INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

HEAVY METALS TOLERANCE AND *IN VITRO* BIOREMEDIATION POTENTIAL *IN PSEUDOMONAS SP R2*

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ABSTRACT

The present study is findings of an investigation of heavy metal tolerance in *Pseudomonas sp R2*- KJ461965, a phenol resistance and organophosphate [17] degrading isolate. Metal impregnated paper discs at 0.2 mg/ml concentration were used to evaluate oligodyanamic action *in vitro*. Growth of the isolate in the paper disc assay and development of biofilm at late stage of culture growth, was a clear evidence of resistance and biosorption mechanism. In liquid culture method, the culture media was spiked with heavy metals viz. lead, mercury, cobalt, copper, zinc, calcium and magnesium, at concentration ranging from 0.5 to 2 mg/ml. The tolerance to metals were ascertained by increase in biomass with increase in metal concentration. Plasmid curing was carried out to locate the genes responsible for tolerance to plasmid or chromosome. Ethidium Bromide (100 µg/ml) and 2 % SDS were used as curing agent. The isolate was found to be resistance to lead, chromium, magnesium zinc, calcium and copper and tolerant to cobalt, mercury and iron. Loss of heavy metal resistance from the isolated strain after plasmid curing confirmed a relationship between heavy metal resistance with plasmid. These findings indicates the novelty of the strain in bioremediation of heavy metals contamination.

Keywords: Heavy metal tolerance, *Pseudomonas sp R2* , Paper disc assay, Plasmid curing.

INTRODUCTION

The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration[1] .Due to various anthropogenic activities, soil and water resources are reported to be contaminated by heavy metals and have become a serious threat to public health and ecosystems[2]. Metals like arsenic, cadmium, lead, mercury, silver etc cause conditions including hypophosphatemia, heart disease and liver damage, cancer and neurological and cardiovascular diseases, central nervous system damage and sensory disturbances. The metals lead, calcium, copper, mercury, cobalt and zinc are being the major hazardous contaminant[3]. Heavy metal contamination have thus evolved into a serious environment problem In geochemical and biological cycles, metals play a vital role in the metabolic processes of the biota. A group of metals whose atomic density is greater than 5 g/cm3 , atomic number above 20, or 5 times or more, greater than water and is toxic or poisonous at low concentrations of 1mg/l[4] and are known as heavy metals. Various strong toxic metal ions such as Hg are very toxic even in lower concentration of 0.001-0.1 mg/ L [5, 6]. When heavy metal concentration is higher (10-100 mg/L) than permissible concentration of less than 1 mg /L, it becomes a cause of concern to environment. Some of the heavy metals are essential and are required by the organisms in trace amounts as micro nutrients (cobalt, chromium, nickel, iron manganese and zinc etc.) and are known as ‘trace elements’[7]. Involved in redox processes, trace elements stabilize molecules through electrostatic interactions and can serve as catalysts in enzymatic reactions and also regulates the osmotic balance [8,6]. While, other heavy metals such as cadmium, mercury and lead do not have any biological role and are detrimental to the organisms even at very low

concentration. However, at high levels both the essential and non essential metals become toxic to the organisms. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage [9]. Of the important metals, Mercury, lead, cadmium, Arsenic and Chromium (VI) are regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns [10,11].

At present the tolerance of soil bacteria to heavy metals has been proposed as an indicator of the potential toxicity of heavy metals to other forms of biota [12]. Microorganisms and their products are known to be highly efficient bioaccumulators and biosorbent tools of soluble and particulate forms of metals. Therefore, In the perspective many approaches have been used to assess the risk posed by the contaminating metals in soil, water bodies etc. [13,14]. It has been reported that there are several potential microbial metal biosorbents, which include Several microbial genera of *Bacillus, Pseudomonas, Streptomyces, Aspergillus, Rhizopus and Penicilium* have Several studies have reported the role of microbes in bioremediation of heavy metals. Bioremediation process uses microorganisms or their enzymes to return to the environment, altered contaminants, back to its original condition [15]. Microbes act as bio sorbent tools by binding contaminants onto their cellular structures and thus can be used in environmental cleanups. Other heavy metal biosorbent species includes fungi, algae and industrial agricultural wastes.

