2015. Acid leaching of heavy metals from contaminated soil collected from Jeddah, Saudi Arabia: kinetic and thermodynamics studies. International Soil and Water Conservation Research, 3(3): 196-208.

44. Turan, V., Khan, S.A., Iqbal, M., Ramzani, P.M.A. and Fatima, M., 2018. Promoting the productivity and quality of brinjal aligned with heavy metals immobilization in a wastewater irrigated heavy metal polluted soil with biochar and chitosan. Ecotoxicology and environmental safety, 161: 409-419.

45. Aparicio, J., Solá, M.Z.S., Benimeli, C.S., Amoroso, M.I. and Politi, M.A., 2015. Versatility of Streptomyces sp. M7 to bioremediate soils co-contaminated with Cr (VI) and lindane. Ecotoxicology and Environmental Safety, 116: 34-39.

46. Fuentes, M.S., Álvarez, A., Sáez, J.M., Benimeli, C.S. and Amoroso, M.J., 2014. Methoxychlor bioremediation by defined consortium of environmental Streptomyces strains. International Journal of Environmental Science and Technology, 11(4): 1147-1156.

47. Saez, J.M., Alvarez, A., Benimeli, C.S. and Amoroso, M.J., 2014. Enhanced lindane removal from soil slurry by immobilized Streptomyces consortium. International Biodeterioration & Biodegradation, 93: 63-69.

48. Singh, R.N., 1961. Role of blue-green algae in nitrogen economy of Indian agriculture. Indian Council of Agricultural Research, New Delhi.
50. Pade, N. and Hagemann, M., 2015. Salt acclimation of cyanobacteria and their application in biotechnology. Life, 5(1): 25-49.

51. Bergi, J. and Trivedi, R., 2020. Bioremediation of Saline Soil by Cyanobacteria. In: Microbial Bioremediation & Biodegradation. Springer, Singapore. pp. 447-465.

52. Sithrer, V. and Tabatabai, B., Morgan University, 2020. Engineered cyanobacteria with enhanced salt tolerance. U.S. Patent 10,626,363.

53. Tsuchimoto, R., Kamiya, N. and Fujita, Y., 2014. Transcriptional regulators ChfR and CuR are essential for diazotrophic growth in nonheterocystous cyanobacteria. Proceedings of the National Academy of Sciences, 111(18): 6762-6767.

54. Stal, L.J. and Heyer, H., 1987. Dark anaerobic nitrogen fixation (acetylene reduction) in the cyanobacterium Oscillatoria sp. FEMS Microbiology Ecology, 3(4): 227-232.

55. Stal, L.J., Paerl, H.W., Bebout, B. and Villbrandt, M., 1994. Heterocystous versus non-heterocystous cyanobacteria in microbial mats. In Microbial Mats. Springer, Berlin, Heidelberg. pp. 403-414.

56. El-Enany, A.E. and Issa, A.A., 2000. Cyanobacteria as a biosorbent of heavy metals in sewage water. Environmental toxicology and pharmacology, 8(2): 95-101.

57. Vanhoudt, N., Vandenhove, H., Leys, N. and Janssen, P., 2018. Potential of higher plants, algae, and cyanobacteria for remediation of radioactively contaminated waters. Chemosphere, 207: 239-254.

58. De Philippis, R., Colica, G. and Micheletti, E., 2011. Exopoly saccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. Applied Microbiology and Biotechnology, 92(4): 697-708.

59. Pereira, S., Micheletti, E., Zille, A., Santos, A., Moradas, P. and De Philippis, R., 2011. Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell?. Microbiology, 157(2): 451-458.

60. Shakhla, D., Vankar, P.S. and Srivastava, S.K., 2012. Bioremediation of hexavalent chromium by a cyanobacterial mat. Applied Water Science, 2(4): 245-251.

61. Shashirekhva, V., Sridharan, M.R. and Swamy, M., 2015. Biochemical response of cyanobacterial species to trivalent selenium stress. Algal Research, 12: 421-430.

62. Faisal, M., Hameed, A. and Hasnain, S., 2005. Chromium-resistant bacteria and cyanobacteria: impact on Cr (VI) reduction potential and plant growth. Journal of Industrial Microbiology and Biotechnology, 32(11-12): 615-621.

63. Kranzler, C., Rudolf, M., Keren, N. and Schleiff, E., 2013. Iron in cyanobacteria. In Advances in Botanical Research (Vol. 65). Press. pp. 57-114.

64. Scanlan, D.J., Ostrowski, M., Mazar, S., Dufresne, A., Garczarek, L., Hess, W.R., Post, A.F., Hagemann, M., Paulsen, I. and Partensky, F., 2009. Ecological genomics of marine picocyanobacteria. Microbiology and Molecular Biology Reviews, 73(2): 249-299.

65. López-Maury, L., Sánchez-Riego, A.M., Reyes, J.C. and Florence, F.J., 2009. The glutathione/glutaredoxin system is essential for arsenate reduction in Synechocystis sp. strain PCC 6803. Journal of Bacteriology, 191(11): 3534-3543.

66. Shahrein, R.I.F.F.A.T., Mahmud, R. and Sen, J., 2007. A study on arsenic decontaminating cyanobacteria of an arsenic affected soil. Journal of Soil and Nature, 1(2): 23-29.

67. Liu, S., Zhang, F., Chen, J. and Sun, G., 2011. Arsenic removal from contaminated soil via bio volatilization by genetically engineered bacteria under laboratory conditions. Journal of Environmental Sciences, 23(9): 1544-1550.

68. Huertas, M.J., López-Maury, L., Giner-Llamia, J., Sánchez-Riego, A.M. and Florence, F.J., 2014. Metals in cyanobacteria: analysis of the copper, nickel, cobalt and arsenic homeostasis mechanisms. Life, 4(4): 865-886.

69. Chen, J., Dong, J., Chang, J., Guo, T., Yang, Q., Jia, W. and Shen, S., 2018. Characterization of an Hg (II)-volatilizing Pseudomonas strain, DC-B1, and its potential for soil remediation when combined with biochar amendment. Ecotoxicology and environmental safety, 163: 172-179.

70. Wang, T., Sun, H., Mao, H., Zhang, Y., Wang, C., Zhang, Z., Wang, B. and Sun, L., 2014. The immobilization of heavy metals in soil by biogeneration of a UV-mutant Bacillus subtilis 38 assisted by NovoGro bio stimulation and changes of soil microbial community. Journal of hazardous materials, 278: 483-490.

71. Sharma, D.C., Sharma, C.P. and Tripathi, R.D., 2003. Phytotoxic lesions of chromium in maize. Chemosphere, 51(1): 63-68.

72. Polti, M.A., Atjián, M.C., Amoroso, M.J. and Abate, C.M., 2011. Soil chromium bioremediation: synergistic activity of actinobacteria and plants. International Biodeterioration & Biodegradation, 65(8): 1175-1181.