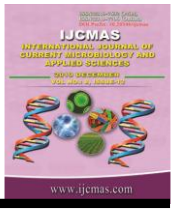

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Biosorption of Heavy Metals by Cyanobacteria

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ABSTRACT

Keywords

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Bio sorption potential of cyanobacteria in absorbing lead and chromium was carried out as an experimental study. The algae collected from the rice field are examined for the lead and chromium absorption potential. Visual changes, followed by disintegration of the cyanobacterial mat had gradually progressed with the increase in concentration of lead and chromium from 0 ppm to 150 ppm which indicates the metal absorption. The FT-IR spectra showed about 10 functional groups are involved in the future reactions of algae. The functional group dominant in lead treated cyanobacterial mat was OH and CO. The O-H Functional group, which shows isomerism of a ketone group C-O which has the highest intensity when it comes to binding and reaction process. The cyanobacteria mat has shown better absorption in Cr (VI) than Pb (IV). From the bio sorption study using the cyanobacterial mats showed promising results that would be efficient in reducing the heavy metal contamination in our ecosystem.

Introduction

Heavy metals occur naturally in the earth's crust and have a specific density of more than 5g/cm^3 (SanaaShanab *et al.,* 2012). These elements are hazardous because of their non-biodegradable nature, as a result of which they tend to accumulate in the tissues of living organisms and cause serious effects like bioaccumulation and bio-magnification in the food chain (Singh *et al.*, 2009; Wuana *et al.*, 2011). The heavy metals in higher concentrations damage the biological systems by affecting the cellular components like the cell membrane, the cell organelles, and some metabolic enzymes. Also, one of the severe effects of heavy metal toxicity involves DNA and protein damage, leading to cancer (Tchounwou *et al.*, 2012).

The need for the removal of heavy metals therefore becomes crucial. Though there are several methods of heavy metal removal like membrane filtration, precipitation of heavy metals using chemicals, reverse osmosis, adsorption on activated carbon, ion exchange; these methods fall short on their economic feasibility and technical practicality (Abdel-Aty *et al*., 2012; Alluri *et al.*, 2007). And therefore, employing biological systems for the treatment of the heavy metal contaminated waters comes to the lime light.

There are various biological organisms; especially microorganisms that show great potential in the biological removal of the heavy metals. Among these microorganisms, cyanobacteria are of a special importance. There have been several studies on heavy metal biosorption using cyanobacteria.

Sharma *et al.*, 2008 worked on the sequestration studies of chromium from dilute aqueous solutions using the exopolysaccharide producing cyanobacteria *Nostoc* and *Gloeocapsa.* Shukla *et al.*, (2012) studied the chromium (CrVI) removing potential of acyanobacterial mat (consortium of *Chlorella sp.*, *Phormidium* sp. and *Oscillatoria* sp.) which was collected from the effluent site itself.

Cyanobacteria mediated biosorption continues even after the cyanobacteria dies; in other words, the dead cells of cyanobacteria can also be employed for the biosorption of heavy metals (Gupta *et al.*, 2015). In addition, the biosorption efficiency of cyanobacteria is relatively higher due to its high binding affinity towards the heavy metals. And lastly, the ease of production of the cyanobacteria with its minimal nutrient needs make it an ideal organism to work on the removal of heavy metals from contaminated wastewater (Dixit and Singh, 2014). Both living and dead biomass as well as cellular products such as polysaccharides can be used which complex metal ions through the action of ligands or functional groups located on the outer surface of the cell (Aksu and Kutsal, 2011).

Materials and Methods

Collection of cyanobacterial mats

Rice ecosystem was chosen for collection due to the abundance and ease in collection. Young and developing mats were collected during high incidence of sunlight during the high noontime. Regular growth monitoring of the culture was done by optical density measurement, pH monitoring and microscopic observation. Pure cyanobacterial mat was maintained in Erlenmeyer flask containing sterilized Bristol medium (composition; $NaNO_3$ – 25g; $CaCl_2{\cdot}2H_2O$ – 2.5g; $MgSO_4{\cdot}7H_2O$ -7.5g; K_2HPO_4 – 7.5g; NaCl – 2.5g dissolved in 1L of Deionized water) under controlled conditions in the growth chamber (Hirata *et al.*, 1996).

