HEAVY METALS BIOMINERALIZATION BY SOME CYANOBACTERIAL ISOLATES

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Abstract

Three cyanobacterial isolates (*Spirulina platensis*, *Nostoc muscorum*, and *Anabaena oryzae)* were used individually or as a mixed culture to precipitate some heavy metals (Hg^{2+}, Cd^{2+}, Cu^{2+} and Pb^{2+}) out of their solutions through using the culture biogas produced during their aerobic growth in a batch bioreactor. Variable capabilities of metal bioprecipitation were recorded by the three algal isolates. FT-IR studies showed the existence of –OH groups in the metal precipitate produced by the algal isolates while –NH groups were identified only in the metal precipitates produced by *N. muscorum*, and *A. oryzae*. In conclusion, this study highlighted a novel approach for heavy metals bioremediation through the transformation of these metals into nitrogen complexes and/or hydroxide complexes via using the culture biogas produced by some cyanobacterial species.

Key words: Cyanobacteria, biogas, heavy metals, bio-mineralization.

Introduction

Metals are directly or indirectly involved in all phases of microbial growth. Many metals such as sodium, potassium, iron, copper, magnesium, calcium, manganese, zinc, nickel and cobalt are vital for biological functions, while others such as aluminum, cadmium, silver, gold, mercury and lead are not known to have necessary biological functions. All these elements can interact with microbial cells and be accumulated as a result of different mechanisms (Gadd, 1992; 2011). Some of these mechanisms have biotechnological importance and can be applied for the bioremediation of metals from industrial effluents.

The capability of some microbial species to adsorb some heavy metals on their surface (Campbell & Martin, 1990; White *et al*., 1995; Figueira *et al*., 2000; Sheng *et al*., 2005; Kumar *et al*., 2009) or accumulate them within their structure (Lamaia *et al*., 2005; Shanab & Essa, 2007; Atici *et al*., 2010; Saravanan *et al*., 2011) is a chief route for the removal of heavy metals from contaminated environment.

Another fashion for the detoxification of heavy metals by microorganisms is the chelation of these metals inside or outside their cells after converting them into other forms to reduce their toxicity. In 2007, Lefebvre *et al*. working with some cyanobacterial strains (*Limnothrix planctonica*, *Synechococcus leopoldiensis*, and *Phormidium limnetica*) demonstrated their ability to convert Hg^{2+} into elemental mercury Hg° and *meta*-cinnabar (β-HgS) under pH controlled and aerated conditions. The transformation of mercury into β-HgS was attributed to the interaction with metal binding sulfhydryl protein as an intermediate step in metal sulfide synthesis.

Moreover, some of the freshwater algae *Limnothrix planctonica* and *Selenastrum minutum* were recorded for their ability to bio-transform Hg^{2+} into a form with the analytical properties of β-HgS under aerobic conditions due to the presence of some protein and non-protein thiol chelators (Kelly *et a*l., 2006). Furthermore, Lengke *et al*. (2006) investigated the gold bioaccumulation by cyanobacterium *Plectonema boryanum* from gold (III)-chloride solutions. They confirmed that the reduction mechanism of gold (III) to metallic gold by this organism involves the formation of an intermediate gold (I)-sulfide due to a chelation process via some thiol compounds.

Thus the aim of this study is to examine the effectiveness of some cyanobacterial isolates (*Nostoc muscorum*, *Spirulina platensis* & *Anabaena oryzae*) to mineralize the metals ions (Hg^{2+}, Cd^{2+}, Pb^{2+} & Cu^{2+}) indirectly through using the volatile metabolites found in the cultural biogas produced during the aerobic growth in a batch bioreactor.

