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# Effect of Heavy Metal Uptake by *E. coli* and *Bacillus* sps

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## Abstract

Over the past century, unrestricted mining, extensive industrialization, modern agricultural practices and faulty waste disposal methods have resulted in the release of unprecedented levels of toxic heavy metals like Cd, Hg, Ag, Sn, Pb, Cu, Co, Mn, Zn, etc into the environment. Many metals are essential for microbial growth in less concentration, yet are toxic in higher concentrations. Biosorption is an attractive alternative approach which involves the binding or adsorption of heavy metals to living or dead cells. Many microbes have the ability to selectively accumulate metals.

The present study is intended to analyze the uptake systems of Bacillus and *E. coli* against different conc. of heavy metals like Zn, Cu, Cd, and Hg in their salt form incorporated into nutrient broth medium observed over a regular interval of time. Analysis was based on how much of the metal from the original conc. Used was left behind in the media after the rest being up taken by the organism. This was done using AAS which was indirectly the representation of percent uptake of heavy metal by the respective organism.

The study showed that Gram –ve organisms like *E. coli* exhibited more resistance to metals like Zn, Cu and Hg in relative comparison with Gram +ve organisms like Bacillus. *Bacillus* sps was less sensitive to effect of Cd than in *E. coli*.

**Keywords:** Bioremediation; Heavy metals; Toxicity; Biosorption; Uptake capacity; AAS

## Introduction

The major sources of water pollution include municipal, industrial and agricultural wastes through which different varieties of pollutants like inorganic and organic pollutants, toxic heavy metals, hazardous wastes, pesticides, herbicides, fertilizers, sediments, petroleum products, detergents etc. are introduced into water bodies. Of all these, toxic heavy metals are of primary concern due to their immediate and devastating effects on biological systems [1].

Looking into the increased environmental awareness, removal of toxic heavy metals is of prime importance and relevance for a healthy environment. Removal strategies are both in terms of conventional and biosorption methods. A wide variety of microbes have good potentials of metal absorption/adsorption. Metal transport systems in microbes are of varying specificity. Rates of uptake can depend on the physiological state of cells, as well as the nature and composition of the environment or growth medium. With toxic heavy metals, permeabilization of cell membranes can result in further exposure of intracellular metal-binding sites and increase passive accumulation. Intracellular uptake may ultimately result in death of sensitive organisms unless a means of detoxification is induced or already possessed [2]. Different microbes have varied capacity of metal uptake in differing concentrations based on their relative tolerance levels. The present work is oriented with the following objectives as to study growth kinetics of bacterial cells under heavy metal environment and to help the formulation of new possibilities in bacterial biosorbents of heavy metals. Hence an attempt was made in the present study to see the biosorption potentials of two selected organisms *Bacillus* sps and *E. coli*

## Materials and Methods

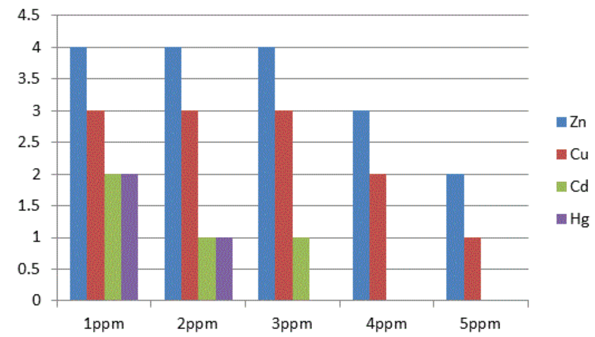
*Bacillus* sps (Gram +ve) and *E. coli* (Gram –ve) were selected for the present study and cultured using Nutrient agar (NA) and Nutrient Broth (NB).

Four heavy metals in their salt form Zinc sulphate ($ZnSO_4.7H_2O$), Copper sulphate ($CuSO_4.5H_2O$), Cadmium chloride ($CdCl_2$) and Mercuric chloride ($HgCl_2$) were used in ppm concentrations.

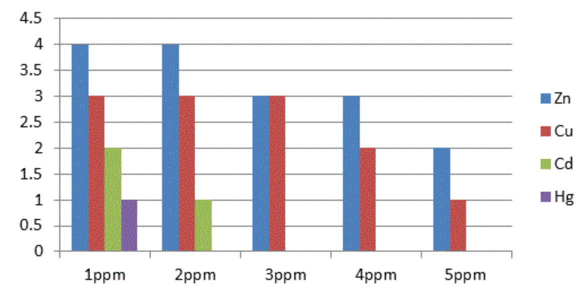
Initial screening with relatively high concentration of each of the heavy metallic salts (1-5 ppm) was carried out to assess the broad range effect of the heavy metal and its tolerance by the organisms with incubation at 37°C for 2, 4, 6 and 8 days period. After every 48 hour incubation, the media was subjected for turbidometric analysis to study the growth of the organism in the presence of the heavy metal under a particular concentration. A set of flasks were also maintained as control with no heavy metal for all the days. Nutrient agar medium was also employed to substantiate the effect of heavy metal on the growth pattern of the organisms. A direct comparison for the effect of a particular concentration of a metal on both the organisms was available from this technique. Equally this method also supported the selection of conc. of heavy metal for its sub lethal dosage. Sub lethal dosages were fixed as 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0 ppm for each metal except for Hg where the concentration was limited to 1 ppm. After incubation and turbidometric analysis, the samples were centrifuged at 4000-5000 rpm for 10 minutes to separate out the cell mass and the broth medium. The cell mass was discarded and the supernatant was used for the heavy metal analysis. The analysis was based on how much of the metal from the original concentration used was left behind in the media after the rest being absorbed/adsorbed by the organism. This was done using Atomic Absorption Spectroscopy (AAS) which was indirectly the representation of percent uptake of heavy metal by the respective organism. The results of percent uptake of heavy metal was calculated and tabulated.