Biosorption studies

The standard chemical used as Cr VI was Potassium dichromate $K_2Cr_2O_7$ and for Lead was Lead Nitrate $Pb(NO_3)_2$. The treatments concentrations ranging from10ppm to125ppm, for both lead and chromium were prepared for 100 mL of volume. For each such treatment the quantity of cyanobacterial mat taken was 4 grams for lead and 1 gram for chromium in a tray and refurbished according to need. Prior to the inoculation of the mats into the treatment flasks, the pH of the solution are to be checked and if deviant, are subjected to adjustments so as to match the optimum growing conditions suitable for the cyanobacteria.

The conical flasks containing the inoculums were sealed with cotton plug and kept under partial sunlight for 48 hours. After the required time, the flasks were subjected to centrifugation and the solutions were filtered using Whatman 41 filter paper. The pH of chromium solutions was adjusted specially in the range of 5.3-6.0 using 2.5% NaOH. Shukla *et al.,* (2012) also reported that biosorption was carried out at a higher pH of 5.5–6.2. The cell pellets after wet digestion using triacid mixture, filtered and is taken to elucidate the concentration of chromium and lead absorbed in the Atomic absorption spectrophotometer (Perkin Elmer AAnalyst 400 AA Spectrometer). With true double-beam Echelle optical system with electrode less discharge lamps with air acetylene flame using the following appropriate instrumental parameters as given in Table 1.

Fourier Transform Infrared Spectroscopic studies (FT-IR)

The cyanobacterial mats which showed dynamic changes in terms of physical changes were taken, air dried for 3 days and grinded to a fine powder using pestle and mortar, and then were given to the FT-IR (Model 8400S of Shimadzu, Japan) using Attenuated Total Reflectance (ATR) technique having wavelength source (400-4000cm^{-1}) to analyze the material (Park *et al.,*2005, Trakal *et al.,* 2014).

Results and Discussion

The cyanobacterial mats taken for the study were associated with the rice fields at the Wetland in Thondamuthur. The mats were a consortium of different cyanobacteria, however, after microscopic observation, the dominant genus was found to be Nostoc. The mats associated at the collar regions of the rice crop were lush green and were chosen. The *Nostoc sp.* Cells were almost cylindrical, arranged as filaments and heterocysts observed were intercalary. For the Bio sorption of lead, the concentrations were fixed in the range of 10ppm to 125 ppm. The reasons for fixing the above concentrations are Lead in the wetland water samples varied between 0.9 and 3.0 mg L^{-1}. All the samples exceeded the maximum permissible limit (0.05 mg L^{-1}) prescribed for drinking water (Pavithapriya *et al.,* 2015). Industrial effluents of chromium are found to be in highly elevated concentrations of 3200 ppm. In Coimbatore, the mean value of Cr (VI) is found to be 141 mg/L. But majority of the isolates are intolerable to grow in such concentrations, hence, the chromium treatments were made to the concentrations of 10, 25, 50, 75, 100, 125, ppm (Pavithapriya *et al.,* 2015). For reference concentration of Pb and Cr was analysed in water and soil samples. In soil the Pb and Cr content was 1.117 ppm and 16.4 ppm respectively and in water it was 0.681ppm and 3.213 ppm respectively.

Visual changes in the cyanobacterial mat were observed in the lead spiked samples after 48 hours. White film formation on the mat and disintegration of the cyanobacterial mat had gradually progressed with the increase in concentration from 0 ppm to 150 ppm. This is clearly evident in the treatments from the treatment100 ppm to 150 ppm. The white film formation, however, was not observed in the Chromium study, but there was the gradual disintegration of the cyanobacterial mat with the increase in the concentration as seen in the Lead treatments. Even at a very high pH of 300 ppm, no white discoloration was observed in the case of Chromium (Plate 1 & 2).