Experimental

Algal cultures and growth conditions

Samples for isolation of *Spirulina* were collected from lakes of Wadi El-Natrun, Egypt while *Anabaena* and *Nostoc* samples were collected from the drains of the cultivated rice fields at El-Fayoum, Egypt during (2010). Samples were filtered through sterilized Whatman No. 41 filter paper and then suspended on 5 ml sterilized BG11 medium (Rippka *et al*., 1979). One to two drops of each suspension were inoculated on solid BG11 and incubated for about two weeks in culture room at 25 ± 1^{o}C under controlled continuous illumination of 40 $\mu Em^{-2}s^{-1}$. The plates were examined and the best colonies were selected, picked up and re-streaked to new agar plates. Re-streaking and sub-culturing were repeated several times to obtain uni-algal cultures. To get axenic

cultures of the test organisms, the tested algae were grown in liquid cultures for 12 days to attain vigorous growth. About 20 ml of each culture were centrifuged at 6000 rpm for 10 min and the algal pellets were then streaked on peptone or yeast extract solid medium. Those which proved not to be axenic, streaks have been repeated till they become axenic. Inocula from axenic cultures were taken into sterilized liquid medium to be ready for the desired experiments. The purified algae were identified as *Spirulina platensis* (Desikachary & Bai, 1996), *Nostoc muscorum* and *Anabaena oryzae* (Prescott, 1978). Batch cultures were grown on BG11 medium for *Nostoc muscorum*, *Anabaena oriza* and Zarrouk medium for *Spirulina platensis* (Zarrouk, 1966).

Growth monitoring

The content of Chlorophyll "a" was used to monitor the algal growth. *Anabaena* & *Nostoc* cultures were centrifuged at 6000 rpm for 10 min to harvest cyanobacterial cells whereas *spirulina* cultures were filtered through sterilized Whatman No.1 filter paper. In this case supernatants were discarded and the chlorophyll "a" content in the biomass was extracted using the standard acetone extraction method described in APHA (1999). After extraction, chlorophyll "a" was determined spectrophotometrically at 750 and 665nm using a PYE Nnicom, SP8-100 UV spectrophotometer.

To estimate the dry weight of the algal biomass, Cells were harvested by centrifugation at 6000 rpm for 10 min. The harvested cells were washed out three times with sterile medium. The pellets formed after the last centrifugation were used for determination of pellet dry weight after drying at 105°C to constant weight.

Operation of the bioreactor

For metal bio-precipitation experiments a batch bioreactor was used according to Essa *et al*. (2005). The bioreactor composed of two chambers; one was used for cyanobacterial growth (2 l) which is maintained under aerobic conditions at 25^{o}C by pumping in filtered compressed air and the other chamber was used for metal precipitation (250 ml) by passing the culture biogas through metal solution via a 0.2 µm membrane. For the metal bio-precipitation experiments, a 1500 ml of each algal culture (about 8.5 mg/l chlorophyll "a") was used in case of the individual algal cultures whereas a 500 ml from each algal culture was used to prepare the mixed algal culture by using cells in the exponential growth phase (Table 1) and algal growth was monitored as described above.

Heavy metals concentrations

Stock solutions of the heavy metals were prepared separately by dissolving 100 milligram of $HgCl_2$, $CdCl_2$, $Pb(NO_3)_2$ & $CuSO_4$ in 100 ml of distilled water and then filtered at 0.2 µm. From these solutions, the different metal concentrations were prepared either separately or in combination.

Heavy metals determination

The collected heavy metal solutions from the precipitation chamber were centrifuged at 10000 rpm for 5 min and the supernatant was used for the determination of the different heavy metals. The mercuric ion concentration was assayed by using Perkinelmer 3300 Spectrometer Atomic Absorption (using hydride system) (Water, Soil and Environment Research Institute, Agriculture Centers, Ministry of Agriculture). While

Cd^{2+}, Pb^{2+} and Cu^{2+} were determined using Unicam Solar 989 AA Spectrometer Atomic Absorption (Faculty of Agriculture, Cairo University).

FTIR Spectroscopy

The precipitates of the different metals in the precipitation chamber were separated from their solutions by centrifugation at 10000 rpm for 5 min. The metal precipitate pellet was washed three times in distilled H_2O and then dried in vacuum to obtain metal precipitate in powder form that was analyzed by Fourier Transform Infrared Spectrophotometer at the wave number range of 400.00 cm^{-1} to 4000 cm^{-1} (Faculty of Science, Fayoum University).

