Gold Bioaccumulation by Filamentous Soil Cyanobacteria Isolated From Mouteh Goldmine (Isfahan Province, Iran)

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Abstract

This study was aimed to investigate the possible exploitation of phototrophic microorganisms for the oxidation of Au^{3+} containing composites as well as recovery and bioaccumulation of Au^{3+} from Mouteh goldmine soil samples. Mechanisms of accumulation including oxidation, dissolution, reduction, leaching and sorption were investigated. After 3 weeks of cyanobacterial growth, gold bioaccumulation from Mouteh gold mine soil solution were determined. Then biomass digested with acidic solutions and subsequently gold amounts were analyzed with Atomic Absorption Spectroscopy. Soil sample with 2 ppm Au^{3+} containing composites ore from Mouteh goldmine were collected. At the end of experiments, the concentration of gold in biomass was almost 6.5 ppm. Results indicated that two soil cyanobacteria species from Mouteh goldmine could bind gold in gold bio-mining process. Bioaccumulation percent was 32% and 30% for *tenue* Gomont and *Osillatoria tenuis* C. Agardh ex Gomont respectively. Cyanobacterial gold accumulation were determined 6.5 gr/kg biomass dried weight.

Keywords: Bio-accumulation, Bio-mining, cyanobacteria, *Phormidium tenue, Osillatoria tenuis*, Mouteh Goldmine

Introduction

The cyanobacteria or blue-green algae are an ancient group of prokaryotic organisms that are found in different environments and are some times in environments that no other vegetation can live in. Their long evolutionary history is considered a reason for the success living of cyanobacteria in different habitats and their wide ecological tolerance. Nowadays cyanobacteria are found in freshwater, marine and terrestrial environments. Cyanobacteria are able to survive at temperatures ranging 45-70 ºC (Castenholz, 1978) and pH lower than 4-5 (Pfennig, 1969, 1974) with optimum range of 7.5-10 (Fogg, 1956).

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Microorganisms such as fungi, yeasts, bacteria and cyanobacteria have been known to have the ability to uptake metal via a process known as bio-mineralization (Dissook et al., 2013).

Bio-mining is now applicable issue and NASA scientific and technical information program, investigated biomining on Earth. It shows that bio-mining is a significant subject. Even NASA is going to investigating bio-mining on Mars and Moon now. Bioleaching of sulfide minerals now represents the largest-scale commercial bioprocessing worldwide. Types of bioleaching in practice (by their industrial names) include dump leaching, heap leaching of copper and gold, biooxidation processing of gold concentrates and BacTech (Bacterial oxidation Technology) moderate thermophile leaching of gold concentrate (Dalton, 2008). NASA concentrated on development of terrestrial bioleaching and mineral biooxidation Processes. Common characteristics of biohydrometallurgy, the industrial application of biomining are that it is water based, it is an aerobic microbial oxidation of iron and sulfur with ferric iron as the prime oxidant of minerals, and generally involves sulfide minerals.

The biggest applications of biomining are currently in mining of precious metals in South Africa, Brazil, Chile, Peru, Australia and China. Industrial methods now include heap bioreactors, heap and dump reactors, and run-of-mine heap reactors. In operations such as the Escondida mine in Chile, as much as 180,000 to 245,000 tons of copper are recovered per annum and 1.56 billion tons of copper reserves remain. The Escondida operators estimate that in next 25 years they will be able to recover 64 million tons per annum. At all these sites, appropriate aeration for microbial growth has been essential whether in a heap process or a tank process with inoculum. The Newmont Mining Corporation using a trade name BIOPRO (bio-oxidation pretreatment) has recovered 12,172 kg of gold over a five-years period from 2000-2005. NASA introduced the Candidate microorganisms like *Anabaena* and other cyanobacteria were suggested with valuable characteristic tolerances, *Anabaena* tolerates low temperature (Dalton, 2008).

The capability of cyanobacteria to remove heavy metals from water solutions is well known (De Philippis et al., 2011) but only a limited number of studies is available on the use of cyanobacteria for the biosorption of Au^{3+} from water solutions (Das, 2010; Syed, 2012).

