http://bma.org.in/ijfas.aspx Int. J. Fund. Appl. Sci. Vol. 4, No. 1 (2015) 9-13

ORIGINAL ARTICLE Open Access

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences

Study of Heavy metal uptake by *Bacillus* sp and *E. coli*

Chandrareddy Vijayadeep [1]*, Pothukuchi Subrahmanya Sastry[2] and Marigowda Jayaramu[3]

[1]Department of Microbiology, Maharani Lakshmi Ammanni College for Women, Bangalore- 560012. India; [2]Department of Botany & Microbiology,Vijaya College, Bangalore- 560011, India; [3]Department of Microbiology, Government Science College, University of Science, Tumkur-572101, India

Manuscript received 28th Nov, 2014, revised , 24th Dec, 2014 & accepted 25th Dec, 2014

Abstract

Background: 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. Various methods are available for the removal and management of heavy metals: Conventional physico-chemical methods and emerging biosorption methods. 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. **Aim:** The present study was intended to analyze the uptake systems of *Bacillus* and *E. coli* against different concentration 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. **Methodology:** Analysis was based on how much of the metal from the original concentration used was left behind in the media after the rest being up taken by the organism. This was done using Atomic Absorption Spectrophotometry (AAS) which was indirectly the representation of percent uptake of heavy metal by the respective organism. **Results:** 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*. **Conclusion:** *Bacillus* sps was less sensitive to effect of Cd than in *E. coli*

Keywords: *Bacillus, E. coli*, Bioremediation, Heavy metals, Toxicity, Biosorption, Uptake capacity, AAS

1. Introduction

The natural water systems of the earth are being converted into a polluted state due to the addition of large amounts of foreign substances which are capable of causing harm to man or any other living organism supported by environment. 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. Heavy metals comprise an ill-defined group of approximately 65 metallic elements of density greater than 5.9 with diverse physical, chemical and biological properties but generally having the ability to exert toxic effects towards microorganisms. Almost every index of microbial metabolism and activity can be adversely affected by elevated concentration of heavy metals.[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

**Corresponding author*
Full Address :
Department of Microbiology, Maharani Lakshmi Ammanni College for Women, Bangalore- 560012. India
Phone no. +919886755403
E-mail: vijayadeep1979@gmail.com

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 was 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 this study to see the biosorption potentials of two selected organisms *Bacillus* sps and *E. coli.*

2. 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 (ZnSO4.7H2O), Copper sulphate (CuSO4.5H2O), Cadmium chloride (CdCl2) and Mercuric chloride (HgCl2) were used in ppm concentrations.

Initial screening with relatively high conc. of each of the heavy metallic salts (1-5ppm) 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 conc. 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.[3]

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.[4]

Table I: Growth pattern of *E. coli* and *Bacillus* on NA

E. coli					
Metal	**1ppm**	**2ppm**	**3ppm**	**4ppm**	**5ppm**
Zn	++++	++++	++++	+++	++
Cu	+++	+++	+++	++	+
Cd	++	+	-	-	-
Hg	++	+	+	-	-
***Bacillus* sp**					
Metal	**1ppm**	**2ppm**	**3ppm**	**4ppm**	**5ppm**
Zn	++++	++++	+++	+++	++
Cu	+++	+++	+++	++	+
Cd	++	+	-	-	-
Hg	++	+	-	-	-

++++ = good growth; +++ =Fair growth; ++ = less growth; + =very little growth; - = no growth

3. Results and discussion

In case of initial screening procedures with 1-5 ppm concentration of selected heavy metals, *Bacillus* sp 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 inhibitory effect at 2 ppm concentration while inhibitory effect of Hg on growth of *Bacillus* was observed with 1 ppm itself as all other concentrations showed very little or no growth of *Bacillus* (Table I, Fig I)

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 there after 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. (Table I, Fig II)

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

It is clear from the results that *E.coli* showed good absorption/adsorption potential wth three of the four heavy metals used in the study that is with Zn, Cu and Hg (Figure III, IV, VI respectively) when compared to *Bacillus* sps (Table II, III, V). Bacillus on the other hand showed a better tolerance and uptake with Cd (Table V, Fig V). Similar findings were reported with respect to Cd bio sorption studies[5,6]. It was also reported that metal binding capacities of *E. coli* was relatively higher than *Bacillus.*[5]