Results

In this study the biogas produced during the growth of the cyanobacterial strains (*Nostoc muscorum*, *Spirulina platensis* & *Anabaena oryzae*) was used to mineralize some metals (Hg^{2+}, Cd^{2+}, Pb^{2+} & Cu^{2+}) from their solutions at different exposure time. Data in Figure (1) showed that *N. muscorum* recorded the highest percentage of mercury removal (90.4% & 93.2% after 2 & 4 days). At the same time, this strain demonstrated a low efficiency for the bio-mineralization of the other metals after 4 days (8.6% for cadmium, 14.3% for lead & 11.6% for copper).

Interestingly, *S. platensis* demonstrated a high capability for the precipitation of Cu^{2+} (23.8% & 43.1% after 2 & 4 days), Pb^{2+} (31.5% & 38.6% after 2 & 4 days) but a low ability of metal removal was recorded for mercury (4.5% & 16.5% after 2 & 4 days) and cadmium (6.7% & 11.8% after 2 & 4 days). At he same time, *A. oryzae* showed a marked potential for the bio-precipitation of copper (40.1 & 52.3% after 2 & 4 days), $lead^{+}$ (18.7 & 36.9% after 2 & 4 days) and mercury (22.4 and 29.3% after 2 & 4 days)

whereas with and cadmium (6.9 & 14.1% after 2 & 4 days) a low percentage of metal removal was demonstrated.

Another experiment was conducted in order to precipitate some heavy metals from a combined metal solution containing Hg^{2+}, Cd^{2+}, Pb^{2+} & Cu^{2+} ions by using the gases produced from the algal cultures after 4 days of exposure time. Results in Figure (2) showed that the three algal cultures have a variable capability for the precipitation of metals from their combined solution. *A. oryzae* recorded a high efficiency for the precipitation of Cu^{2+} (96.4%), Hg^{2+} (89.1) & Pb^{2+} (83.6%). At the same time, *S. platensis* recorded (85.8%, 83.6% & 81.9%, for the bio-precipitation of Cu^{2+}, Pb^{2+}& Hg^{2+}, respectively. In case of *N. muscorum*, a high percentage of metal bio-removal from the combined solution was obtained (94.3% for Hg^{2+}, 48.9% for Pb^{2+}& 13.6% for Cu^{2+}). Apparently, the three algal strains recorded low efficiency for the bio-removal of cadmium from the mixed metal solution when they were used as individual cultures (13.7% with *S. platensis,* 13.6% with *A. oryza*e & 11.7% with *N. muscorum*).

Unexpectedly, the biogas produced from a mixed algal culture composing of *N. muscorum*, *S. platensis* & *A. oryza*e that contains almost the same concentration of the algal biomass (about 8.5 mg/l chlorophyll "a") showed a low efficiency of metals bio-removal (0.5%) for cadmium, (0.6%) for copper, (4.5%) for lead whereas with mercury a high percentage of metal removal was recorded (41.1%).

Data in Table (2) showed that the culture headspace gases produced from *A. oryzae*, *S. platensis*, *N. muscorum* cultures have shifted the pH value of the single or the

combined metal solutions inside the precipitation camber towards alkalinity (7.8 – 8.4) whereas the mixed cyanobacterial population recorded pH value (7.3).

Infrared spectral studies were conducted on the metal complexes obtained from the precipitating chamber. Data in Figure (3) showed the presence of amine groups (-NH) with absorption peak at 3300 – 3500 cm^{-1} and hydroxide groups (-OH) with absorption peak at 3500 – 3700 cm^{-1} in the metal precipitate produced by the biogas of *A. oryzae* and *N. muscorum* but in case of *S. platensis* the hydroxide group only was demonstrated.

Discussion

Although the adsorption of metal ions on living and non-living cell surface is very rapid process and not interconnected with the metabolic activity of the microbial cells, the application of bio-sorbents in the environment and industry has not been clear yet. Therefore in this study the cyanobacterial strains were used to complex heavy metals out of their solutions through using their natural volatile compounds (VCs) that were produced during their aerobic growth.