By uptaking the dissolved toxic metal ion and accumulate it as solid form in their cells (Lamaia et al., 2005; Campbell and Martin, 1990), cyanobacteria can reduce the metal ion stress from the environment. Common applications that take advantage of this process include bioremediation where microorganisms are used to accumulate heavy metal found in contaminated sites. Another emerging application is in precious metal recovery where microorganisms are used to recollect expensive metal such as gold that have been dissolved during the treatment process (Dissook et al., 2013).

Some microorganisms isolated from

gold-bearing deposits are capable dissolving gold; dissolution was aided by presence of aspartic acid, histidine, serine, alanine, glycine and metal oxidants. Bacteriform gold is well known uptaking Au^{3+} from chloride solutions which was documented for at least seven freshwater cyanobacteria genera. Some bacteriform gold is biogenic may be useful indicator of gold deposits and of processes gold accumulation (Dyer et al., 1994). Bio-sorbent material rich in exopolysaccharides from the acid mine drainage site bound Au^{3+} three times more effectively than did other materials and removed 100% Au^{3+} from solutions. Algal cell alive or dead, rapidly accumulated Au^{3+} and begin to reduce it to Au^0 and Au^+ within 2 days (Robinson et al., 1997). For understanding the mechanisms of bioaccumulation by cyanobacteria, which include oxidation, reduction, dissolution, leaching and sorption, additional research efforts should be conducted all around the world. In determining the abilities of microorganisms to remove gold ions from different solutions, a number of investigators have shown that Au^{3+} ions bind to cell walls of bacteria, fungi, and algae. A number of studies have focused on gold recovery and mining processes by algae and micro-organisms (Kuyucak and Volesky, 1989; Mack et al., 2007; Mata et al., 2008; Niu and Volesky, 2000; Tsuruta, 2004; Lin et al., 2005; Das, 2010), but among them only few reports documented the biosorption of gold ion with cyanobacteria (Savvaidis, 1998; Lengke et al., 2006; Chakraborty et al., 2008).

Kamennaya et al. (2012) showed that, many cyanobacteria, especially those forming colonies or biofilms, can excrete organic polymeric substances to form extracellular formations e.g., sheaths or capsules. The function of these exopolymeric substances (EPS) may be to allow association of cells, facilitate gliding, support uptake of micronutrients and absorb heavy metals from the solution (Sutherland, 2001; Nicolaus et al., 1999). Also gold biosorption by exopolysaccharide producing cyanobacteria was investigated by Colica et al. (2012). To the best of the author’s knowledge no research has yet been carried out to confirm use of cyanobacteria from goldmine soils in Iran and all over the world, despite their important role in gold production. Cyanobacteria can be used instead of sodium cyanide and activated carbon in gold processing. The main aims of this research are the identification of cyanobacteria, dissolution of gold by cyanobacteria, and finally bioaccumulation assumption of gold in cyanobacterial biomass.

Material and Methods

Sampling

The Mouteh Gold Mine, located 270 km to the south-west of Tehran, and 10 km north-west of Mouteh village, is the main gold producer in Iran, with an annual production of 300 kg Au^{3+}. Mineralogical analysis of the ore shows that the ore is refractory and contains free gold and gold telluride associated with pyrite (and chalcopyrite). Gold grains are very small, mostly 5 μm, and are seldom larger than 15 μm in diameter (Dehghani et al., 2009). In this study soil samples were collected from Mouteh Goldmine Soil during September 2018 (Table 1).

Culture and isolation

The collected soil samples were cultured in BG11 medium and incubated at 28 ± 2° C with continuous illumination at 60 μmol photon $m^{-2}s^{-1}$ (Rippka et al., 1979). After isolation, samples were purified by several subcultures on solid BG11 medium.

Morphological identification

Identification of samples was carried out based on accepted criteria (Prescott, 1962; Desikachary, 1959; Komarek and Anagnostidis, 2005). Taxonomic determination was carried out by light microscopy (400–1,000×) and photographs were taken with a Canon camera 1200D. Axenic condition was checked daily by microscopic observation. the variable vegetative and reproductive characteristics used in the taxonomic determination were: presence or absence of a sheath, the appearance of the filament and colour of cells, cell size and constriction at the cross walls, cellular granulation and cell shape.