Different microbial sources like the green *algae Closterium moniliferum* (*Bory*) *ehrenb*[7]and several fungi[8,9] are indeed good biosorbents like bacteria. It was reported earlier increased metal uptake by bacteria led to

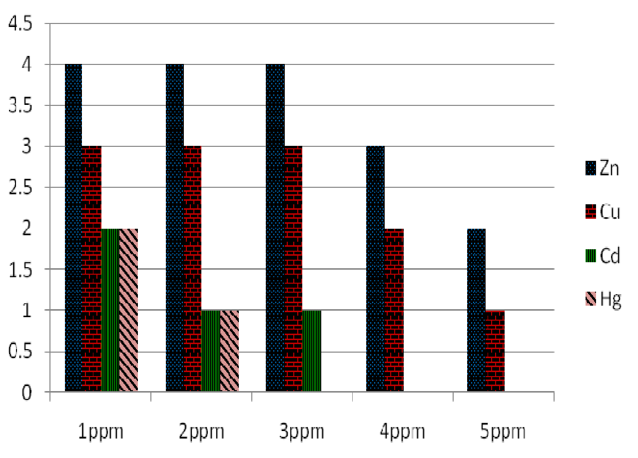

Figure I: Growth pattern of *E. coli* on NA

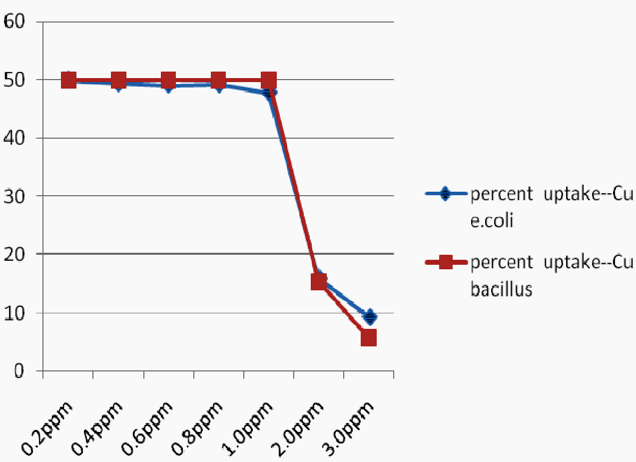

Figure IV: % absorption of Cu by *E. coli* and *Bacillus* sp

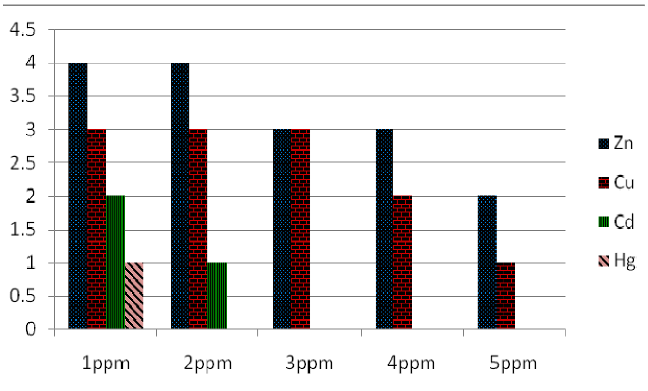

Figure II: Growth pattern of *Bacillus* on NA

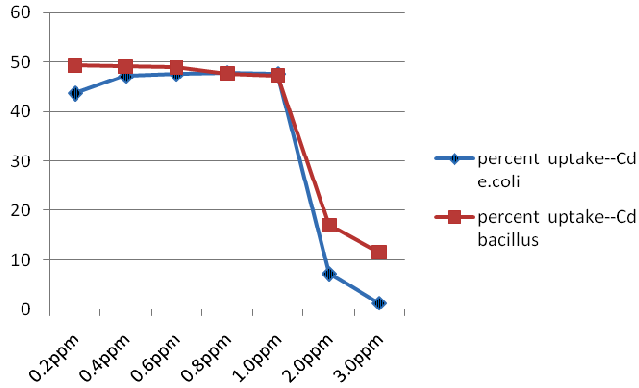

Figure V: % absorption of Cd by *E. coli* and *Bacillus* sp

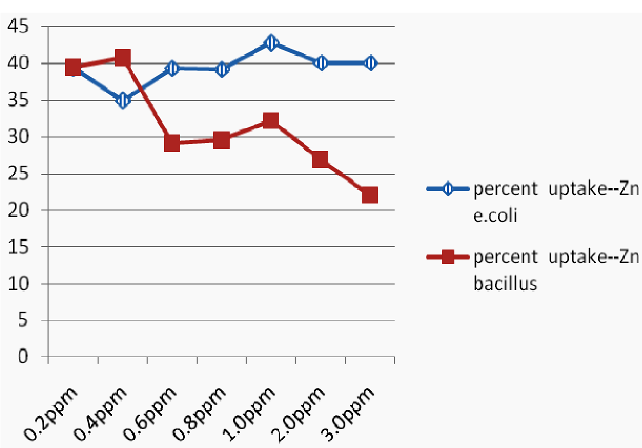

Figure III: % absorption of Zn by *E. coli* and *Bacillus* sp

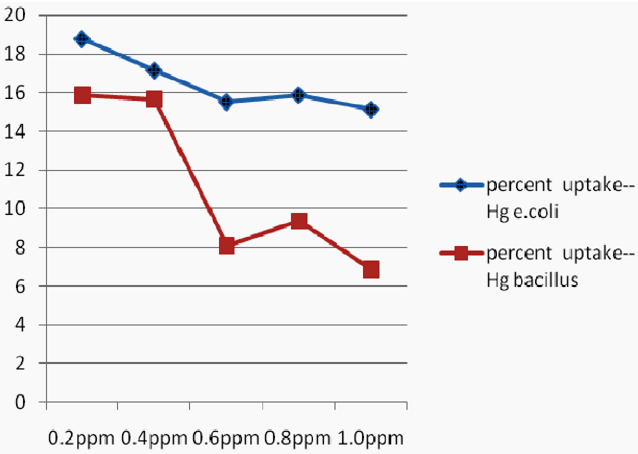

Figure VI: % absorption of Hg by *E. coli* and *Bacillus* sp

Table II: % absorption of Zn by *E. coli* and *Bacillus* sp

E. coli	2nd day	4th day	6th day	8th day	avg	*Bacillus*	2nd day	4th day	6th day	8th day	avg