Since heavy metals cannot be destroyed, the remediation of heavy metal pollution becomes difficult. However, the unique phenotypes and properties of Bacteria have enabled their application in bioremediation of these metals. It is a well known fact that *P. aeruginosa* cells grown in biofilms accumulate higher amounts of metals than planktonic cells [15,16].

The isolate *Pseudomonas sp. R2* obtained in screening process of phenol degraders, was tested for its ability to degrade organophosphates [17]. As Pseudomonas species are well known for removal of a number of contaminant, in the present investigation, the isolated strain was subjected to heavy metal tolerance. Responses of cells against various metal ions are not yet completely understood. Stress conditions induce a variety of responses inside bacterial cells which may result in the change of various proteins in the cell. An attempt was made to identify plasmid or chromosome mediated determinants of Pseudomonas sp. R2 to confer resistance to heavy metals.

MATERIALS AND METHODS

Microorganism used- *Pseudomonas sp R2* capable of phenol and organophosphate degradation.

Media used- Tris buffered Minimal salt medium (MSM) And Luria Bertaini (LB) broth (High Media- Mumbai)

Heavy metal-copper (cu), zinc (zn), Iron (Fe), magnesium (Mg), Lead (Pb), Cobalt (Co), Calcium (Ca) and mercury (Hg) all were of analytical grade obtained from Merck Chemical Co., Germany.

Medium and metal solution preparation

All the metal solutions were prepared in sterilized glassware treated with 0.1 M HCl before and after the experiments, to avoid binding of metals to it. 1L of the medium were prepared and was sterilized at 15 lb/in2 pressure and 121^{0}C for 30 minutes [18]. A loopful of pure bacterial culture was transferred to 10 ml nutrient broth medium to obtain log phase culture. Microbial culture of 0.1 ml volume was inoculated into the medium and incubated at 37^{0}C in orbital shaker at 120 rpm for one week interval. This culture was used as inoculums for biomass development for another 24 hrs.

Different concentrations of metal solution viz. 0.5 mg/ml to 2 mg/ml concentration were prepared from stock of 1000 mg/L in respective reagent and diluted in sterile double distilled water to required volume. Log phase culture of 10 mg biomass equivalent to 5X 10^5 cells were added to each concentration of 100 ml medium in an Erlenmeyer flask. The flasks were incubated as described previously placed on a shaker with a constant speed of 80 rpm and left to equilibriate for 1 week period.

Biomass was collected by centrifugation at 5000 rpm for 5 min. The supernatant was discarded and the cells were re-suspended in purified water for washing and again centrifuged as above to make sure that no media remain on the cell surface. The cell pellet was dried 24 hr at 80 oC then the total bacterial cell dry weight was determined as g/L[19,20].

Estimation of tolerance and effect on growth were determined after 7 days enrichment. The biomass in different samples were collected in form of pellet at 24 h intervals, by centrifuging at 5000 rpm at 4^{0}C for 10 minutes. The pellet was dissolved in sterile LB media and the optical density was measured at 600 nm against uninoculated LB as blank. **The growth and increase in mass of cells was considered as an evidence for the tolerance property of the**

isolate towards adoption to higher metal toxicity.

Isolation of plasmid

Pure cultures of the isolate were grown overnight in 25 ml of sterile Luria-Bertani broth (Hi-Media) and cell pellet was harvested by centrifugation at 5000 rpm for 5 min at 4°C. The cell plasmid DNA were isolated using Birnboim and Doly's Alkaline Lysis method (Miniprep method)[21]. The extracted plasmid was suspended in 20 µL of TE buffer and was further analyzed by 1% Agarose gel electrophoresis .