Metal solution utilizing

For the first set of experiments and screening of the strains, gold pure solution (3.5 ppm) were utilized. For the second set of experiment, Mouteh goldmine soil containing (2 ppm) Au^{3+} in 1000 Litter culture medium was used.

Inocoulation of cyanobacteria

300 gr soils inoculated with 0.5 gr *Phormidium tenue* and *Oscillatoria tenuis* separately (Seiderer et al., 2017). The physico-chemical characteristics of the soil, before and after the inoculation of soil were determined during two weeks.

Recovery of Au^{3+} from the biomass

Au^{3+} bioaccumulation was investigated using blue-green algae biomass ash. After an appropriate time contact with the Au^{3+} containing soil, the biomass was recovered by centrifugation (500 g for 20 min at 20 °C) and the pellet was incinerated in oven at 200 °C for 1 hour (Fig. 1).

The ashes obtained at the end of experiment was solved in nitric acid and chloridric acid (3:1) in high temperature and stiring. After acid digest, the sample filtered in order to determinate amount of gold in solution samples, by Atomic absorption spectrometry (AAS) (Colica et al., 2012).

Data analysis methods

The concentration of metals present in solution before and after of inoculation and the cyanobacteria biomass were determined by an Atomic Adsorption Spectrophotometer (AAS) (Analyst 400; Perkin Elmer, Waltham, MA, USA).

The soil samples and two cyanobacteria were

Table 1. Gold mine geographical data.

Location	Latitude/Longitude
Chah-Khatun, Mouteh gold complex	50° 45' N, 33° 34' E

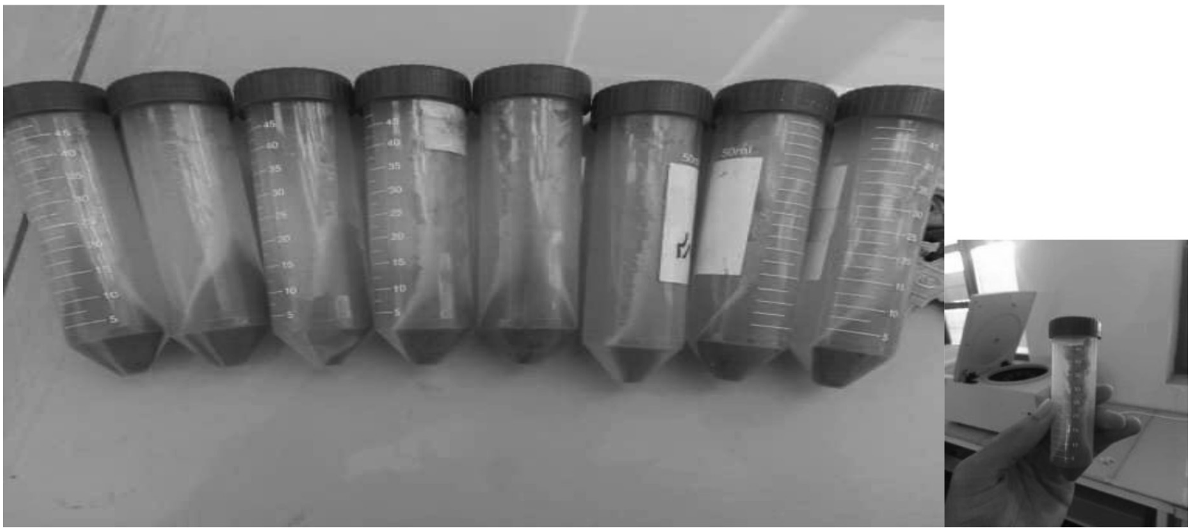

Fig. 1. Centrifuged samples and the separated cyanobacterial mat.

processed for analysis of Au^{3+} contents. The metal uptake at the below equilibrium (qe), expressed as miligram of metal removed per gram of dry biomass, was calculated as:

$$q_e = (c_i - c_f) / m$$

Where q_e is mg of metal removed per gram of dry biomass,V is the sample volume (l), Ci and Cf are the initial and final metal concentrations (mg/l), respectively, and m is the amount of dry biomass (g) utilized (Volesky and May-Phillips, 1995). In the experiments, C_i was calculated taking in consideration the metal removal observed in controls carried out without the biomass.