0.2ppm	90	85	80	60	39.37	**0.2ppm**	75	75	80	85	39.37
0.4ppm	72	70	70	67	34.87	**0.4ppm**	65	78	90	93	40.75
0.6ppm	79	79	79	77	39.25	**0.6ppm**	50	53	60	70	29.12
0.8ppm	85	79	75	74	39.12	**0.8ppm**	56	56	60	64	29.5
1.0ppm	91	83	89	79	42.75	**1.0ppm**	60	62	65	70	32.12
2.0ppm	95	90	72	63	40	**2.0ppm**	52	52	55	56	26.87
3.0ppm	99	84	84	53	40	**3.0ppm**	43	44	44	45	22

Table III: % absorption of Cu by *E. coli* and *Bacillus* sp

E. coli	2nd day	4th day	6th day	8th day	avg	*Bacillus*	2nd day	4th day	6th day	8th day	avg
0.2ppm	100	100	100	100	50	**0.2ppm**	100	100	100	100	50
0.4ppm	95	100	100	100	49.37	**0.4ppm**	100	100	100	100	50
0.6ppm	93	100	100	100	49.12	**0.6ppm**	100	100	100	100	50
0.8ppm	94	100	100	100	49.25	**0.8ppm**	100	100	100	100	50
1.0ppm	95	95	96	96	47.75	**1.0ppm**	100	100	100	100	50
2.0ppm	17	27	41	41	15.75	**2.0ppm**	23	26	25	49	15.37
3.0ppm	12	20	21	21	9.25	**3.0ppm**	5	12	12	17	5.75