Plasmid curing by various chemicals

The 24 hrs old cultures of the isolate were grown in sterile Luria-Bertani broth containing various chemical agents *viz,*Ethidium Bromide (100 µg/ ml) and 2% Sodium dodecyl sulphate (SDS)[22]. The tubes were incubated at 37°C for 24 hrs. After the incubation the isolates were reinoculated in sterile Luria-Bertani broth and incubated further for 24 hrs. The cured isolates were checked for their heavy metal tolerating capacity, by turbidity method.

RESULTS AND DISCUSSIONS

The isolates exhibited confluent growth in Zinc, Copper, Calcium and magnesium containing liquid media. The concentration of 0.2 mg/ml, used in this study have proven to be growth promotive (Fig 1c). While at the concentration 2 mg/ml, iron exhibits oligodyanamic action in solid agar plate metal disc assay (Fig 1b) indicative of biofilm- like formation at late phase of cell growth. The disc plate assay is indicative of biofilm- like formation at late phase of cell growth, as a mechanism of heavy metal resistance (Fig 1c). The microbes have evolved several types of mechanisms to tolerate the uptake of heavy metal ions, particularly involving the efflux of metal ions outside the cell by either active bioaccumulation and/or passively (biosorption). Apart from cellular metabolic stage activity, there is involvement of molecular component, particularly genes. Microorganisms have developed chromosomally or extra chromosomally controlled detoxification mechanisms to overcome the detrimental effects of heavy metals. The genes for this general type of mechanism have been found on both chromosomes and plasmids[23]. Minimal inhibitory concentrations (MICs) of several different metal ions for *Escherichia coli* on agar medium, and the most toxic metal (with the lowest MIC) was mercury, whereas the least toxic metal tested was Manganese[24]. These mechanisms include the complexation of the metal ions inside the cell, after efflux of metal ions and accumulation. Finally, the heavy metal ions are reduced to a less toxic state[6].

In the liquid medium assay, 1 mg/ml biomass of the isolate, when used in different metal salt formulated medium showed varied results in the growth pattern. The isolate is able to tolerate heavy metal lead and calcium salts with a maximum growth at 1.0 mg/ml concentration (Graph 4, Graph1). In mercury (HgCl2), containing medium, a maximum concentration of 1.5 mg/ml enhances growth (Graph 2). With Cobalt (CoCl2) and Magnesium (MgSO4) incorporation, the medium at 2 mg/ml concentration provides optimum growth as indicated by turbidity (Graph 3 and Graph 5 respectively). The metals copper (CuSo4), Iron (FeSO4), and Zinc (ZnSO4) showed optimum growth at 0.5 mg/ml concentration (Graph 7, Graph 6 and Graph 8 respectively). For the biosorption of Cu(II) and Cd(II) by inactivated cells of *Pseudomonas aeruginosa* PU 21, the maximal capacity occurred at pH 6.0[25]. The lower charge density of mercury when compared to Chromium, was shown to be responsible for higher affinity towards mercury, in gram negative *Pseudomonas aeruginosa,* involving the cell surface complexation mechanism than ion exchange or affinity. In gram negative bacteria most of their lipopolysaccharide (LPS), phospholipids and proteins are exposed on the cell surface and involves in cell surface complexation. On the other hand, Gram positve bacteria normally show low levels of surface complexation due to heavily cross linked peptidoglycan layer[26]. Study carried out elsewhere reported a minimum inhibitory concentration of 3.5 mM of Cu2+ on p*aenibacillus polymixa*. The levels of Zn2+ tolerance among the bacterial isolates were different from one to the other showing the reduction of their growth to 50 % in the presence of 2.98 mg/L of Zn2+ by *B.thureingiensis* and 7.02 mg/L of Zn2+ by *Paenibacillus sp.*[27]. The metals absorbed by the *Pseudomonas sp R2* are in relation to reports of Vinod N *et al*., 2009[28]., where in it is revealed that for the 250 ppm its 0.25 mg &0.28mg, for the 500 ppm its 0.41 &0.51 mg similarly for the 1000 ppm its 1.33mg &.67mg. When exposed to metals at 0. 2 mg/ml concentration, *Pseudomonas sp. R2* exhibits resistance to lead, cobalt and mercury, which is prominent with stationary phase cells and increased biomass content. 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. Initially as