Statistical analysis

The treatments of this research was *Phormidium, Oscillatoria* and gold mine soil solution as control. Data are the means and standard deviation of at least three replicates. Data were analyzed using one way analysis of variance via SPSS (ver. 18). Mean values were compared using Duncan multiple comparison procedure.

Results

Isolation and identification of algae

Cyanobacteria *Phormidium tenue* Gomont, *Osillatoria tenuis* C.Agardh ex Gomont, *Lyngbya aestuarii* Liebman ex Gomontand green algae, *Scenedesmus obliquus* (Turpin) Kützing were identified (Fig. 2).

Determination of Au^{3+} bioaccumulation performances of selected cyanobacteria

In a preliminary screening, four cyanobacteria were cultivated on Mouteh gold-mine soil cyanobacteria for their metal uptake capability (Fig. 3). Two cyanobacteria *Phormidium* and *Oscillatoria* was produced enough biomass on soil culture, and then selected for gold bioaccumulation test and their effects were compared to control. Analysis of variance indicated that treatments used in current experiment had a significant effect ($P < 0.01$) on amount of gold bioaccumulation (Table 2).

Based on the data presented in Figure 4 both cyanobacteria increase the bioaccumulation

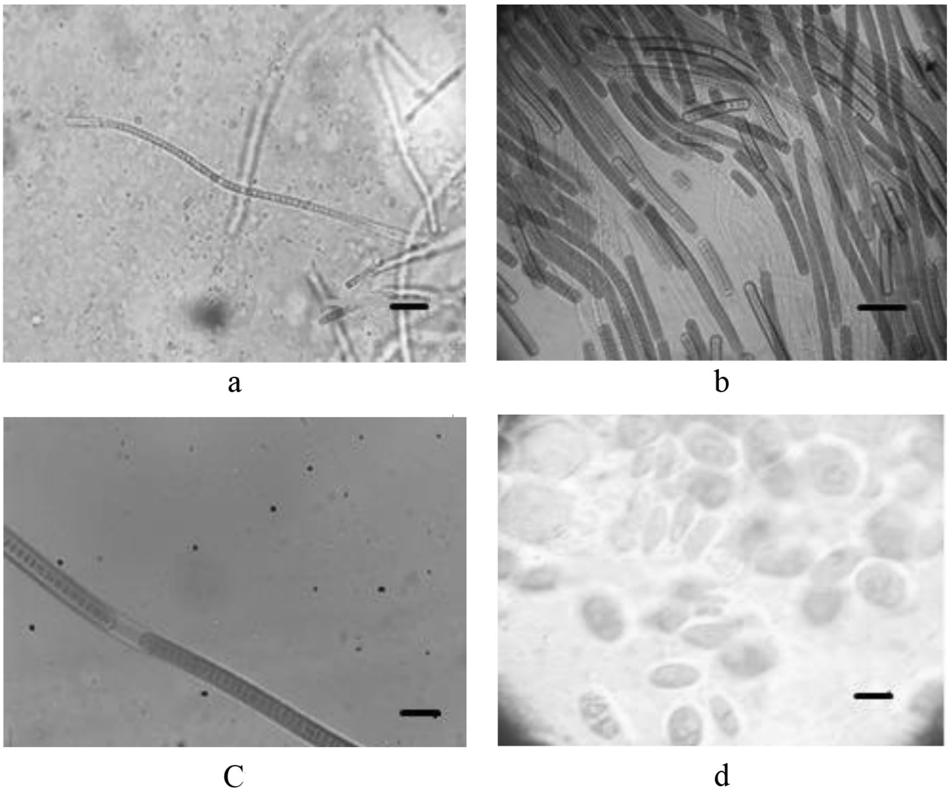

Fig. 2. Cyanobacterial species and the green algae from Mouteh Goldmine soil samples. a. *Phormidium tenue*; b. *Lyngbya aestuarii*; c. *Osillatoria tenuis*; d. *Scenedesmus obliquus* (Bar = 0.02 mm).

