Biosorption of Heavy Metals from Soil by Pseudomonas Aeruginosa

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Abstract: Pseudomonas aeruginosa is known to accumulate metal in their cell by the process called Biosorption. This property is used here for removal of metals from the soil to reduce its toxicity. Soil sample collected from Durgapur, West Bengal was mixed with different concentration of metals; chromium, copper & zinc in ppm level and was inoculated with bacterial sample. After incubation the metals were extracted and were compared with different standards using spectrophotometric and titration based methods. Metals are indispensable constituents of approximately one third of all proteins. As such, metals are involved in virtually all biological processes, including metabolism, energy transduction, gene expression, cell signalling, and formation of endo- and exoskeletons, and electron transfer. Among the techniques suitable for the quantification of metal ions in soil sample, inductively coupled plasma mass spectrometry are likely to be the most widely employed. However, although these techniques are reliable and sensitive, they suffer from the limitation of being rather costly (considering instrument acquisition and maintenance), time-consuming (with respect to sample preparation), and not always readily available. Therefore a general spectrophotometric and titration based analysis is been performed. The results are quite promissable, Metal absorbed by pseudomonas aeruginosa i.e., Cr, Zn and Cu in 1000 ppm were 1.07mg, 1.33mg and 0.67mg when compared to control soil sample in 1000 ppm with metals, but without Pseudomonas aeruginosa , compared were 3.7mg respectively. The present study depicts that bacteria removes chromium efficiently and this could be used for industrial waste management and other environmental contaminants.

Key words: Biosorption, Pseudomonas aeruginosa, chromium, copper & zinc in ppm level, Industrial waste Management.

I. INTRODUCTION

Effluents released from the textile industries contain various organic dyestuffs, chrome dyes and other chemicals during various operations and produce a large quantity of solid and liquid waste containing hexavalent chromium, salts of zinc, sulphates, copper, sodium and potassium etc. Pollution is correlated with the degree of industrialization and the intensity of chemical usage (Kabata-Pendias et al 1999).

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Past and present industrial activities have often resulted in the pollution of underlying soils where these activities take place, either by leaching of water from landfills or direct discharge of industrial wastewater into soil (Kolembkiewicz et al 1999).

These industrial wastewaters are produced mainly and in large quantities by textile industries, due to the nature of their operations, which require high volume of water that eventually results in high wastewater generation (Cwietlik et al 2004). The most common toxic soil pollutants include heavy metals and their compounds, organic chemicals, oils, tars and pesticides. Soil pollution by heavy metals such as mercury, copper, chromium and lead are of great concern to public health. The content of chromium in soils is generally low. The range of mean concentrations of chromium varies from 7 to 150 ppm (Dudka et al 1992). Its content and distribution in the soil largely depends on the type of the soil's mother rock. The presence of additional amounts of the element in the soil is caused by human activity, including emission of furnace or coke dust or combustion of fluid fuel or tannery waste water or communal solid waste. The migration of chromium takes place only in a strongly acid and reducing medium, where it occurs in the form of the $Cr3+$ cation, or a strongly basic and oxidizing medium, where it occurs as the CrO 2- anion. Due to low solubility of its compounds and their high availability to plants, chromium plays an important role in the soil processes. To date, no standards are in force in Poland concerning the permissible levels of heavy metals, including chromium, in the soil. It is accepted that the natural content of chromium in the surface layer (0-15 cm) of sandy soil is 2.0-30.0 ppm, in dusty and loamy soil it is 14.8 – 81.0 ppm. The Directive of the Minister of the Environment states that the acceptable content of chromium in built-up and urban areas (at a depth of 0.3-15.0 m) should not exceed 150-190 ppm. [5]The present work is a continuation of the wider study on the determination of the content of heavy metals (Cd, Pb, Cu, Fe, Co) in the soils of the urban agglomeration. Several studies have reported the role of microbes in bioremediation of heavy metals. Bioremediation is the process that uses microorganisms or their enzymes to return the environment, altered by contaminants, back to its original condition (Jankiewicz B et al 1999). Bio sorbents, like microbes, can bind contaminants onto their cellular structures and have been used in environmental cleanups. They can be used to bind with heavy metal pollutants like: bacteria, fungi, algae and industrial agricultural wastes. It has been reported that there may be several potential microbial metal biosorbents.

