



BIOACCUMULATION OF THE HEAVY AND TOXIC METALS BY THE NOVEL MICROORGANISMS

¹Anup Baranwal, ¹Jeevana Praharsha, ¹Ramanuj Rauniyar, ¹Rabindra Khadka, ²Ramprasad Kuncham & ³Vinod Nagashetti

1 Centre for R &D in Life Sciences; Biotechnology Research Laboratory #Post Graduate-Department of Biotechnology, Dayananda Sagar Institutions, Dr.C.D.Sagar Centre for Life Sciences, SM Hills, Kumaraswamy Layout, Bangalore-560078.

2 Eurofins Genomics India Pvt Ltd., 183, Gayathri Tech Park, 1st Floor, Whitefield, Bangalore, Karnataka- 560066, India.

3 Dept of Chemical Engineering, Dayananda Sagar College of Engineering, Bangalore-560078, India.

Abstract

The Globe has been encountering a large number of problems related to the various ill management of various resources due to extensive industrialization, increasing population density and a highly urbanized society because of this there is a large amount of effluents is responsible for the degradation of water bodies, Soils and the environment In this study the Novel microorganism are used for the removal of the toxic and heavy metals, despite of being their own relevant properties. Various microorganisms is known to accumulate metal in their cell or cell walls either in the viable or attenuated state by the process called Biosorption .In this study we have targeted the three metals ions such as the Lead, Cobalt and Chromium .The Microorganism used are the *Bacteria E.coli-ATCC12458* and the *Pseudomonas aeruginosa ATCC-15457* and the *Fungi are the Saccromycea .cerevisiae NCL-3570, Pichia stipitis NCL-3497 and the Tricoderma resi NICM-4596*. Among the techniques appropriate for the quantification of metal ions in broth sample, inductively coupled plasma mass spectrometry are likely to be the most widely employed. However, although these techniques are unfailling and sensitive, they endure 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 titration based analysis is been performed for the lead, cobalt and spectroscopic analysis for the chromium. The results are quite promising, Metal absorbed by *E.coli* ,*Pseudomonas aeruginosa* , *Tricoderma.resi*, *S. cerevisiae* and *Pichia stipitis* i.e., Cr, Co and Pb in 1000 ppm was 735ppm, 715ppm,736ppm,745ppm &630ppm for the Chromium ,for the lead it is 750ppm,748ppm,785ppm,755ppm &715ppm and for the cobalt it is 698ppm,715ppm,765ppm,715ppm &690ppm and respectively for the others when compared to control broth sample in 500ppm &250ppm . The present study depicts that bacteria and fungus removes chromium, cobalt and lead efficiently and this could be used for industrial waste management and other environmental contaminants. Therefore it is been concluded that apart from their Novel activities these microorganism can be used as a toxic and metal removing agent.

Keywords: Biosorption, Chromium, Cobalt & Lead in Ppm Level, Industrial Waste Management, Novel Microorganism