Table 2. Analysis of variance of the effect of cyanobacteria on gold bioaccumulation of Mouteh gold-mine soil.

Source of variation	Sum of Squares	df	Mean of squares	significant
Treatment	42.652	2	21.326	39.322**
Error	3.254	6	.542	
Total	45.906	8		

** significant at 1% level, ($P < 0.01$)

of gold when compare to control. The mean of bioaccumulation was 1.59, 6.4 and 6 ppm for control, *Phormidium tenue* and *Oscillatoria tenuis*, respectively. According to our results bioaccumulation efficiency of *Phormidium tenue* and *Oscillatoria tenuis* was determined about 32 and 30, respectively (Table 3). So *Oscillatoria tenuis* and *Phormidium tenue* with high gold bioaccumulation could play an important role as biomonitoring agents. Also these algal species can use instead sodium cyanide and activated carbon in gold factory (Table 3).

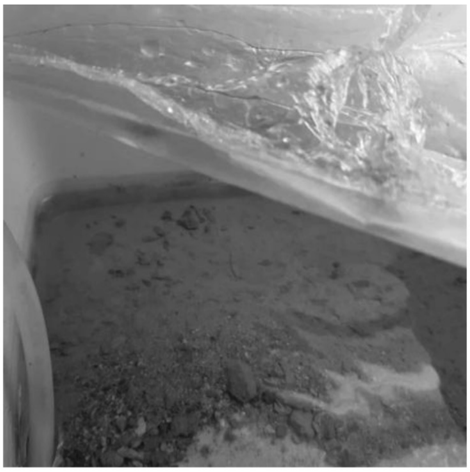

Fig. 3. The cyanobacterial growth on Mouteh gold mine soil, after 2 weeks.

Table 3. Descriptive statistics of different treatments used in experiment

Gold containing soil/Au^{3+} content	Mean (ppm)	Efficiency of Au^{3+} biorecovery
Control	1.595±0.20	-
Phormidium tenue	6.4±1.25	32%
Oscillatoria tenuis	6± 0.15	30%
Total	4.6650	

Discussion

Cyanobacteria commonly known as blue-green-algae, are not truly eukaryotic algae. They are Gram-negative prokaryotes, perform oxygenic photosynthesis, and also fix atmospheric N_2. They are ubiquitous in ponds, lakes, water streams, rivers, and wetlands. They can easily survive the extreme environments such as hot springs, hyper-saline waters, freezing environments, and arid deserts (Singh, 2014). Bioleaching as one of the important part of metal bioaccumulation by cyanobacteria has been implemented as an efficient and low-cost method to extract metals such as copper, cobalt, and gold from sulphide or iron containing ores and mineral concentrates in a number of countries around the globe. Bioleaching is an innovative way to recover minerals from ores using relatively low-capital-cost and non-polluting technology. Although some minerals such as gold are inert to biological reactions, they can be liberated using bacteria-cyanobacteria consortium that acts on certain types of ores and other minerals that co-occur with these minerals. The presence of a large set of genome sequence data covering diverse species of cyanobacteria makes them attractive targets to screen for gold biomineralization potential. Dissook et al. (2013) was founded that the potential candidate of cyanobacteria

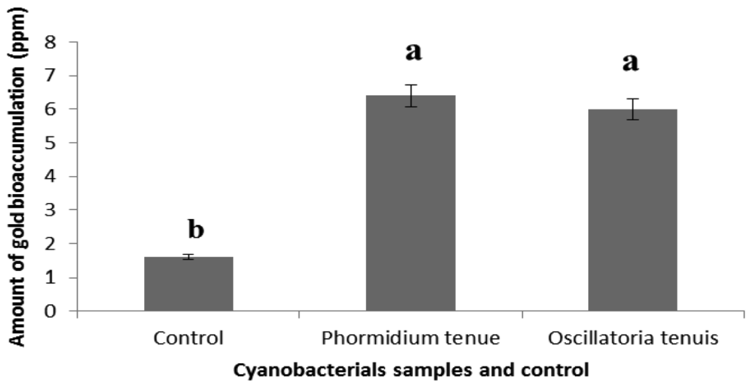

Fig. 4. Effect of Mouteh gold-mine soil treatments on gold bioaccumulation. (mean that at least one letter in common, no significant difference Duncan test at 5% level).