These include genera of Bacillus, Pseudomonas, Streptomyces, Aspergillus, Rhizopus and Penicilium. A study was done in Michigan's Copper County and the abandoned mining area. Miners left behind a lot of red metal; this was not good because waste from the mining still contained high fractions of copper, so high that almost nothing could grow on it. This study identified high levels of zinc, cesium, lead, and arsenate and mercury resistance in eight copper resistant Pseudomonas strains. These metal resistant strains were capable of bioaccumulation of multiple metals and solubilization of copper. These results may show potential applications for remediation of metal contaminated soils. Since heavy metals cannot be destroyed this makes remediating the heavy metal pollution difficult. Bacteria have unique phenotypes and properties that could change this. It was observed that P. aeruginosa cells grown in biofilms accumulate higher amounts of metals than planktonic cells (Jankiewicz B et al 2000). There was a study done on the multi resistant bacteria Bacillus sp. L14 and its potential application on bioremediation of heavy metals. It was observed in the presence of the heavy metals copper II, cadmium II, and lead II. There was indication that its efficiency to remediate may be prompted through inhibiting the activities of ATPase. With its adaption abilities and promising remediation efficiency of heavy metal bioremediation at low concentrations, this could be very useful for developing efficient metal removal system. This investigation will focus on the bioremediation of heavy metal contaminated soil so that traces of heavy metals won't be found in plants grown in that soil. This will decrease the amount of humans exposed to these toxic chemicals through digestion. Microbes would be the ideal organisms used to bioaccumulate the heavy metals found in contaminated soils due to their abundance on earth. This is a cost effective method of bioremediation of heavy metals

II. MATERIAL AND METHODS

Microorganism used**-** *Pseudomonas aeruginosa* **(**source, from Sagar Hospital, Bangalore)
Soil sample- collected from Durgapur, West Bengal.
Media used-LB broth (HighMedia- Mumbai)
Heavy metal-copper (cu), zinc (zn) and chromium (cr), all are in powder form.

Estimation of Copper (Cu)

Reagents:

0.01 M l copper solution, 0.01 M EDTA standard solution, NaOH, Erio T indicator powder: Tartaric acidophil 10 buffer NH_4Cl (Turek A., et al 2000)

Procedure:

1. Place 10-30 ml (exactly measured) 0.01 M Copper solution in 250 ml flask
2. Add a spatula end of tartaric acid.
3. Add 5 ml of buffer pH 10 and dilute to about 50-100 ml. If turbidity occurs (CuSO) 4) add more tartaric acid.
4. Add Erio T (too much will change the color intensity, so start small).
5. Titrate until the colour changes from violet just too clear blue.
6. Repeat twice to be able to report the rsd of the method.

Estimation of Zinc (Zn)

Reagents: Sodium Acetate, Sodium Hydroxide, Xylenol Orange, EDTA Solution (Jankiewicz B et al 2001)

Procedure:

1. Add 10 mL of 4 M sodium acetate to each unknown Zn^{2+} solution and, using a pH meter, adjust the pH to 5.8 with 3 M sodium hydroxide.
2. Heat the solution to approximately 95EC using the hot plate/stirrer (DO NOT BOIL!).
3. Add 5 drops of xylenol orange indicator (0.2 g/100 mL, 50% alcohol) and a stirring bar to the solution, and titrate **immediately** with EDTA solution.
4. Maintain the solution temperature in the range of 85-95EC. The endpoint is a sudden color change from violet to yellow-pink.

Estimation of Chromium (Cr):

Reagents: 3% Hydrochloric Acid ,Concentrated Orthophosphoric Acid ,Concentrated Sulfuric Acid ,Stock Standard Solution Of Cr (VI)- Obtained By Dissolving 0.2829 G Of Potassium Permagnate , 1,5-Diphenylcarbazide ,Ammonium Peroxydisulphate(Ramakrishna, et al 2011)

Procedure:

1. In order to plot the calibration line, 0.0, 0.2, 0.4, 0.8, 1.4, 2.0, 3.0, 4.0 and 5.0 ml of the working Cr (VI) solutions were measured in turns into 50 ml flasks, which corresponded with the Cr (VI) content in a sample ranging from 0.0 to 1000ppm . One ml of H_2SO_4 (1:1) and 0.3 ml of concentrated H_3PO_4were added and the solution was diluted with distilled water up to the mark.
2. After 5 minutes, 1.0 ml of 1,5- diphenylcarbazide was added to each sample. After another 10 minutes, the absorbance of the solutions was measured in 5 cm cells at λ=543.5 nm against blank test.
3. The dependence between the concentration of chromium (VI) and absorbance is rectilinear over the whole range of the examined concentrations

