Screening for Heavy Metal Bioaccumulating Bacteria from an Urban Wetland

Sushma M[1]. and Amudan R[2].

Department of Biotechnology, S.I.E.S. College of Arts Science and Commerce,

Sion (West), Mumbai 400022, INDIA - ramudhan@gmail.com, corresponding author

Abstract

Wetlands in urban settings are subject to huge amounts of heavy metal pollution due to the presence of various industries. The micro-organisms present in such waters naturally develop resistance to such high concentration of metals. These micro-organisms can be used in bioaccumulation and bioremediations studies. In the present study, bacteria were isolated from the Mithi river and their tolerance levels against three metals viz. Chromium (Cr), Copper (Cu) and Lead (Pb) were quantified. Their capabilities to bio accumulate and bio remediate these metals were studied by UV-VIS spectroscopy. The bacteria showed a high level of tolerance ranging between 3000-5000 ppm for these metals. Algae showed tolerance in low concentrations of Chromium and Copper upto250ppm and higher concentrations of around 500ppm of Lead. The ability of algae to bio accumulate these metals were studied by U.V-VIS spectroscopy.

Key words: Bio remediation, Bioaccumulation, Heavy metals, Bacteria, Algae

Introduction:

The discharge of waste water containing heavy metals into natural water bodies has adverse environmental effects. These heavy metals are accumulated in the environment as they cannot be degraded and pose a threat when they find their way into the food chain. In order to survive in heavy metal polluted environments, many micro-organisms have developed resistance to toxic metal ions (Nies and Silver, 1995～ Nies,1999). The property of some species of bacteria to extract metals from their surroundings, have been utilized to purify industrial effluents (Chatterjee *et al*., 2012).

Sources of lead in pollution are the automobile industry, petroleum and paints. Exposure to lead causes lead poisoning and may result in cardio-vascular disorders, mental impairment in children, kidney malfunction, etc. Copper is widely used metal in electrical and plumbing industries, agriculture, fungicides, tanning, ceramic and other chemical industries. Exposure to copper can cause irritation of the nose, mouth and eyes, headaches, stomach aches, dizziness, vomiting and diarrhea, liver and kidney damage and even death. Long term exposure causes Wilson's disease, an inherited (genetic) disorder in which copper builds up in the liver. The major sources of Chromium are Steel industry, construction, leather tanning, dye & pigment and wood preservative industries. Exposure to chromium can cause dermatitis. Hexavalent chromium in solution is a carcinogen.

Material and methods:

2.1 Screening for heavy metal accumulators: Water sample was collected from the Mithi river. The organisms were isolated on Nutrient agar plates. MIC of the isolated organisms was performed. The media used for MIC was Nutrient broth containing 500ppm-3000ppm of individual heavy metals Cupric Sulphate, Potasium Dichromate and Lead (II) acetate.

2.2 Identification:The selected organisms were identified using biochemical methods as specified in Bergey's Manual of Systematic Bacteriology Volume 1 & 2, (Garrity et al., 2005).

2.3 U.V. Spectrometry of certain isolated bacteria M5. X8,X9,X11: The bacteria were grown in 0.1% yeast extract water containing the individual heavy metals. The concentration of Lead (II) acetate ,Cupric Sulphate and Potasium Dichromate was 100 ppm. A control without any metal was also maintained. 0.1 ml of culture of 0.05O.D at 530nm was inoculated and all the media were incubated for 72 hours at room temperature. The concentration of the heavy metals before and after growth of organisms was determined by UV spectrometry. The instrument used was Nanophotometer P-class by Implen.

2.4 Enrichment of algae: A consortium of algae was enriched in Alan-Chu medium and inoculated in different media containing individual metals viz 100–500 ppm of Lead (II) acetate,Copper sulphate and Potassium dichromate respectively. A control without any metal was also maintained.1 gm of algae was inoculated in the media and incubated at room temperature in the presence of light for 30 days.