Biosorption of Pb

The cyanobacteria showed increased absorption of the metal Pb ions from the solution. The rate of absorption begins at a steady pace till the treatment of 25ppm.The concentration absorbed by the treatment of 25ppm was 28 ppm, which was followed by a significant increase in the absorption in the subsequent treatments of 50 ppm and 75 ppm has shown absorption of 46 and 78 ppm respectively which shows 50% increase when compared with the former to the latter. After the increase, in higher concentration such as 100 ppm, there is a sharp decline of the biosorption in the concentration to 40 ppm. Further treatments weren't added because the Pb was in the verge of achieving its Maximum Threshold Concentration, the concentration above which the cyanobacteria cannot survive. The highest absorption which was seen in treatment 5(70ppm) and the Biosorption capacity of the cyanobacteria in lead is found to be 0.70mg/g of cyanobacteria (Fig. 1).

Biosorption of Cr

In case of Chromium, similar as that of Lead, the number of treatments were the same but the inoculums added to each flask was fixed as 1 g of wet weight of the cyanobacterial mat. The biosorption capacity is seen in treatment 6 (100ppm) which is found to be 1.428mg/g of cyanobacteria.

The graph (Fig. 2) represents the absorption of Hexavalent Chromium by the cyanobacteria in the treatments ranging from 10 ppm to 125 ppm.

The absorption rate increases at a steady pace from 10 ppm to 50 ppm and then in the concentration of 75 ppm the absorption rate and subsequently more increase in the 100 ppm concentration. After the higher sorption trend the rate of absorption plummets to 787 ppm in the subsequent treatment of 125 ppm. Therefore it denotes the approaching maximum threshold concentration of the element (Table 1).

Bio removal efficiency of cyanobacteria

The lower concentrations initially show very less removal percentage but as concentration increases bio removal percentage also increases and attains a stable phase (above 90%) till 125 ppm concentration. Among all the treatments the concentration of 100 ppm has the highest efficiency of 96.92%. The cyanobacteria under different treatments have absorbed to their capacity in the given time of 48 hours. In order to know how much heavy metal is removed from the solutions, it is found out by calculating the bio removal efficiency. Highest removal efficiency is seen in 25 ppm concentration where 97.79% of the heavy metal was removed (Fig. 3 and 4).

Changes in pH due to absorption

The pH changes in the solution are the result of many cumulative factors of the cyanobacterial metabolism and the initial pH. Overall there has been a slight increase of the pH among all the concentrations except in the solution of the blank. In our experiment, maximum biosorption could not be achieved fully. Abdel –Aty *et al.*, (2012) reported the maximum biosorption at a pH of 3 but in the study the pH was slightly alkaline (to simulate the effluent pH) and therefore the full capacity of the cyanobacteria to absorb the heavy metals was not visualized. As far as the pH of Chromium is concerned, the solution in accordance with the trend of the Lead treatments, follows an increase in the pH too (Table 2). There is a positive trend in the increase of pH in all treatments regardless of the concentrations. This is due to the carbonic anhydrase enzyme present in the cyanobacteria which is a basically a zinc metallo enzyme which facilitates the reversible hydration of carbon dioxide which plays a significant role, like that in the manipulation of pH, as in this case resulted in an increase (Soltes-Rak *et al.*,1997).

Changes in functional groups

The FTIR finds a change in the absorption pattern indicates a change in the material, by this principle the functional group of the heavy metals can be understood.

Table.1 Instrumental parameters for Pb and Cr analysis

S.No	Conditions	Pb	Cr
1.	Lamp current (mA)	5	4
2.	Fuel	Acetylene	Acetylene
3.	Fuel support	Air	Air
4.	Flame stoichiometry	Oxidizing	Oxidizing
5.	Optimum working range (µg/mL)	0.1 - 30	0.1 - 30
6.	Wavelength (nm)	217.0	357.6
7.	Slit width (nm)	1.0	2.7/0.8
8.	Flame support	Nitrous oxide	Nitrous oxide