Indroduction

Organic micropollutants such as pesticides will only cause detrimental effects to organisms if they are taken up by the organism and can reach a target site where they can do harm. The processes of uptake, biotransformation, and elimination, also termed bioaccumulation or toxicokinetics, modify the concentration of organic chemicals in organisms, and kinetic rate constant models of these processes quantify and yield the time course of internal concentrations. Bioaccumulation and biotransformation are key factors modifying toxicity, and bioaccumulation itself is one of the assessment end points in risk assessment of chemicals Bioaccumulation and biotransformation are key toxicokinetic processes that modify toxicity of chemicals and sensitivity of organisms (Vieira R et al 2000). Bioaccumulation kinetics vary greatly among organisms and chemicals Toxicity in *Escherichia coli* resulting from high concentrations of cobalt has been explained by competition of cobalt with iron in various metabolic processes including Fe-S cluster assembly, sulfur assimilation, production of free radicals and reduction of free thiol pool. Here we present another aspect of increased cobalt concentrations in the culture medium resulting in the production of cobalt protoporphyrin IX (CoPPIX), which was incorporated into heme proteins including membrane-bound cytochromes and an expressed human cystathionine beta-synthase (CBS). The presence of CoPPIX in cytochromes inhibited their electron transport capacity and resulted in a substantially decreased respiration. Bacterial cells adapted to the increased cobalt concentration by inducing a modified mixed acid fermentative pathway under aerobiosis. We capitalized on the ability of *E. coli* to insert cobalt into PPIX to carry out an expression of CoPPIX-substituted heme proteins. Transition metals such as zinc, copper, cobalt or manganese, often incorporated in heme analogs used in porphyrin replacement studies, are considered toxic at elevated concentrations (Gadd, G.M et 1990). Their coordination chemistry and redox properties can lead to non-specific binding to various proteins, displacement of other metals (usually iron) from their natural binding sites and generation of free radicals (reviewed in . Recent studies showed that cobalt toxicity in *E. coli* and *S. enterica* is mainly due to its direct competition with iron especially affecting the synthesis of Fe-S clusters or indirectly via cobalt-mediated oxidative depletion of free thiols pool. On the other hand, the concentrations of cobalt chloride ranging from 100 µM up to 400 µM were found suitable for optimal *E. coli* growth and expression of cobalt-substituted iron-type nitrile hydratase Cobalt is an essential trace element for many living organisms, as it plays a key biological role as the

centrally coordinated ion in cyclic tetrapyrroles known as corrin rings. Corrinoids, including the coenzyme vitamin B₁₂(adenosylcobalamin [AdoCbl]) and its cobalamin (Cbl) derivatives, are coenzymes in a number of central metabolic reactions. Cobalt can also be associated directly with cobalt-dependent enzymes (noncorrin enzymes). To acquire sufficient cobalt for metabolism, bacteria have high-affinity uptake systems to scavenge Co²⁺ from the environment, where it is often available only in trace amounts (Singh et al 2010). When external metal concentrations are very high, Co²⁺ accumulation may become toxic, and excess Co²⁺ can be removed from cells by efflux systems. Chromium is the seventh most abundant metal in the earth's crust and is naturally occurring in soil, but can be found in all phases of the environment. In fresh water concentrations range from 0.1 to 117 µg l⁻¹, while concentrations in serpentine soils can reach up to 125 g kg⁻¹. Chromium has a wide industrial use and is released into the environment by processes such as electroplating, tanning, polishing, painting, pigment manufacture and wood preservation. These anthropogenic activities have led to a widespread contamination of the environment. Chromium is not an essential element for plant nutrition, but may nevertheless be taken up by plants (Verma et al 2001). Only two oxidative forms Cr III and Cr VI are stable enough to occur naturally, but they are drastically different in charge, physiochemical properties as well as chemical and biochemical reactivity. Overall, Cr VI is considered to be the more toxic than Cr III. As an anion it is negatively charged and highly soluble in water and thus has a better bioavailability and is more mobile than the cationic form Cr III. Like other heavy metals chromium is phytotoxic and can result in growth inhibition, degrade photosynthetic pigments, lead to nutrient and water imbalance and induce oxidative stress (McLean J et al 2001). Terrestrial plants take up essential and non-essential elements from the soil, while aquatic plants take up ions from all their surroundings. There are many studies of the effects of chromium on higher plants, but in respect to algae most research focuses on biosorption abilities of certain species for phytoremediation to remove chromium from contaminated water (Hussein H et al 2004). Only few studies investigate the effects of chromium on physiological processes in the algal cells and none seem to determine where chromium is located intracellular (Subudhi E et al 2008). Nevertheless it is of relevance to study not only how much metal can be accumulated, but also to understand how the contaminant is entering a plant cell, what effects it causes on cell physiology, as well as development, whether it is compartmentalized and which detoxification mechanisms exist. This is particularly important since plants are an essential source of food to animals and humans and they are also used as resource for medical drugs and other commonly used products (Chatterjee et al 2011). When plants are cultivated in contaminated areas there is a risk of heavy metal accumulation, allowing contaminants such as chromium to enter the food chain. Cr VI is not only considered highly toxic to plants but also to mammals and humans, due to its detrimental effects on several organs and tissues, it is a potential carcinogen (Parvathi et al 2007).