shown in Figure 1b there was inhibition to lead but in late growth phase (stationary to death phase) there is appearance of confluent zone of growth, indicating biofilm formation. The concentrations selected in the study indicates intense growth in all the metals tested. Above this concentration these metals have proven to be oligodyanamic in action. The findings of this study indicates high and multiple heavy metal tolerance capacity of the *Pseudomonas sp R2* as indicated in most studies in Pseudomonas species.

A study done in Michigan's Copper County and the abandoned mining area, with a lot of red metal left behind, as a waste from the mining, indicated the presence of high fractions of copper, 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. In the present investigation, the findings of the heavy metal tolerance in the bacterial isolate, before and after plasmid curing was assessed by comparing the ability of the isolate to grow in metal containing medium. The effect of curing agents in removing the plasmid was studied by disappearance of intact three forms of plasmids in the isolate. The result of gel doc and ability of heavy metal tolerance being lost, clearly indicates role of plasmid in heavy metal tolerance in this isolate. Also, ethidium bromides proves to be an efficient agent for curing the plasmid (Figure 2a and Figure 2b). The plasmid-cured variant, were obtained by culturing in LB broth. However the plasmid-cured variant did not grow on metal supplemented media. Moreover, resistance to a wide range of heavy metals such as Mg2+, Hg2+, Cu2+, Co2+, Zn2+, Fe+2, Ca2+ and Pb2+ was mediated by a plasmid of size 20-23-kb.

The metals copper, calcium, magnesium and Zinc, as confirmed from both liquid culture and Disc impregnated metal assay, are growth promotive. And in lead, mercury and cobalt salt containing media there is a variation in the growth rate and intensity of biomass at different concentration of metal salts used.

Plasmid Curing results suggests that lead and mercury resistance genes were conferred by plasmid DNA. The development of the mercury resistance can result from vertical gene transfer (reproduction), horizontal gene transfer, including transposons and broad host range plasmids and selection pressures on spontaneous mutants due to the presence of heavy metals[29]. These resistance determinants are often found on plasmids and transposons[30]. Regulations of cellular processes following exposure to metal ions at both transcriptional and translational levels have also been reported [31-33]. A number of genes, located on bacterial plasmids and chromosomes, have been identified that encode specific resistance to a number of heavy metal ions, including Ag^{+}, $AsO2^{-}$, $AsO4^{3-}$, Cd(II), Co(II), $CrO4^{2-}$, Cu(II), Hg(II), Ni(II), Sb(III), $TeO3^{2-}$ and Zn^{2+}[34]. The best-known mechanism involves metal binding and sequestration by proteins like metallothioneins (MT). The bacterial genetic systems for resistances to mercury (*mer*), lead (*pbr*) and cadmium, zinc and cobalt (*czc, cad*) have been linked to metal tolerance. Indeed, further studies have to be performed to elucidate bioremediation of heavy metals and detoxification processes operating in this system. Some heavy metal resistance determinants move from plasmid to chromosome or in the reverse direction. However, the molecular mechanisms and underlying principles as explained for detoxification reveals the plasmid coded genes involvement in removal of heavy metals[35, 36]. This makes plasmid encoding heavy metal resistance and important aspect of environmental research.