Table.2 Changes in pH due to absorption

Changes in pH before and after absorption of Pb			**Changes in pH before and after absorption of Cr**		
Concentration (ppm)	pH before inoculation	pH after inoculation	Concentration (ppm)	pH before inoculation	pH after inoculation
Blank	6.90	6.73	Blank	5.71	5.73
10	6.60	6.61	10	5.82	5.91
25	6.62	6.67	25	5.59	5.61
50	6.54	6.55	50	5.81	5.91
75	6.30	6.72	75	5.38	5.47
100	6.55	6.60	100	5.50	5.95
125	6.50	6.51	125	5.42	5.84

Fig.1 Biosorption of Lead by cyanobacteria

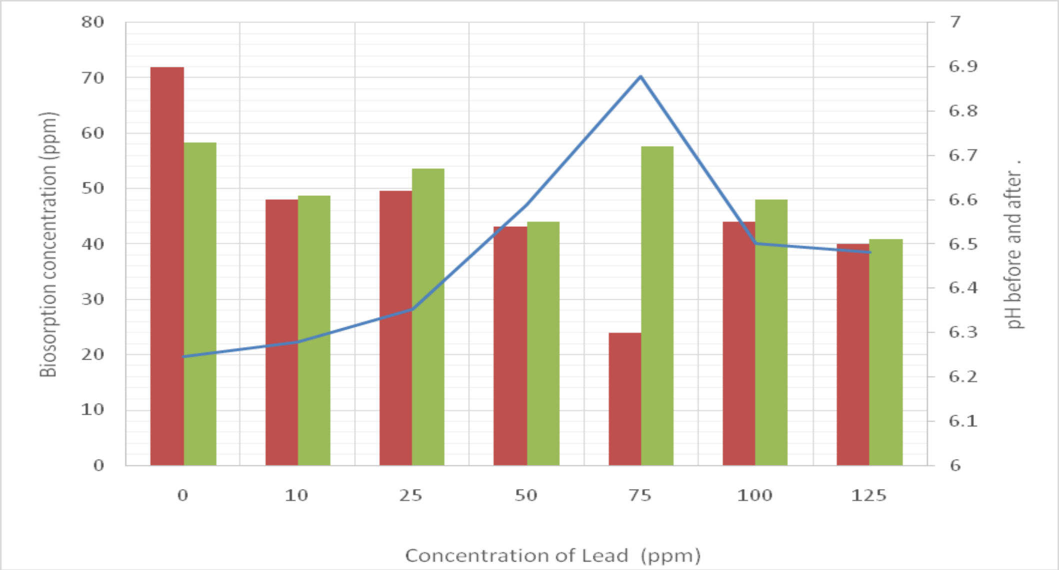
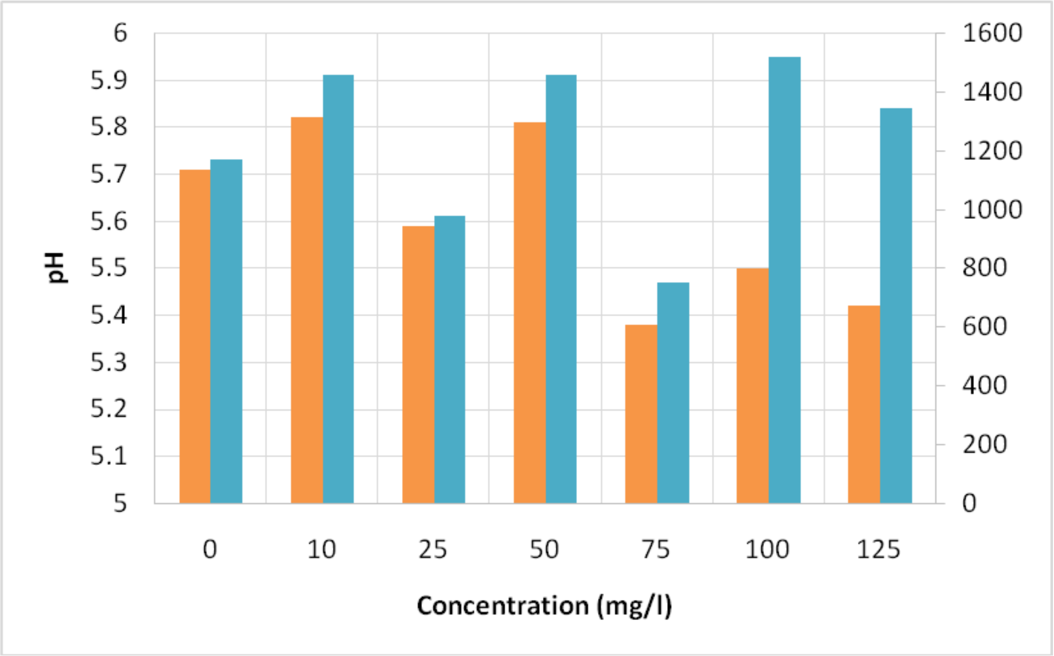