III. RESULT AND DISCUSSION

Microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater.The bacterial strain was tested for metal tolerance with wide range of hexavalent chromium ,coper and Zinc concentrations (250,500 and 1000 ppm). The results indicated that after 7 days incubation the growth of bacterium was good up to 1000 ppm hexavalent chromium ,coper and Zinc concentration. Based on the metal tolerance level, the strain was subjected to selected concentrations of hexavalent chromium. The experiment shows the percent removal of hexavalent chromium, coper and Zinc after treatment with all microorganisms. Based on the Table 1 for the chromium estimation the chromium absorbed by the pseudomonas aeruginosa are as follows the metal absorbed at 250ppm is 0.31 mg ,similarly for the 500ppm it is found to be 0.42mg while for the 1000ppm its quite promising that is 1.07 mg. However for the titration based analysis for the zinc and the copper

(Table 2 &3) the metals absorbed by the pseudomonas aeruginosa are as follows for the 250ppm its 0.25 mg &0.28mg, for the 500ppm its 0.41 &0.51 mg similarly for the 1000 ppm its 1.33mg &.67mg .Therefore it is been observed that the highest absorption for the zinc at 1000 ppm is found to be 1.33mg while the lowest absorption was found to be for the copper at 1000 ppm was 0.67 mg(Bio resource Technology 2010).

Colorimetric Estimation of Chromium-Table1

Concentration of 'cr'	Absorbance of control soil(540nm)	Absorbance of test soil(540nm)	'Cr' removal by pseudomonas strain
250ppm	0.16	0.12	0.31mg
500ppm	0.35	0.29	0.42mg
1000ppm	0.73	0.52	1.07mg

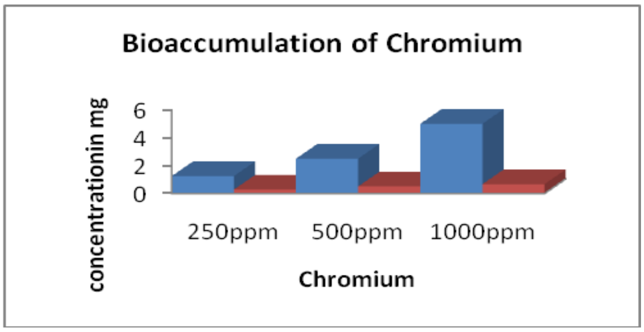

Fig:1

Estimation of Zinc (zn) –Table-2

Concentration of zinc in soil	Absorbance of control soil(610nm)	Absorbance of test soil(610nm)	Zinc(zn) removal by pseudomonas strain
250ppm	0.15	0.12	0.25mg
500ppm	0.31	0.26	0.41mg
1000ppm	0.65	0.42	1.33mg

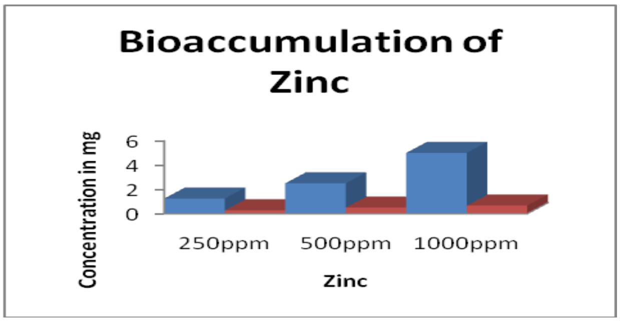

Fig:2

Estimation of Copper (cu) –Table3

Concentration of copper(cu) in soil	Absorbance of control soil(460nm)	Absorbance of test soil(460nm)	Copper(cu) removal by pseudomonas strain
250ppm	0.27	0.21	0.28mg
500ppm	0.53	0.42	0.51mg
1000ppm	0.89	0.73	0.67mg

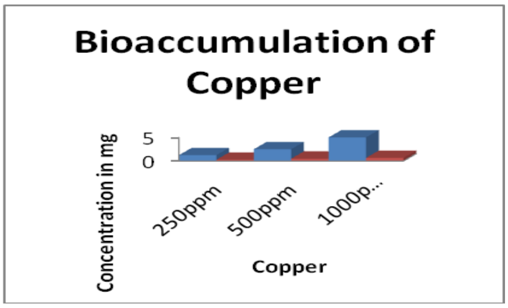

Fig:3

IV. CONCLUSION

It can be concluded from the present study that Pseudomonas aeruginosa has a great potential to remove hexavalent chromium, copper and zinc from aqueous solution as well as effluent and soil. From the above results it is clear that it is a good biosorbents and can be used for removal of heavy metals from industrial wastes and that further study for application of this technology in field is recommended. Pseudomonas aeruginosa can be commercially exploited for the chromium, cooper and Zinc removal efficiently. (Guo, H et al 2010)

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