2.5 U.V. Spectrometry of algal cells: The concentration of the heavy metal in the algal cells was determined by U.V. spectrometry. The algal cells of known weight were taken and crushed and mixed in 5ml of distilled water. The pellet

was centrifuged and heavy metal concentration was determined by U.V. spectroscopy. The instrument used was Nanophotometer P-class by Implen.

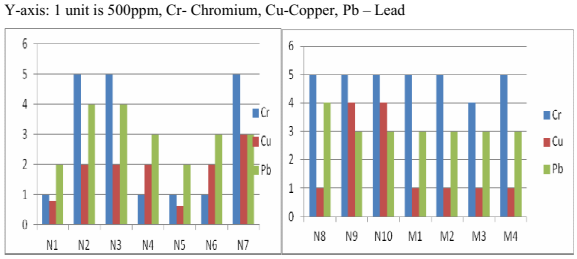
Results and Discussion:

3.1 Screening for heavy metal bioaccumulators

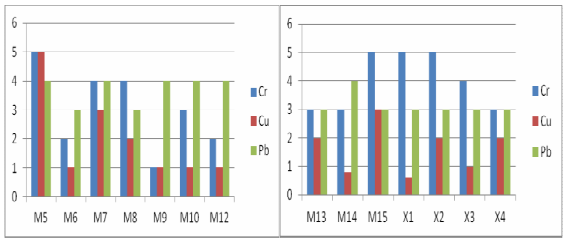
Graphs 1-5: Screening for heavy metal bioaccumulators by MIC

Graphs 1-5: X-axis: N1-10, M1-15, X1-11-Thirty five isolates obtained from urban wetland

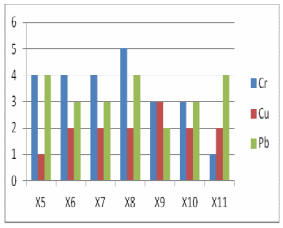
Y-axis: 1 unit is 500ppm, Cr- Chromium, Cu-Copper, Pb – Lead



Graph 1 Graph 2



Graph3 Graph 4

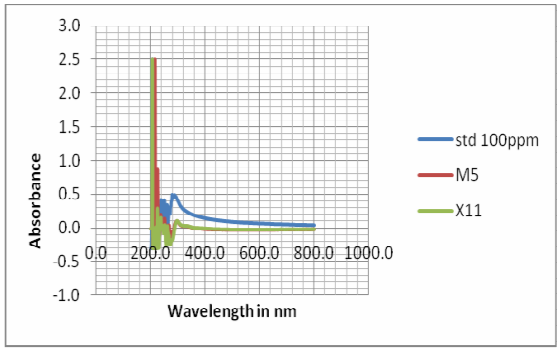


Graph 5

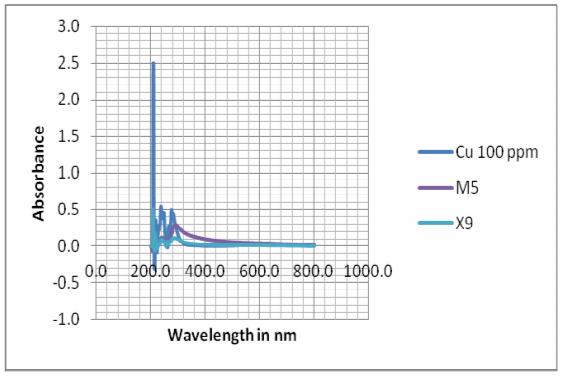
3.2 Identification

The organisms were identified as M5 – *Enterobacter aerogenes* (grows both in presence of Copper and Lead); X8 – *Bacillus pumilus* (grows in presence of only Chromium); X9 – *Bacillus alvei* (grows in presence of only Copper); X11 – *Bacillus cereus* (grows in presence of only Lead).