Fig.2 Biosorption of Cr by cyanobacteria



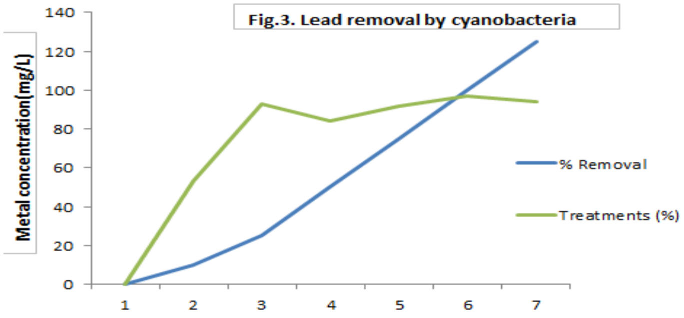


Fig.3. Lead removal by cyanobacteria

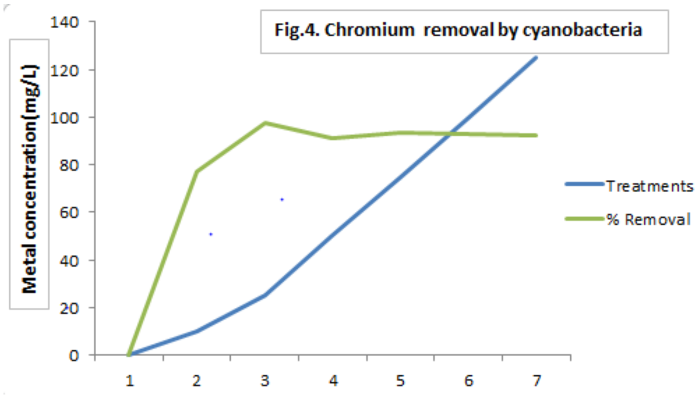


Fig.4. Chromium removal by cyanobacteria

Fig.5 FT-IR spectra of lead treated cyanobacterial mat

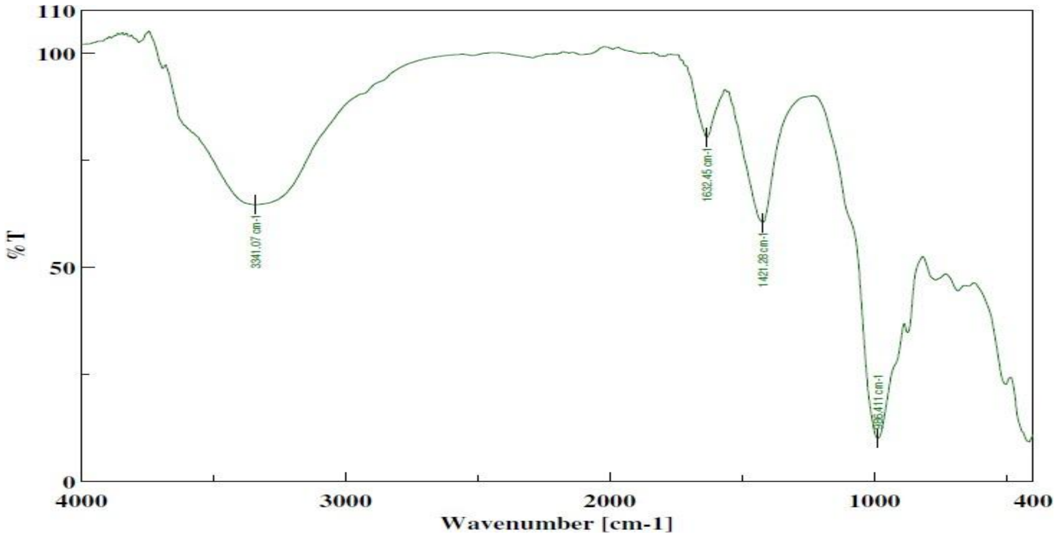