CONCLUSION

The present study ascertains the future application for multicontaminant bioremediation sites by Pseudomonas sp R2. The aim of the work was to study the bioprospectives of *Pseudomonas sp R2* in heavy metals treatment as well, by biosorption and microbial leaching process. Based on turbidometry method, disc impregnated method and plasmid curing, the findings reveals and provide evidence for heavy metal tolerance to lead, mercury, cobalt, copper, Zinc, calcium and magnesium. Further cloning of these genes along with molecular analysis of other additional genes involved, would certainly elaborate its usefulness as a bioprospective strain. Since the optimal conditions for phenol degradation and organophosphate degradation are near pH 7± 2[17]. The study for pH optimization and temperature has not been undertaken in the present investigation. The purpose of the study was basically to evaluate the potential of the strain for *in situ* bioremediation of phenol and or organophosphate. These results are evident of potential applications for remediation of metal contaminated soils. Since heavy metals contributes as a major concerned pollutant in soil and water, the novel isolate can be a bioprospective strain for removal of phenol and

or organophosphate in heavy metal contaminant sites as well. A detail study of three factors need to be evaluated in further investigation.

ACKNOWLEDGEMENT

The author wish to thank the management of Dr. L. Bullayya P.G. College, particularly Dr. G. Madhu Kumar for supporting the research and providing moral support throughout the study.

RESULTS

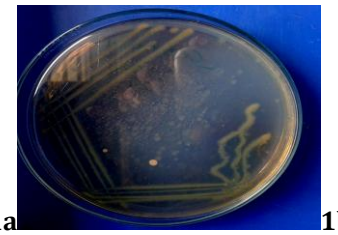
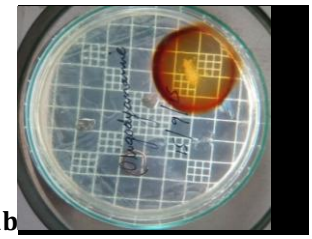
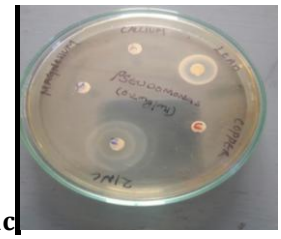
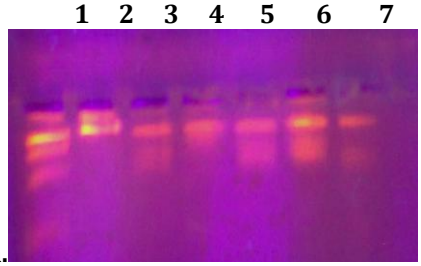
1a  1b  1c 

Fig. 1a: *Pseudomonas sp*. R2 pure culture
Fig. 1b: Oligodyanamic action of Iron at 0.5mg/ml
Fig. 1C: Metal tolerance in Pseudomonas sp R2 (24 hrs) isolated strain after 72 hrs

2a. 

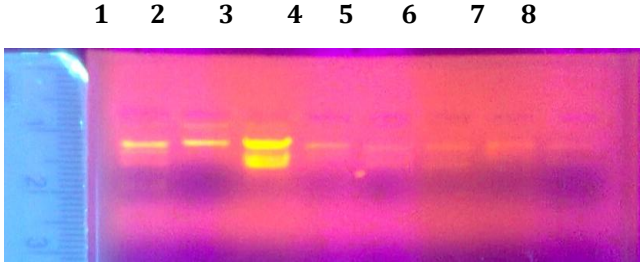
2b 

Fig. 2a: Plasmid DNA from Pb, Zn, Cu, Hg, Mg, Co containing media. Approx. 20- 23 Kb. Lane 1: Marker
Fig 2b. Plasmid after curing in different metal salt conditions lane 1: Marker- lambda DNA Hind III and ØX174 DNA- Hae III digest (lane 2. Pb,3-control Pb) 4, 5,6,7 and 8-Zn, Cu, Hg, Mg, Ca and Co respecively)

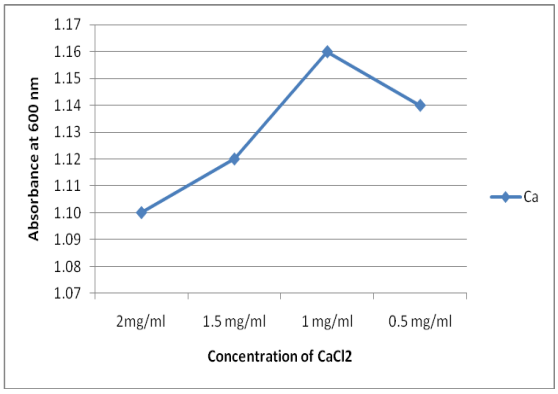
Table 1: Metal tolerance Before Curing agent EtBr treatment in Pseudomonas sp R2 at 0.5 mg/ml