Material and Methods

Estimation of Lead (Pb)

Synopsis: Lead is held in solution by weakly chelating tartrate so that it may react with Erio T to form a bluish violet color. The lead is titrated with standard EDTA. The end point is observed by a loss of the bluish violet color as the last of the lead-Erio-T complex is consumed (D.Hooyberghs et al 1992, & Cabuk A et al 2005 & Y. Zhan et al 2009).

Reagents:

0.01 M lead solution, 0.01 M EDTA standard solution, NaOH, Erio T indicator powder, Tartaric acid, pH 10 buffer NH₄Cl

Procedure:

1. Place 10-30 ml (exactly measured) 0.01 M lead 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 a turbidity occurs (Pb(OH)₂) 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 to clear blue.
6. Repeat twice to be able to report the rsd of the method.

Estimation of Cobalt (Co)

Synopsis: The quantitative determination of many metal ions in solution can be achieved by titrating with a standard solution of a Lewis base (ligand). A necessary requirement is that the ligand combines (complexes) quantitatively with a particular metal ion under the solution conditions (P. R. Norris et al 1977). The most common ligand is the anion of ethylenediaminetetraacetic acid (EDTA, H₄Y). The titrant is usually prepared by dissolving the disodium salt of this acid, Na₂H₂Y, since the acid is only slightly soluble in water.

Co²⁺(aq) + Y⁴⁻(aq) → CoY²⁻(aq)

CoIn²⁺(aq) + Y⁴⁻(aq) → CoY²⁻(aq) + In(aq) violet yellow-pink

Reagents: Sodium Acetate, Sodium Hydroxide, Xylenol Orange, EDTA Solution

Procedure:

1. Add 10 mL of 4 M sodium acetate to each unknown Co²⁺ solution and, using a pH meter, adjust the pH to 5.8 with 3 M sodium hydroxide.
2. Heat the solution to approximately 95°C 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.

- 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):

Synopsis: The diphenylcarbazide method involves the formation of a purple-red complex of chromium (VI) with 1,5- diphenylcarbazide in the acid medium. Its molar absorption coefficient at $\lambda=545$ nm is $4.3 \cdot 10^4$. The obtained color is a result of a reaction of chromium (III) in statu nascendi, still not hydrated with water molecules, produced in the process of the reduction of Cr (VI) with 1, 5- diphenylcarbazide, which is at the same time oxidized to form the diphenylcarbazone (Volesky et al 1995 & Khambhaty Y et al 2009).

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

Procedure:

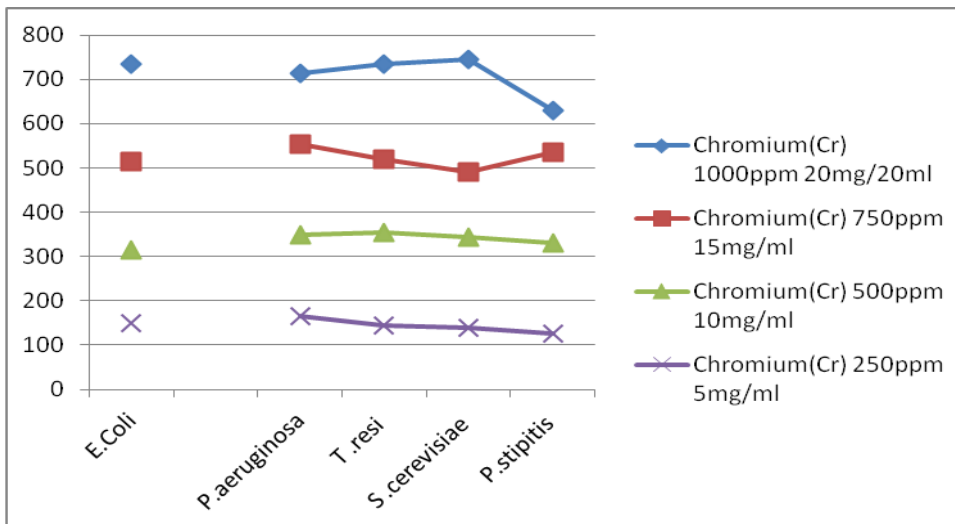
- In order to plot the calibration line, 0.0, 0.2, 0.4, 0.8, 1.4, 2.0, 3.0, 4.0 and 5.0 ml of the working Cr (VI) solutions were measured in turns into 50 ml flasks, which corresponded with the Cr (VI) content in a sample ranging from 0.0 to 1000ppm . One ml of H₂SO₄ (1:1) and 0.3 ml of concentrated H₃PO₄ were added and the solution was diluted with distilled water up to the mark.
- 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 $\lambda=543.5$ nm against blank test.
- The dependence between the concentration of chromium (VI) and absorbance is rectilinear over the whole range of the examined concentrations