The diversity of VCs produced in nature is enormous. VCs from microorganisms are released mainly as metabolic products during growth, as secondary metabolites for protection against antagonists and competitors, or as signaling molecules in cell-to-cell communication (Kai *et al*., 2007; Ryu *et al*., 2004; Wheatley, 2002). The production of VCs has been determined for various microorganisms such as fungi (Ezra *et al*., 2004; Buzzini *et al*., 2003), bacteria (Farag *et al*., 2006; Wood *et al*., 2006; Bunge *et al*., 2008) and cyanobacteria (Mario *et al*., 2001; Hockelmann & Juttner, 2004).

The outlet gases produced during the actively growing cultures of *S. platensis, N. muscorum* and *A. oryzae* demonstrated a great efficiency for the precipitation of metals ions (Hg^{2+}, Cd^{2+}, Cu^{2+} and Pb^{2+}) from their individual synthetic solutions and from the mixed metal solution as well. These results are inconsistent with our previous work (Essa *et al*., 2005; 2006) that clarified the huge capability of *klebsiella pneumoniae* to precipitate different metals (mercury, cadmium & lead) from synthetic solutions via using the culture headspace gas. The presence of some volatile organo-sulfur species (dimethyl disulfide) in the culture off-gases of this bacterium was assumed to play a principle role of the metal bioprecipitation process.

Infrared spectral analysis showed the presence of amine groups (-NH) in the metal precipitate produced only by *N. muscorum* and *A. oryzae* while hydroxide groups (-OH) were identified in the metal precipitates produced by the three algal strains. These results are consistent with those reported with Macaskie *et al*. (2007) who used the volatile gases produced from *Klebsiella pneumoniae* to selectively precipitate gold (III) ions out of an industrial wastewater as Au-complexes. The formation of the insoluble gold-amine complexes was attributed to the presence of some volatile biogenic metabolites in the biogas produced during the aerobic growth of this organism.

The presence of amine groups in the metal precipitate produced by the cyanobacterial biogas could be formed as a result of the interaction of the metal ions and ammonia gas expected to be found in the culture outlet biogas. The presence of ammonia in the culture biogases was confirmed by using Nessler's reagent (alkaline solution of mercuric potassium iodide) produces heavy dark yellow precipitate in presence of ammonia (not published data).

It is well known that, some cyanobacteria are characterized by their ability to convert the atmospheric nitrogen into ammonia gas or ammonium ions during the nitrogen fixation process (Kerby *et al*., 1983; Wang *et al*., 1991; Abdlhameed & Hammouda, 2007). At the same time, they can assimilate different nitrogen sources such as nitrate, nitrite, ammonia or urea into ammonium (Herrero *et al*., 2001).

The release of ammonia during the growth of the cyanobacterial strains could explain the change of the pH value of metal solutions in the precipitation chamber towards alkalinity and was assumed to participate in the transformation of soluble metals into insoluble metal complexes. Under alkaline conditions, due to the presence of ammonia, a soluble metal hydroxide ions is formed which is then precipitated as a neutral metal hydroxide molecule. The hydroxide ions attached to metal ions may act as bridging group to join two or more metals together in a process called dehydration-dimerization (Manahan, 2000). Excess ammonia in the metal precipitation chamber may react with the metal hydroxide precipitates leading to the formation of metal-amine complexes such as the precipitate of mercury, cadmium and lead ions (Hughes & Poole, 1991). At the same time, the complexation of the metal ions into nitrogen based precipitates could be formed due to the presence of some biogenic volatile amines in the culture outlet gases (Ozugual, 2004) which can chelate some metal ions (Hughes & Poole, 1991).

Although the utilization of the cyanobacterial biogases for heavy metal bioremediation is a promising mechanism because the metals are kept separate form the algal biomass and can be handled independently, further analysis are required to identify the cyanobacterial biogases and to figure out their role in the metal chelation process.