Metals	Lead	Zinc	Cobalt	Mercury	Magnesium	Copper	Calcium	Iron
Growth	±	±	+	+	+	+	+	±

Table 2: Metal tolerance after Curing agent EtBr treatment in Pseudomonas sp R2 at 0.5 mg/ml

Metals	Lead	Zinc	Cobalt	Mercury	Magnesium	Copper	Calcium	Iron
Growth	_	_	±	±	_	+	_	_

Key: ± : Indicates very less turbidity or no turbidity in liquid media or growth inhibition (oligodyanamic action) at higher concentration above 2mg/ml and appearance of growth, as cells gets older, near death phase. + : Indicates growth and appearance of turbidity.



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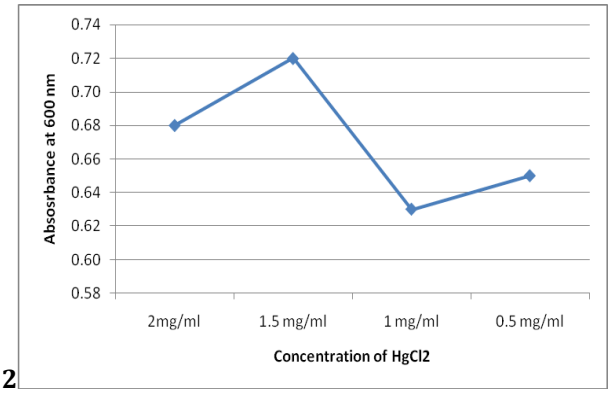

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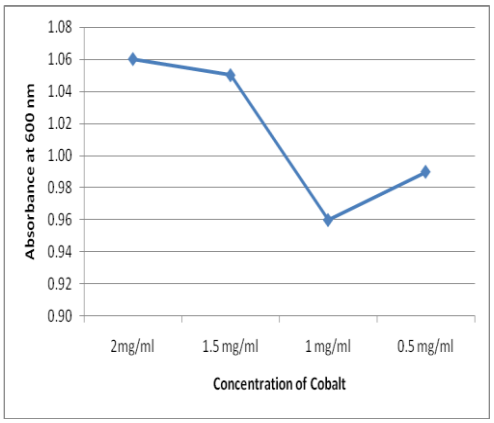

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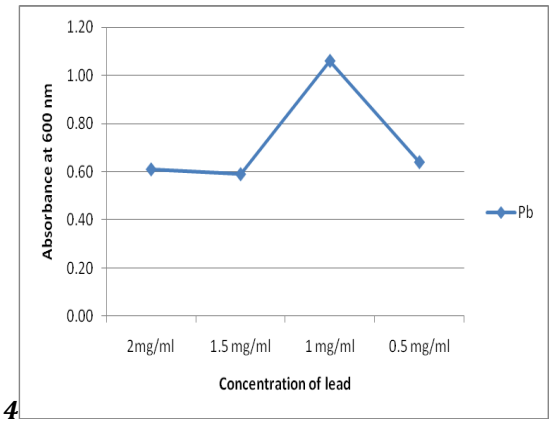

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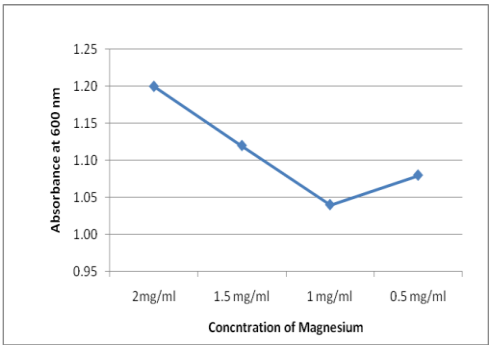