## Results

In case of initial screening procedures with 1-5 ppm concentration of selected heavy metals, *Bacillus* sps showed good growth with 1 ppm and 2ppm of Zn and1 ppm of Cu. With increase in the concentration the growth of *Bacillus* decreased indicative of toxic effect of the metal above 3 ppm levels of Zn and Cu. Cadmium had an effect at 2 ppm conc. while Hg effect on growth of *Bacillus* was observed with 1 ppm itself as all other concentrations showed very little or no growth of *Bacillus* (Graph 1) *E. coli* showed a higher rate of tolerance as compared to that of *Bacillus*. With Zn and Cu, the growth rate of was fair until 3 ppm and thereafter showed a little decrease. With Cd and Hg, the growth was stable till 2 ppm but decreased with a high rate as the concentration was increased (Graph 2).

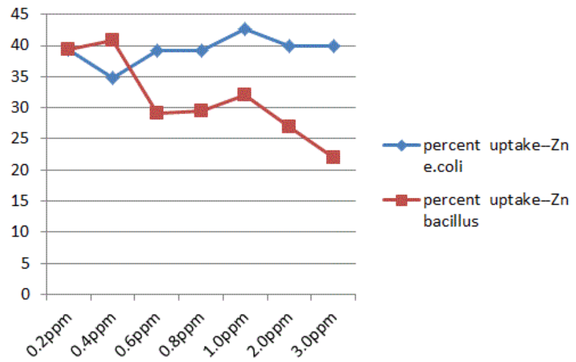



**Graph 1:** Growth pattern wrt *E .coli* on NA

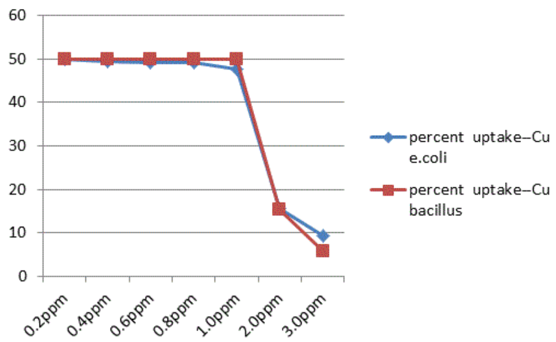



**Graph 2:** Growth pattern wrt *Bacillus* on NA

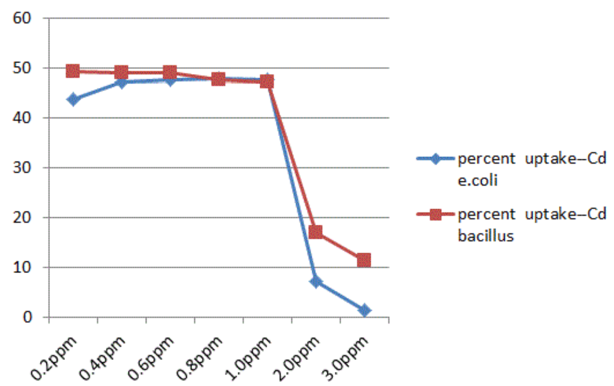
Based on these results of broad range concentration of the selected heavy metals, further analysis was done with sub lethal dosages and effect of percent uptake of heavy metal was compared between *E. coli* and *Bacillus.*



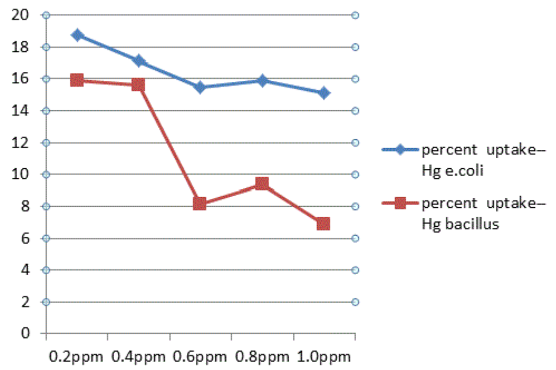

**Graph 3:** % absorption of Zn by *E. coli* and *Bacillus* sps




**Graph 4:** % absorption of Cu by *E. coli* and *Bacillus* sps




**Graph 5:** % absorption of Cd by *E. coli* and *Bacillus* sps




**Graph 6:** % absorption of Hg by *E. coli* and *Bacillus* sps

## Discussion

It is clear from the results that *E. coli* showed good absorption/adsorption potential with three of the four heavy metals used in the study that is with Zn, Cu and Hg (Graphs 3,5 and 6 respectively)when compared to *Bacillus* sps. Bacillus on the other hand showed a better tolerance and uptake with Cd (Graph 5). Similar findings were reported with respect to Cd biosorption studies [3,4]. It was also reported that metal binding capacities of *E. coli* was relatively higher than *Bacillus* by [5]. Different microbial sources like the green algae *Closterium moniliferum* (Bory) ehrenb [6] and several fungi [7,8] are indeed good biosorbents like bacteria. It was reported earlier increased metal uptake by bacteria led to their death by various cell mediated mechanisms [3,8]. The above said mechanisms could have been true in the present study since the death rate of the bacterial cells was observed to be increasing with the increasing conc. of metal taken into the cells.

In conclusion the present study highlights the microbes possess a high potential for metal remediation strategies and can minimize the bioavailability and biotoxicity of heavy metals. Gram negative bacteria like *E. coli* have proved to be good biosorbents for metals like Zn, Cu and Hg, while Bacillus can be a biosorbent of choice against Cd toxicity. These organisms can be exploited as ecological indicators for bioremedial purposes.

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