Fig.6 FT-IR spectra of chromium treated cyanobacterial mat

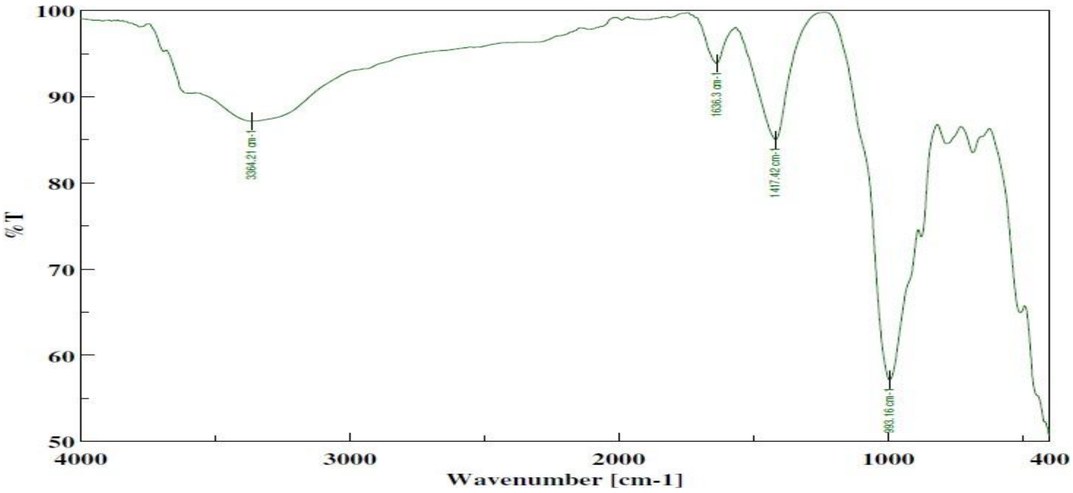


Plate.1a Cyanobacterial growth after spiking with lead at different concentration (0 to 50ppm)

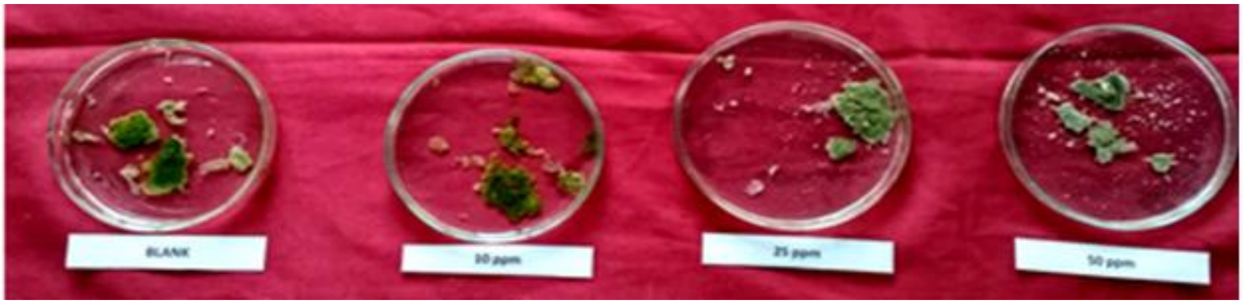


Plate.1b Cyanobacterial growth after spiking with lead at different concentration (75 to 150ppm)