Result and Discussion

The bacterial strain and fungal strains was tested for metal tolerance with wide range of hexavalent chromium, cobalt and lead concentrations (250,500,750 and 1000 ppm). The results indicated that after 24 hours incubation the growth of bacterium was good up to 1000 ppm hexavalent chromium cobalt and lead concentration. Based on the metal tolerance level, the strain was subjected to selected concentrations of hexavalent chromium (200, 400, 600, and 800 and 1000 ppm) for absorption up to seven days. When different concentrations of hexavalent chromium, cobalt and lead were plated with nutrient agar, the microorganisms were able to resist up to 1000 ppm of hexavalent chromium, cobalt and lead. The biochemical tests show the percent removal of chromium, cobalt and lead after treatment with all microorganisms (Table1).

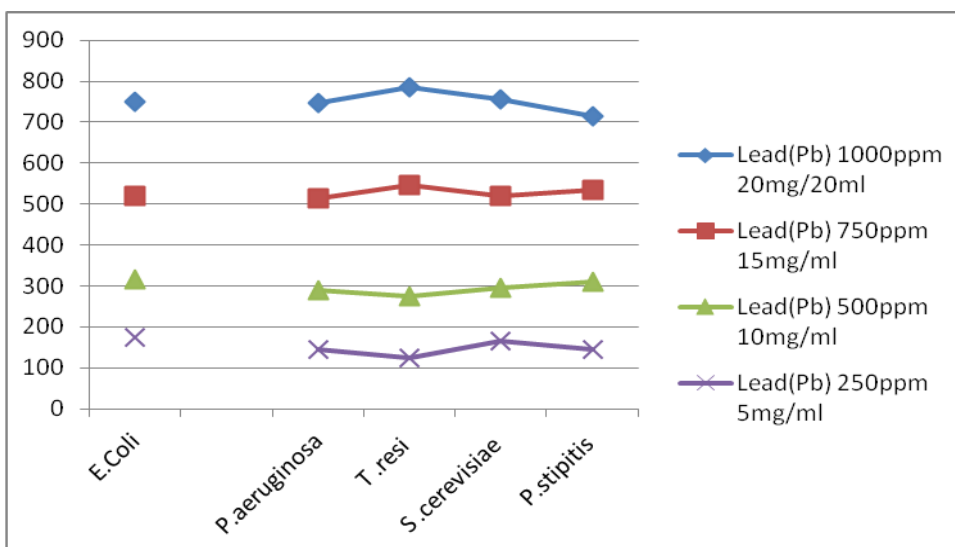
Table1: Chromium, Lead & Cobalt Bio-Absorption