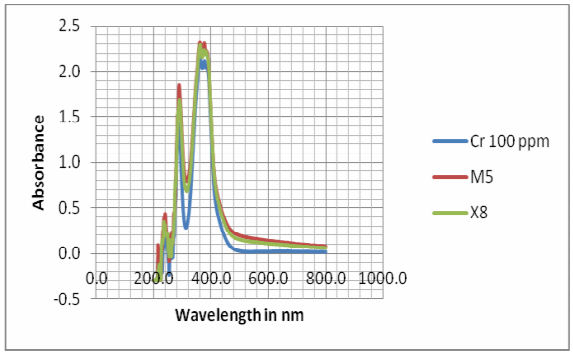
3.3 UV Spectrometry of isolates M5, X8, X9, X11



Graph 6: UV Spectrometry of isolates M5, X11 grown in lead



Graph7: UV Spectrometry of isolates M5, X9 grown in copper



Graph 8: UV Spectrometry of isolates M5, X8 grown in chromium

From the MIC data, it can be said that organisms growing in waters polluted with heavy metals develop a natural resistance towards these metals. Some organisms were able to tolerate up to only 250ppm while others were able to tolerate up to 3000ppm of the metals individually. M5 showed flourishing growth in the presence of all the three metals individually. The mechanisms used by the micro-organisms may include metal exclusion by permeability barriers, active transport of the metal away from the cell organism, intracellular sequestration of the metal by protein binding, extracellular sequestration, enzymatic detoxification of the metal to a less toxic form and reduction in metal sensitivity of cellular targets (Bruins

et al., 2000~ Nies and Silver, 1995~ Silver, 1996). The isolated organisms were identified using biochemical methods as follows, M5as *Enterobacter aerogenes,* X9 as *Bacillus alvei* and X8 and X11 as *Bacillus cereus.* M5 – *Enterobacter aerogenes* (tolerated up to 1500ppm of Chromium, Copper and Lead), X-8 (tolerated up to 2500ppm of Chromium), X9 – *Bacillus alvei* (tolerated up to 1500ppm Copper),and X11 – *Bacillus cereus* (tolerated up to 2000ppm of Lead). *Bacillus species* are generally known to be good at uptake of metals as reported in various surveys (Zekri , *et al.*,2005).

The absorption maxima for Potassium dichromate was found to be at 363 nm. There was no observed difference between the concentration of standard and the concentration of the broth. Cupric sulphate showed two absorption maxima at 237 nm and 278 nm respectively. A shift in the peak towards 291 nm was observed after incubation. The absorption maxima of Lead (II) acetate before incubation was at 285 nm. A shift in the peak towards 295 nm was observed after incubation. The UV spectroscopy results show a drift in the absorption maxima in the media after incubation with the bacteria indicating that the bacteria may be bio transforming the metal into some other form. There is also a dip in the height of the peak indicating a reduction in the concentration of the metal. The percent reduction in the concentration of the metal could not be determined due to the shift in the wavelength. However, it can be said that the organism might have bioaccumulated the heavy metal.

3.4 Enrichment and microscopic observation of algae in Allan and Chu media in the presence and absence of chromium, copper and lead.