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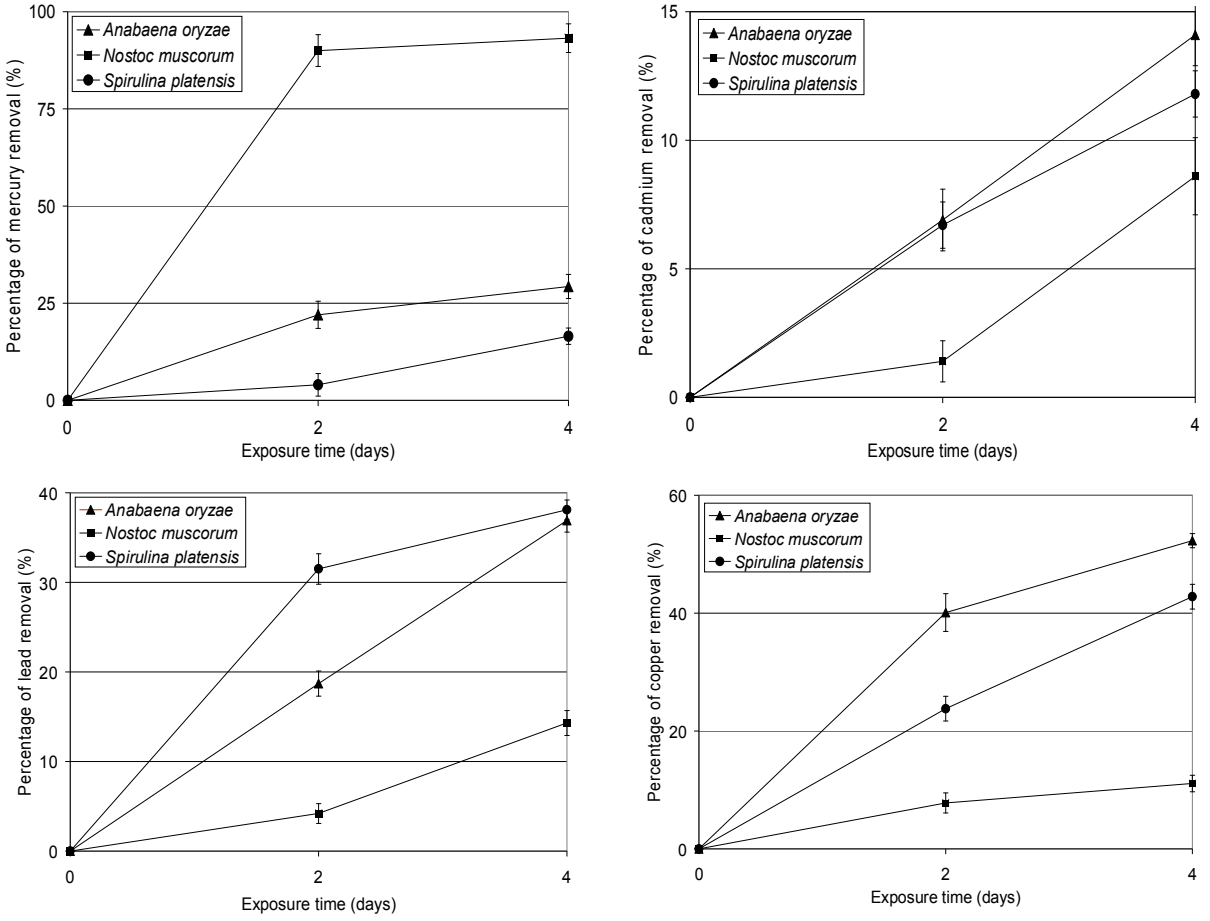

Figure (1): The capability of the different cyanobacterial isolates (*S. platensis*, *A. oryzae* and *N. muscorum*) for bioprecipitation of some heavy metals (Hg^{2+}, Cd^{2+}, Pd^{2+} & Cu^{2+}) via using the culture biogas produced during their aerobic growth. Initial metals concentration were 28.5, 29.0, 28.8, & 30.9 mg/ml for Hg^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}, respectively. Data are the means of three replicates.