for gold biomineralization are *Chloroflexus*, *Anabaena*, *Calothrix*, *Chamaesiphon*, *Nostoc* and *Rivularia*. The cyanobacteria that don't appear to be good candidates for gold biomineralization are *Synechococcus* and *Cyanothece* genus. Barnett et al. (2012) investigated mining genomes of marine cyanobacteria, biological and chemical co-evolution and metal requirements.

It has been hypothesized that metal ion bioavailability presented an evolutionary selection pressure on the "choice" of metals within metalloenzymes (Williams and Da Silva, 2003). Conversely, biological evolution and the emergence of life has changed the chemical composition, or more precisely, the speciation of the atmosphere, the lithosphere and of course the hydrosphere. Probably, cyanobacteria might be deemed responsible for the greatest change of all by inventing oxygenic photosynthesis (Raymond and Blankenship, 2004). Consequently, they were amongst the first organisms that encountered, and had to cope with, the changes in the chemical composition of their environment that oxygenation brought about (Cavet et al., 2003). Thus, the "co-evolution of biology and chemistry" is imprinted on both the metallome and the metalloproteome. In the case of cyanobacteria, metal ion requirements and sensitivities, as far as they have been experimentally determined, agree with the notion that they evolved in an environment with metal ion concentrations typical of a sulfidic or a ferrous ocean (Saito et al., 2003).

Hokmollahi et al. (2015) studied Yazd province terrestrial ecosystems and observed the positive correlation between metal contents in soil samples and cyanobacterial species which indicated these algal species were able to absorb metals from soil and play an important role as biomonitoring agents. In their research *Oscillatoria tenuis* with high biosorption capacity of metals (77.33%), which exist in the station along with high amounts of Pb^{2+} could play a key role as biomonitoring agent and also demonstrated an evolutionary selection pressure on the "choice" of metals by cyanobacteria so that, biological and chemical co-evolution, and cyanobacterial metal requirements is acceptable hypothesize. Darnall et al.)1986 (investigation different metal ions bounding by *Chlorella vulgaris*. *Chlorella* has an unusually high affinity for the binding of Au^{3+}, Ag^{+} and Hg^{2+}, gold appears to be the moist strongly bound of all. In fact, over 90% of the gold from a solution containing as little as gold (II1) can be removed by *C. vulgaris* cells (at a concentration of 5 mg/ml). This process may also be useful in wastewater treatment process and the metal ions can be easily absorbed. Gold biosorption by exopolysaccharide producing cyanobacteria by Colica et al. (2012) demonstrated that in a preliminary screening, four cyanobacteria were tested for their metal uptake capability using pure solutions of and of Cu, Ni and Zn, the three metal contaminants most frequently found in Au^{3+} wastewaters, the highest Au^{3+} uptake was observed by *Cyanothece* sp. CE4 which showed q values of 318 ± 42 mg/g. Primary Au^{3+} containing solution amount was 2828 ppm, which efficiency of Au^{3+} biorecovery is 16 percent. This research show that the efficiency of Au^{3+} biorecovery of gold by *Cyanothece* CE4 is half of present research, and two cyanobacterial species from mouteh goldmine soil samples are useful for gold bio-mining. These two cyanobacterium bioaccumulate almost 32% of gold from 2 ppm mouteh goldmine soil solution samples and Au^{3+} uptake showed q values of 6.5 g/kg. Eisler (2003) investigated recovery of gold from a wide variety of solutions by selected species of bacteria, yeast, fungi, algae and higher plants. Gold accumulation by *spirulina platensis* was 7 gr/kg biomass dried weight. So the data of preset research is almost equal and recovery of gold by *Phormidium tenue* was 32% and this species accumulated 6.5 g/kg gold in dried weight.