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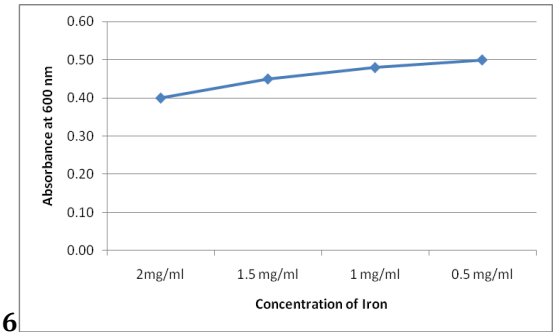

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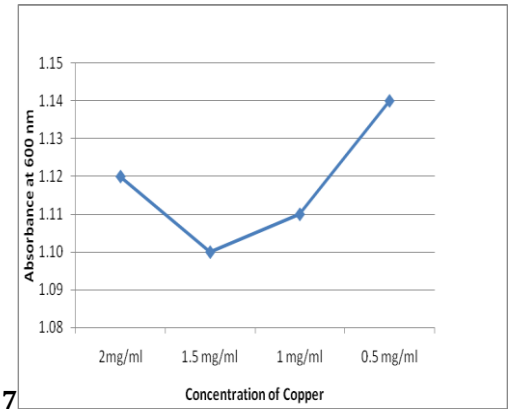

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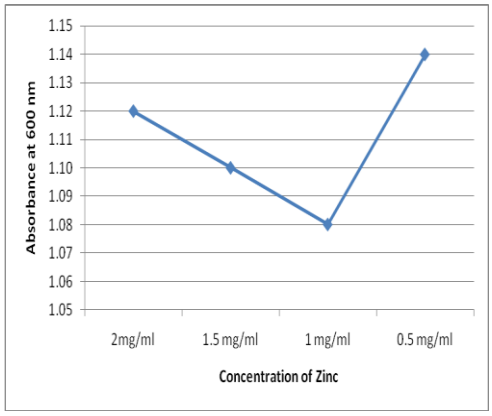

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Graph. 1: Effect of Calcium on the growth of *Pseudomonas sp R2*
Graph. 2: Effect of Mercury on the growth of *Pseudomonas sp R2*
Graph. 3: Effect of cobalt on growth of *Pseudomonas sp R2*
Graph. 4: Effect of lead on the growth of *Pseudomonas sp R2*
Graph. 5 : Effect of Magnesium on growth of *Pseudomonas spR2*
Graph. 6 : Effect of Iron on growth of *Pseudomonas spR2*
Graph. 7 : Effect of Copper on growth of *Pseudomonas spR2*
Graph. 8 : Effect of Zinc on growth of *Pseudomonas spR2*