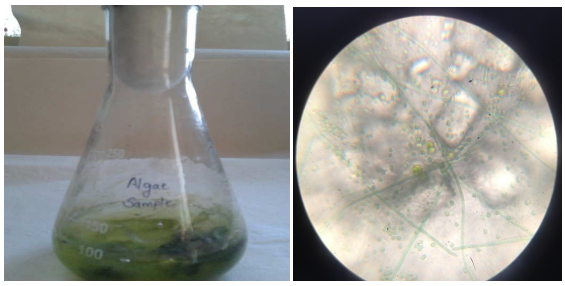


Figure 1: Microscopic observation of algae under normal conditions

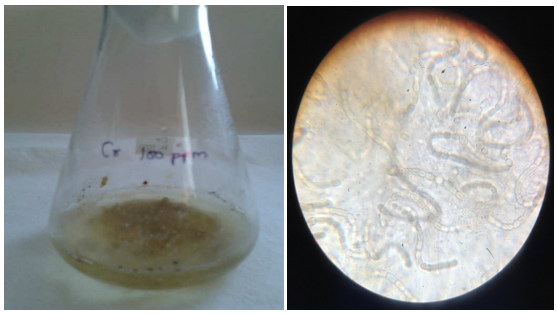


Figure 2: Microscopic observation of algae in 100ppm chromium

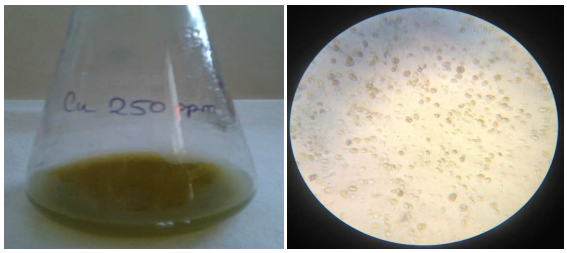


Figure3: Microscopic observation of algae in 250ppm of Copper

Figure 4: Microscopic observation of algae in 500 ppm of lead

3.5 UV Spectrometry of algal isolates:

Concentration of metal used in media (ppm)	Amount of metal in the cells mg/gm (273nm)	Amount of metal in the cells mg/gm (373nm)
50	0.296	0.119
100	0.401	0.154
250	0.835	0.154
350	1.100	1.048
500	1.141	1.202

Table 1 :Amount of chromium bioaccumulation by algal cells

No growth was observed in 250ppm, 350ppm and 500 ppm.

Concentration of metal used in media (ppm)	Amount of metal in the cells mg/gm (206nm)
50	2.821
100	4.383
250	6.646
350	10.527
500	10.455

Table 2: Amount of copper bioaccumulation by algal cells

No growth was observed in 350ppm and 500 ppm

Concentration of metal used in media (ppm)	Amount of metal in the cells mg/gm (209nm)
50	3.14
100	2.75
250	4.67
350	8.75
500	2.6

Table 3: Amount of lead bioaccumulation by algal cells

Varied morphological changes are seen in the case of the algal cells grown in the presence of the metals as against control. The U.V. spectroscopy studies indicate the presence of heavy metals in the algal cells. This indicates the bioaccumulation of the heavy metals in the algal cells. The presence of two peaks at 273 nm and 373nm in the case of chromium indicates a possible change in the structure of chromium in the presence of the algae. This may have been possibly brought about by the algal cells. Table 1 indicates a low presence of chromium of around 0.296mg/gm-1.141 mg/gm in the algal cells. Table 2 indicates an uptake of nearly 2.821mg/gm- 10.527mg/gm in the case of copper with the highest uptake seen between 350ppm and 500 ppm. While, Table 3 shows the presence of nearly 2.086mg/gm-9.925mg/gm the highest being in 350ppm. The cells show the presence of the heavy metals chromium and copper at higher concentrations indicating uptake by the inoculated cells. At these concentrations, however growth of algae was inhibited. This indicates that algae are unable to grow at higher concentrations of chromium and copper but the inoculated cells are able to take up the metals. Hence algal cells can be used as bio indicators for various heavy metals and also help in accumulating and possibly bio remediating the metals. Algae have been used as bio indicators for pollution in fresh water bodies (Bellinger E.G., *et al*., 2010). Metal compounds such as Cr, Cu, Pb, Cd, Mn, As, Fe, Ni, Hg, and Zn can also be bio remediated by microalgae. Microalgae such as *Chlorella* and *Scenedesmus* have shown tolerance and bioremediation capabilities to certain heavy metals (Pinto E., *et al*., 2003).

Acknowledgements:

We would like to express my gratitude to Dr. Prabhakar Dongre, HOD, Department of Biophysics, Mumbai University and Ms. Jessy John, Department of Biophysics, Mumbai University for their for their invaluable help.

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