Table IV: % absorption of Cd by *E. coli* and *Bacillus* sp

E. coli	2nd day	4th day	6th day	8th day	avg	*Bacillus*	2nd day	4th day	6th day	8th day	avg
0.2ppm	80	85	85	100	43.75	**0.2ppm**	95	100	100	100	49.37
0.4ppm	90	93	95	100	47.25	**0.4ppm**	93	100	100	100	49.12
0.6ppm	93	93	95	100	47.62	**0.6ppm**	92	100	100	100	49
0.8ppm	94	94	95	100	47.87	**0.8ppm**	85	96	100	100	47.62
1.0ppm	92	95	97	100	47.75	**1.0ppm**	84	94	100	100	47.25
2.0ppm	3	10	13	32	7.25	**2.0ppm**	30	30	37	39	17
3.0ppm	2	2.7	2.7	3	1.35	**3.0ppm**	13	23	23	33	11.5

Table V: % absorption of Hg by *E. coli* and *Bacillus* sp

E. coli	2nd day	4th day	6th day	8th day	avg	*Bacillus*	2nd day	4th day	6th day	8th day	avg
0.2ppm	15	25	50	60	18.75	**0.2ppm**	6	11	23	25	8.12
0.4ppm	20	30	40	47	17.12	**0.4ppm**	30	35	40	48	19.12
0.6ppm	28	35	45	60	15.5	**0.6ppm**	30	33	37	43	15.62
0.8ppm	30	28	33	36	15.87	**0.8ppm**	14	16	20	25	9.37
1.0ppm	25	29	32	35	15.12	**1.0ppm**	5	10	15	25	6.87

their death by various cell mediated mechanisms[10]. 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.

Conclusion

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.

Acknowledgement

I gratefully acknowledge the assistance rendered by Department of Botany, Microbiology and Biotechnology of my current workplace .I also thank Shashirekha, Chief Chemist, Institute of Geology and Mines, Government of Karnataka, Bangalore and Karthikeyan. S, Chief Analyst Shukla B.P, Zonal Officer, Central Pollution Control Board, Bangalore for their regardful assistance in analysis of results.

Conflict of interest

The author's declares none.

References

1. Gadd G. M. Heavy metal pollutants: Environmental and Biotechnological aspects. *Encyclopedia of Microbiology*, (1992) **2:**342-360.
2. Gadd G. M. Heavy metal accumulation by bacteria and other microorganisms. *Experntia*, (1990) **46**:834-840.
3. Tamilselvan N,Saurav K and Kannabiran K. Biosorption

of selected toxic heavy metals using algal species *Acanthopora spicefera*. *Pharmacologyonline*, (2011) **1**: 518-528

4. Sravani M, Sridevi V, K. Vijay Kumar and Harsha N. A Comparative Study on Determination of Physico Chemical parameters of Bio sorption of Lead (II) by *Aspergillus niger*, NCIM 616 using AAS & ICPMS. *IOSR Journal of Pharmacy*, (2012) **2**(**6**):60-64

5. Gourdon R, BhendeS, Rus E and Sofer S.S. Comparison of Cadmium bio sorption by Gram-positive and Gram-negative bacteria from activated sludge. *Biotechnology Letters,* (1990) **12(11)**: 839-842.

6. Gelmi M, Apostoli P, Cabibo E, Porrru S,Alessio L and Turano A. Resistance to cadmium salts and metal absorption by different microbial species. *Current Microbiology*, (1994)**29**:335-341.

7. Sastry P.S and Choudhary B.R. Uptake of heavy metals by unicellular green alga *Closterium moniliferum*. *Phyko,* (1988) **27**:140-145.

8. Hoyle B.D and BeveridgeT.J.Metal binding by the peptidoglycan sacculus of *Escherichia coli* K-12. *Canadian Journal of Microbiology*,(1984) **30:**204-211.

9. Ross I.S. Uptake of zinc by fungi. Stress Tolerance in fungi. Marcel Dekker, New York, 1993(b) 237-257.

10. Volesky B, Phillips M.H.A.Biosorption of heavy metals by *Saccharomyces cerevesiae*. *Applied Microbiology Biotechnology,* 1995, **42:**797-806.