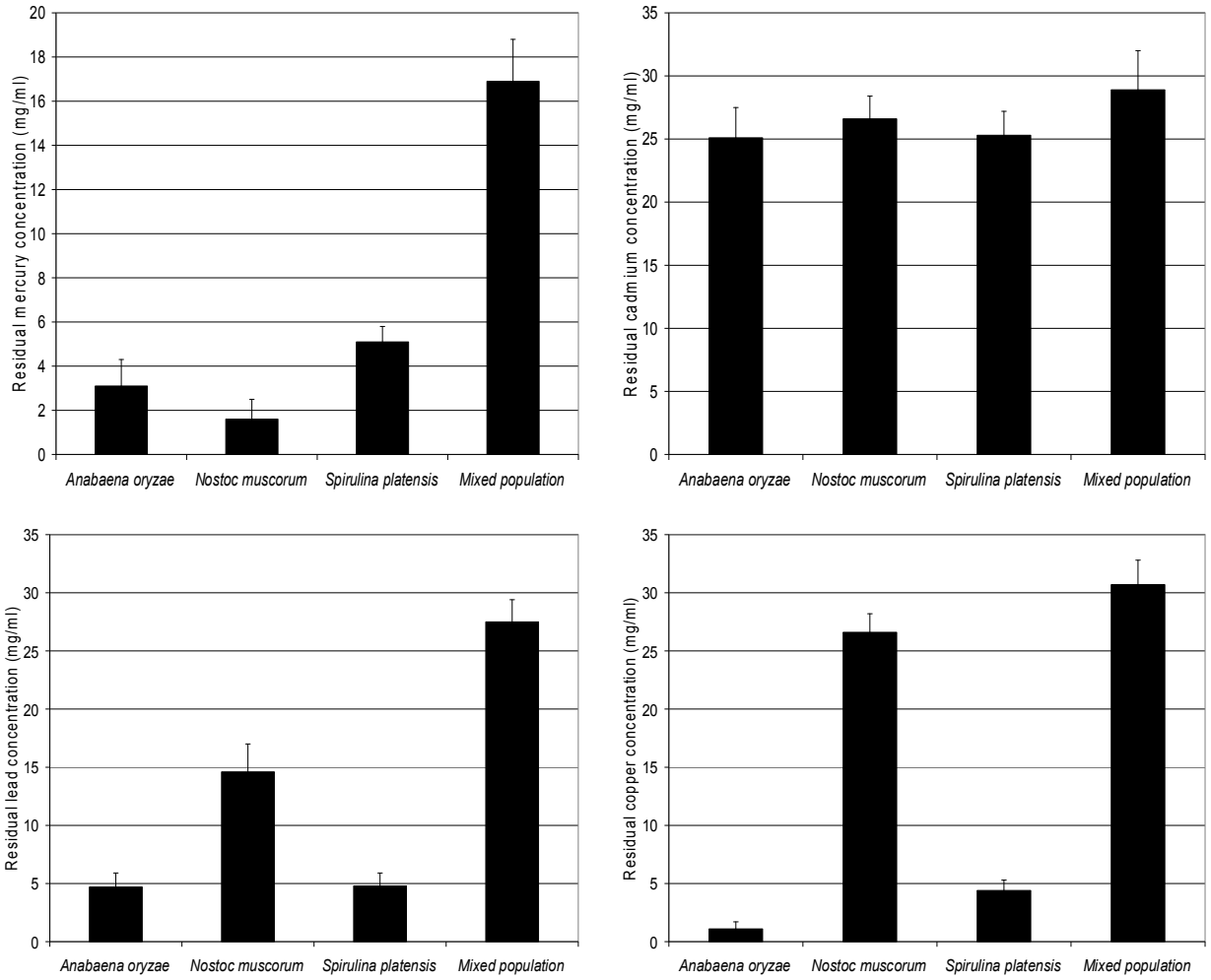

Figure (2): Effect of the culture biogas of *S. platensis*, *A. oryzae, N. muscorum* and a mixed culture of the three strains on metal bioprecipitation from a combined heavy metals solution after 4 days of exposure time. Initial metal concentrations were 28.5, 29.0, 28.8, & 30.9 for Hg^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}, respectively. Data are the means of three replicates. Error bars represent the standard errors of the means.