	Chromium(Cr)				Lead(Pb)				Cobalt(Co)			
	1000ppm 20mg/20ml	750ppm 15mg/ml	500ppm 10mg/ml	250ppm 5mg/ml	1000ppm 20mg/20ml	750ppm 15mg/ml	500ppm 10mg/ml	250ppm 5mg/ml	1000ppm 20mg/20ml	750ppm 15mg/ml	500ppm 10mg/ml	250ppm 5mg/ml
<i>E.Coli</i>	735	515	315	150	750	520	315	175	698	515	315	155
<i>P.Aeruginosa</i>	715	555	350	165	748	515	290	145	715	545	285	125
<i>T.resi</i>	736	520	355	145	785	545	275	124	765	520	295	135
<i>S.Cerevisiae</i>	745	490	345	140	755	520	295	165	715	575	300	145
<i>P.Stipitis</i>	630	535	330	125	715	535	310	145	690	562	310	165



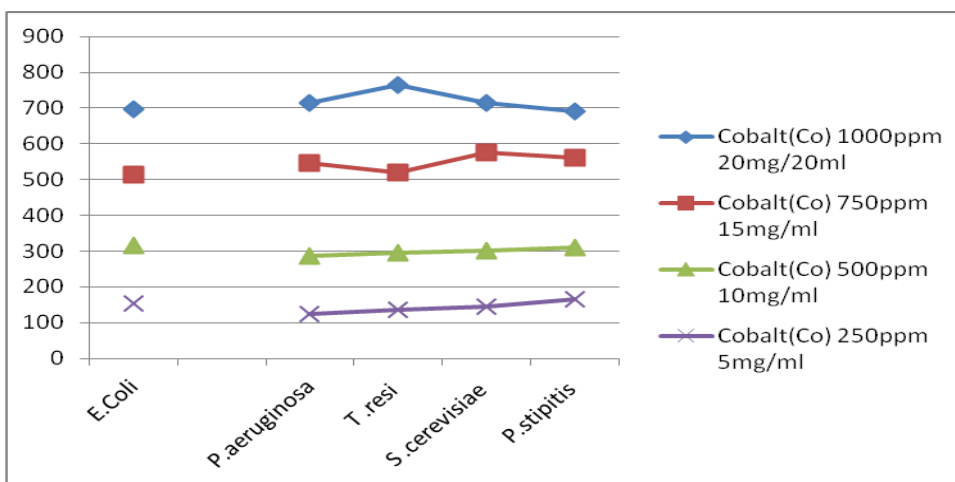
Chromium-Absorption Fig: 1

Out of five microorganisms Chromium was absorbed maximum by the *S.Cerevisiae*, which is 753/1000ppm, which will be 75.3 % and the minimum Bio-absorption was seen in the microorganism *P.stipitis* which is 50.0%, that is 125/250ppm



Lead-Absorption Fig: 2

Out of five microorganisms Lead was absorbed maximum by the *T.resi*, which is 785/1000ppm, which will be 78.5 % and the minimum Bio-absorption was seen in the microorganism *P.stipitis* which is 49.6%, that is 124/250ppm



Cobalt-Absorption Fig: 3

Out of five microorganisms Cobalt was absorbed maximum by the *S.Cerevisiae* which is 575/750ppm, which will be 76.67 % and the minimum Bio-absorption was seen in the microorganism *P.aeruginosa* which is 50.0%, that is 125/250ppm

Conclusion

The experiments conducted on the bioaccumulation of heavy metals (Cobalt, Chromium, and Lead) from aqueous solution by *S.cerevisiae* NCL-3570, *Pichia stipitis* NCL-3497, *Trichoderma reesei*, *E.coli* and *Pseudomonas aeruginosa* has shown that these organisms are having high potential of removing heavy metals through biosorption mechanism. Microorganism inoculated with different concentration of heavy metals (1000ppm, 750ppm, 500ppm and 250ppm) after 7 days incubation has shown to accumulate heavy metals of varied concentration among which *S.cerevisiae* NCL-3570, *Trichoderma reesei* and *Pseudomonas aeruginosa* are the best for the bioaccumulation of heavy metals from aqueous solution. Thus bioaccumulation of metals contaminated aqueous solution using *S.cerevisiae* NCL-3570, *Trichoderma reesei* and *Pseudomonas aeruginosa* can be considered as the most cost effective means to remediate aqueous solution.

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