In conclusion, this study showed the good potential of some EPS-producing cyanobacteria for the biooxidation of Au^{3+}-containing deposits and bio-recovery and bio-accumulation of Au^{3+} from the Mouteh gold-mine soil samples, but also pointed out further investigation is needed to design an efficient technology for metal recovery from the cyanobacterial biomass.

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References

Barnett JP, Millard A, Ksibe AZ, Scanlan DJ, Schmid R, Blindauer CA. (2012). Mining genomes of marine cyanobacteria for elements of zinchome- ostasis. Frontiers in Microbiology. 3: 142.

Campbell lR and Martin MH. (1990). Continuous flow fermentation to purify wastewater by the removal of cadmium. Water Air and Soil Pollution. 37: 394-403.

Castenholz RW. (1978). The biogeography of hot spring algal through enrichment cultures. Internationale Vereinigung für Theoretische und Angewandte Limnologie: Mitteilungen. 21: 296-315.

Cavet JS, Borrelly GPM, Robin- son NJ. (2003). Zn, Cu and Co in cyanobacteria: selective control of metal availability. Federation of European Microbiological Societies Microbiology Reviews. 27: 165-181.

Chakraborty N, Banerjee A, Lahiri S, Panda A, Ghosh AN, Pal R. (2008). Biorecovery of gold using cyanobacteria and an eukaryotic alga with special reference to nanogold formation – a novel phenomenon. Journal of Applied Phycology. 21: 145-152.

Colica G, Caparrotta S, Bertini G, De Phillipis R. (2012). Gold biosorption by exopolysaccharide producing cyanobacteria and purple nonsulphur bacteria. Journal of Applied Microbiology. 113: 1380-1388.

Dalton B.P. (2008). Lunar Regolith Biomining Workshop Report. NASA Ames Research Center. 32 pp.

Darnall DW, Greene B, Mlchael T, Henzl J, Michael H, Robert A, Pherson Mc., Sneddon J, Alexander MD. (1986). Selective Recovery of Gold and Other Metal Ions from an Algal Biomass. Environmental Science and Technology. 20: 206-208.

Das N. (2010). Recovery of precious metals through biosorption– a review. Hydrometallurgy. 103: 180-189.

Dehghani A, Ostad-rahimi M, Mojtahedzadeh SH, Gharibi KhK. (2009). Journal of the Southern African Institute of Mining and Metallurgy. 10: 417-421.

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

Desikachary TV. (1959). Cyanophyta. Indian Council of Agricultural Research, New Delhi, 686 pp.

Dissook S, Anekthanakul A, Kittichotirat W. (2013). Screening of gold biomineralization mechanism in cyanobacteria. Procedia Computer Science. 23: 129-136.

Dyer BD, Krumbein WE, Mossman DJ. (1994). Accumulation of gold in the sheath of Plectonema terebrans (filamentous marine cyanobacteria). Geomicrobiology Journal. 12: 91-98.

Eisler R. (2003). Biorecovery of gold. Indian Journal of Experimental Biology. 41: 967-971.

Fogg GE. (1956). The comparative physiology and biochemistry of the blue-green algae. Bacteriological Reveiews. 20: 148-165.

Hokmollahi F, Riahi H, Soltani N, Shariatmadari Z, Hakimi-Meybodi MH. (2015). A taxonomic study of blue-green algae based on morphological, physiological and molecular characterization in Yazd province terrestrial ecosystems (Iran). Rostaniha. 16 (2): 152-163.

Kamennaya NA, MAjo-Franklin C, Trent N, Christer J. (2012). Cyanobacteria as Biocatalysts for Carbonate Mineralization. Minerals. 2: 338-364.

Komarek J and Anagnostidis K. (2005). Cyanoprokaryota 2. Teil/2nd Part: Oscillatoriales. p.

759.

Kuyucak N and Volesky B. (1989). The mechanism of gold biosorption. Biorecovery. 1: 219-235.