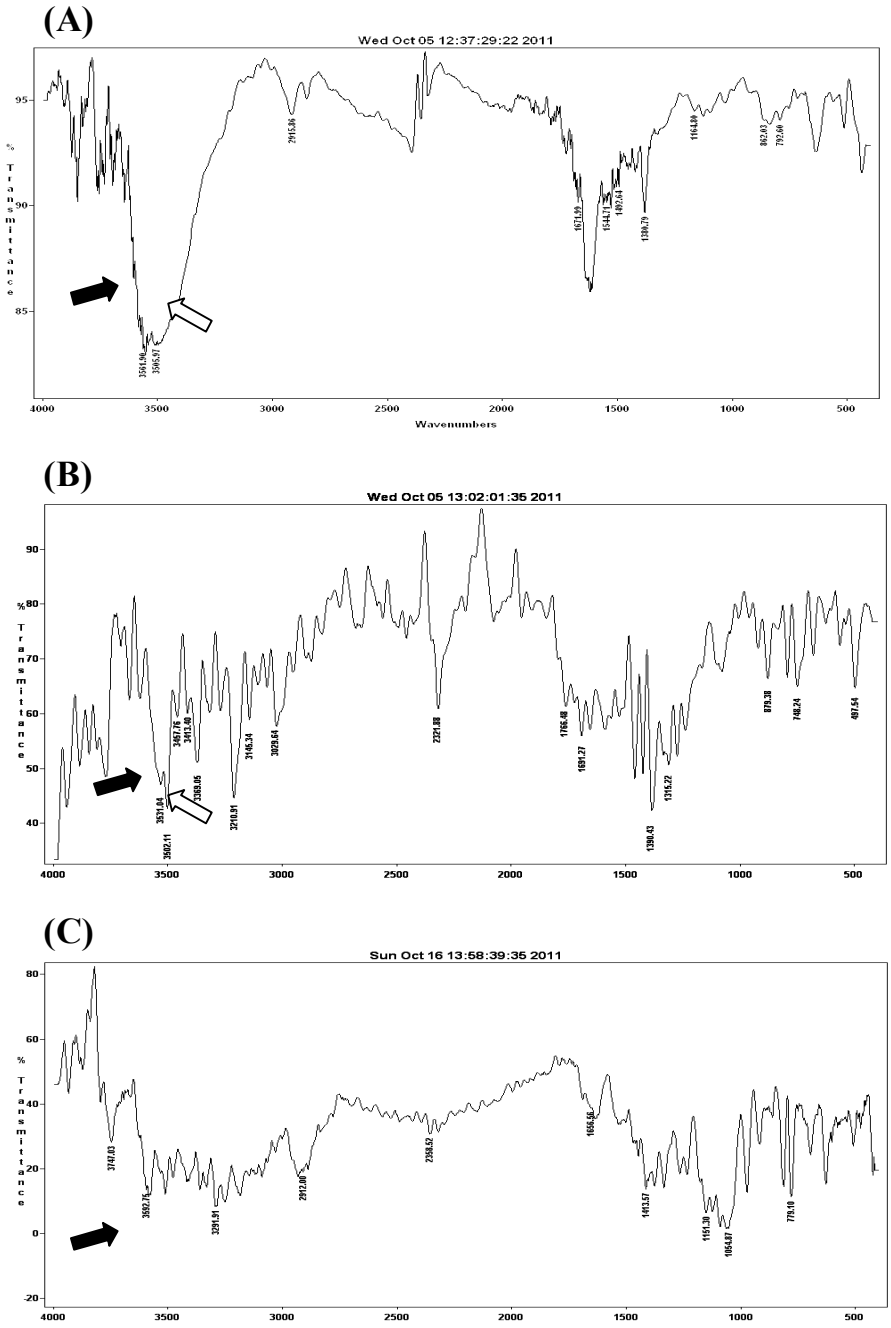

Figure (3): FTIRS spectra of the metal precipitates obtained via the interaction between culture biogases of *A. oryzae* (A), *N. muscorum* (B) and *S. platensis* (C) and the combined metals solution in the precipitation chamber after 4 days of exposure time. White arrows indicate the –NH groups while black arrows indicate the -OH groups.