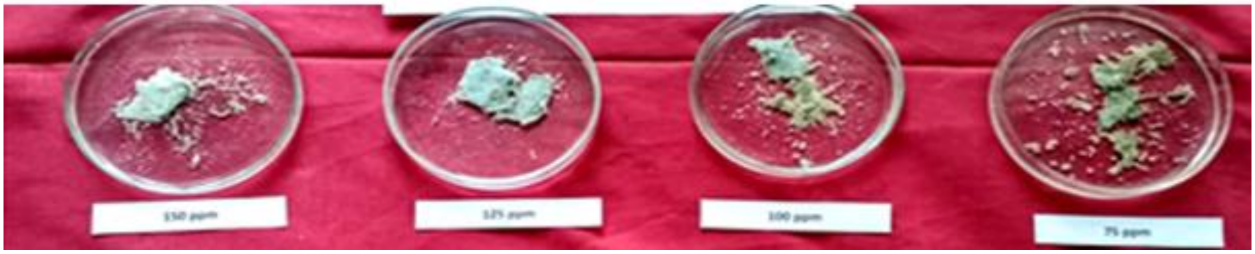
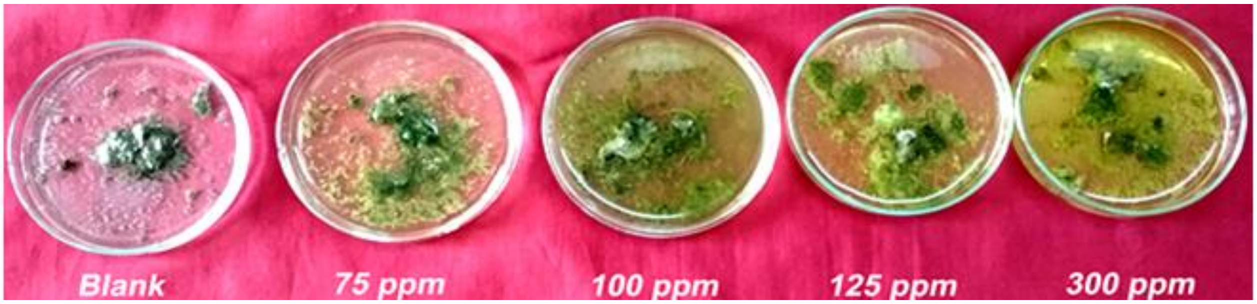


Plate.2 Cyanobacterial growth after spiking with lead at different concentration



By nature, the total spectrum generated is a series function of absorbed energy response they are only somewhat discrete and degenerative. The graph is interpreted by the particular “peak” of energy at a certain wave number can move around based on other chemical and matrix factors (Fig. 5 and 6).

Generally, microorganisms show potential removal of heavy metals through bioadsorption or bioaccumulation, and sometimes by both mechanisms. The former mechanism involves largely in physical adsorption whereas the later, involves chemical bondage and is zero energy process. So it’s the passive accumulation process which includes several processes like complexation, chelation and micro-precipitation. The positively charged metal ions are sequestered primarily on the surfaces of the negative ions resulting in polysaccharide coating that is found on many microbes. The functional group dominant in lead treated cyanobacterial mat was OH and CO. The functional group dominant in chromium treated cyanobacterial mat was OH and CO and an open chain silicon compound (Si-O-Si).

The functional group dominant in lead treated cyanobacterial mat is OH and CO. They show maximum stretching and strong intensity. There weren’t any overlapping of NH and OH groups in *Anabaena* as reported by Abdel – Aty *et al.*, (2012). Similarly, the functional group dominant in chromium treated cyanobacterial matis OH and CO and an open chain silicon compound (Si-O-Si). The peak in intensity is observed at 3400-3200 cm^{-1}. Based on a study by Shukla *et al.*, (2012), the increase in the OH groups is primarily due to the hydrolysis of cell wall polysaccharides to shorter saccharides under the acidic pH. As the ill effects of the heavy metals in our environment are prevailing, it is high time for environmental activists and researchers to find ways and means to put an end to the increasing heavy metal contamination. For this situation, the biosorption of heavy metals like lead and chromium using cyanobacteria would be economically feasible and practical. From the biosorption study using the cyanobacterial mats we have witnessed promising results that would be efficient in reducing the heavy metal contamination in our ecosystem. The study can be extended further by determining the time needed for maximum absorption and open field biosorption studies using the mats can be experimented.

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