Lamaia C, Kruatrachuea M, Pokethitiyooka P, Upathamb ES, Soonthornsarathoola V. (2005). Toxicity and accumulation of lead and cadmium in the filamentous green alga *Cladophora fracta* (O.F. Muller ex Vahl) Kutzing: A laboratory study. Science Asia. 31: 121-127.

Lengke MF, Ravel B, Fleet ME, Southam G. (2006). Mechanisms of Gold Bioaccumulation by Filamentous Cyanobacteria from Gold (III)−Chloride Complex. Environmental Science and Technology. 40 (20): 6304-9.

Lin Z, Wu J, Xue R, Yang Y. (2005). Spectroscopic characterization of Au^{3+} biosorption by waste biomass of *Saccharomyces cerevisiae*. Spectrochim Acta. 61: 761-765.

Mack C, Wilhelmi B, Duncan JR, Burgess JE. (2007). Biosorption of precious metals. Biotechnology Advances. 25: 264-271.

Mata YN, Torres E, Bla´zquez ML, Ballester A, Gonza´lez F, Mun˜oz JA. (2008). Gold (III) biosorption and bioreduction with the brown alga *Fucus vesiculosus*. Journal of Hazardous Materials. 166: 612-618.

Nicolaus B, Panico A, Lama L, Romano I, Manca MC, de Giulio A, Gambacorta A. (1999). Chemical composition and production of exopolysaccharides from representative members of heterocystous and non-heterocystous cyanobacteria. Phytochemistry. 52: 639-64.

Niu H and Volesky B. (2000). Gold-cyanide biosorption with L-cycsteine. Journal of Chemical Technology & Biotechnology. 75: 436-442.

Pfennig N. (1969). *Rhodopseudomonas acidophila*, sp. nov. a new species of the budding purple nonsulfur bacteria. Journal of Bacteriology. 99: 597-602.

Pfennig N. (1974). *Rhodopseudomonas globformis*, sp. nov. a new species of the Rhodospirillaceae. Archives of Microbiology. 100: 197-206.

Prescott GW. (1962). Algae of western great lakes area. WMC. Brown Company Publishing, Iowa, USA. p. 933.

Raymond J and Blankenship RE. (2004). Biosynthetic pathways, gene replacement and the antiquity of life. Geobiology. 2: 199-203.

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. (1979). Generic assignments, strain histories and properties of pure culture of cyanobacteria. Journal of General Microbiology. 111: 1-61.

Robinson MG, Brown LN, Hall BD. (1997). Effect of gold (Ш) on the fouling diatom *Amphora coffeiformis*; Uptade, toxicity and intractions with copper. Biofouling. 11 (59). Saito MA, Sigman DM, Morel FMM. (2003). The bioinorganic chemistry of the ancient ocean: the co-evolution of cyanobacterial metal requirements and biogeochemical cycles at the Archean-Proterozoic boundary? Inorganica Chimica Acta. 356: 308-318.

Savvaidis I. (1998). Recovery of gold from thiourea solutions using microorganisms. Biometals. 11: 145-151.

Seiderer T, Venter A, Van Wyk F, Levanets A, Jordaan A. (2017). Growth of soil algae and cyanobacteria on gold mine tailings material. South African Journal of Science. 113: 11-

12.

Singh JS. (2014). Cyanobacteria: a vital bio-agent in eco-restoration of degraded lands and sustainable agriculture. Climate Change and Environmental Sustainability. 2: 133-137.

Sutherland IW. (2001). Exopolysaccharides in biofilms, flocs and related structures. Water Science and Technology. 43: 77-86.

Syed S. (2012). Recovery of gold from secondary sources – a review. Hydrometallurgy. 115: 30-51.

Tsuruta T. (2004). Biosorption and recycling of gold using various micro-organisms. Journal of General and Applied Microbiology. 50: 221-228.

Volesky B, May-Phillips HA. (1995). Biosorption of heavy metals by Saccharomyces cerevisiae. Applied Microbiology and Biotechnology 42: 797-806.

Williams RJP and Da Silva JJ RF. (2003). Evolution was chemically constrained. Journal of Theoretical Biology. 220: 323-343.