Table (1): Growth parameters of the cyanobacterial cultures *S. platensis*, *A. oryzae* and *N. muscorum* at the exponential growth phase (14 days) and after using them for the bioprecipitation of heavy metals from their solutions inside the bioreactor for 4 days of exposure time.

Growth parameters	Initial analysis			Final analysis		
	A. oryzae	*S. platensis*	*N. muscorum*	*A. oryzae*	*S. platensis*	*N. muscorum*
Culture pH	9.73 ± 0.17	6.69 ± 0.11	6.58 ± 0.66	9.81 ± 0.04	6.76 ± 0.18	6.91 ± 0.21
Ch"a" (mg/l)	8.46 ± 0.34	8.31 ± 0.15	6.89 ± 0.09	10.66 ± 0.08	9.63 ± 0.15	7.66 ± 0.33
Dryweight (g/l)	1.55 ± 0.21	1.42 ± 0.11	1.19 ± 0.05	1.6 ± 0.31	1.53 ± 0.07	1.28 ± 0.06

Table (2): Changes of pH values of the individual and combined heavy metals solution inside the precipitation chamber as a result of using the biogas produced by the cyanobacterial cultures (*S. platensis*, *A. oryzae* and *N. muscorum*) after 4 days of exposure time.

Individual metal solutions	Initial pH value	Final pH value		
		A. oryzae	*S. platensis*	*N. muscorum*
Mercury	6.34 ± 0.13	٨.11 ± 0.14	8.21 ± 0.06	8.28 ± 0.09
Cadmium	6.86 ± 0.06	8.46 ± 0.18	7.79 ± 0.13	8.19 ± 0.12
Lead	6.77 ± 0.11	8.34 ± 0.06	7.77 ± 0.15	8.42 ± 0.08
Copper	6.53 ± 0.09	8.19 ± 0.12	7.99 ± 0.08	8.27 ± 0.11
Cyanobacterial culture	**Combined metal solution**			
	Mixed population	*A. oryzae*	*S. platensis*	*N. muscorum*
Initial pH value	6.45 ± 0.09			
Final pH value	7.29 ± 0.09	7.78 ± 0.07	8.31 ± 0.16	8.12 ± 0.11

الترسيب الحيوي لبعض العناصر الثقيلة باستخدام الطحالب الخضراء المزرقة

أشرف محمد محمد عيسى* - سها سيد محمد مصطفى**

*** قسم النبات– كلية العلوم – جامعة الفيوم – مصر**
**** قسم الميكروبيولوجيا - معهد بحوث الأراضي و المياه والبيئة – مركز البحوث الزراعية – الجيزة - مصر**

تناولت هذه الدراسة استخدام ثلاث عزلات من الطحالب الخضراء المزرقة (*Spirulin platensis* *Nostoc muscorum* and *Anabaena oryzae*) في صورة منفردة أو في صورة خليط لترسيب بعض العناصر الثقيلة (الزئبق و الكادميوم و الرصاص والنحاس) من محاليلها الصناعية حيث تم تنمية هذه العزلات داخل مخمر هوائى واستخدمت الغازات المصاحبة لنموها في ترسيب هذه العناصر. أوضحت نتائج تحليل FT-IR إلى وجود مجموعات الأمين (-NH) في رواسب المعادن الناتجة عن السلالتين (*Nostoc muscorum* and *Anabaena oryza*e) ومجموعات الهيدروكسيل (-OH) في رواسب المعادن الناتجة عن الثلاث عزلات وقد خلصت هذه الدراسة الى نجاح استخدام هذا الإتجاه الجديد في التخلص من سمية بعض العناصر الثقيلة عن طريق ترسيبها في صورة مركبات نيتروجينيه ومركبات هيدروكسيله لهذه العناصر.