REFERENCES

1. Lenntech Water Treatment and Air Purification. Water Treatment, Published by Lenntech, Rotterdamseweg, Netherlands (www.excelwater.com/thp/filters/Water-Purification.htm). 2004.
2. Mohammad Oves, Mohammad Saghir Khan, Almas Zaidi and Ees Ahmad. "Soil Contamination, Nutritive Value, and Human Health Risk Assessment of Heavy Metals: AnOverview", chapter. Toxicity of Heavy Metals to Legumes and Bioremediation. 2012;1–27.
3. Roane TM and Pepper IL. Microorganisms and Metal Pollutants in Environmental Microbiology Maier, R., M, Gerba, C.P., Pepper, I.A. (Eds.),. Academic press, San Diago, California, London. 1999; 403- 423.
4. Yilmaz EI. Metal tolerance and biosorption capacity of Bacillus circulans strain EB1", Research in Microbiology. 154;409-415,2003.
5. Gadd GM. Metals and microorganisms: A problem definition". FEMS Microbiology Letters. 1992; 100:197-204.
6. Nies DH. Microbial heavy metal resistance. Applied microbiology and biotechnology. 1999;51: 730-750.
7. Bruins MR, Kapil S and Oehme FW. Microbial resistance to metals in the environment". Ecotoxicology and Environmental Safety. 2000;45:198-207.
8. Hussein H, Farag S, Kandil K and Moawad H. Tolerance and uptake of heavy metals by pseudomonads. Process Biochemistry. 2005;40:955-961.
9. Jefferies DJ and Firestone P. Chemical analysis of some coarse fish from a Suffolk river carried out as part of the preparation for the first release of captive-bred otters. J Otter Trust. 1984;1:17–22.
10. Brown B and Absanullah M. Effects of heavy metals on mortality and growth. Mar Pollut Bull. 1971;2:182–187.
11. Moore JW. Inorganic Contaminants of Surface Water Residuals and MonitoringMuraleedharan, TR and Venkobachar, C. "Mechanism of cobalt biosorption. Biotechnol Bioeng. 1990; 33:823-831.
12. Olson BH and Thronton I. The resistance patterens to metals of bacterial populations in contaminated land. Journal of Soil Science. 1982;33:271-277.
13. Hassen A, Saidi N, Cherif M and Boudabous A. Effects of heavy metals on Pseudomonas aeruginosa and Bacillus thuringiensis. Bioresource Technology. 1998;65:73-82.
14. Hassen A, Saidi N, Cherif M and Boudabous A. Resistance of environmental bacteria to heavy metals. Bioresource Technology. 1998;64:7-15.
15. Jankiewicz B, Ptaszyński B and Turek A. Spectrophotometric Determination of Copper(II) in Samples of Soil from Selected Allotment Gardens in Lodz. Polish Journal of Environmental Studies 1999;8(1):35-38.
16. Hussein H, Farag S, Kandil K and Moawad H. Tolerance and uptake of heavy metals by pseudomonads. Process Biochemistry. 2005;40:955-961.
17. Sharmila Begum S and Arundhati A. A study of Bioremediation of Methyl Parathion In Vitro using Potential Pseudomonas sp. Isolated from Agricultural Soil, Visakhapatnam. Int J Curr Microbiol App Sci. 2016;5(2):464-474.
18. Bahig El-Deeb. Plasmid mediated tolerance and removal of heavy metals by American Journal of Biochemistry and Biotechnology. 2009;5(1):47-53.
19. Ishizaki A and Tanaka K. Production of poly-ß -hydroxybutytic acid from carbon dioxide by Alcaligenes eutrophus ATCC17697. J Ferm and Bioeg. 1991;71(4):254-257.
20. Du G, Chen J, Yu J and Lun S. Continuous production of poly-3- hydroxybutyrate by Ralstonia eutropha in a two stage culture system. J Biotech. 2001;88:59-65.
21. Sambrook J, Fritsch EF and Maniatis T. Molecular cloning: A laboratory manual, 2nd. Ed. Cold spring harbour, NY: Cold spring harbour laboratory press. 1989.
22. Edward Raja C and Selvam GS. Plasmid profile and curing analysis of Pseudomonas aeruginosa as metal resistant. International Journal of Environmental Science & Technology. 2009;6(2): 259 -266.
23. Ehrlich HL. Microbes and metals. Applied Microbial Biotehnology. 1997;48:687-692.
24. Mergeay M, Nies D, Schlegal HG, Gertis J, Charles P and Van Gijsegam F.

Alcaligenes eutrophus CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. Journal of Bacteriology. 1985;162(1):328-324.
25. Nies DH. Microbial Heavy-metal Resistance. Appl Microbiol Biotechnol. 1999;51:730-750.
26. Chang JS, Law R and Chang CC. Biosorption of lead, copper and cadmium by biomass of Pseudomonas aeruginosa PU21, Water Res. 1997;31:1651–1658.
27. Gupta R, Ahuja P, Khan S, Saxena RK and Mohapatra H. Microbial biosorbents:meeting challenges of heavy metal pollution in aqueous solutions. Current Science. 2000;78:967–973.
28. Guo H, Luo S, Chen L, Xiao X, Xi Q, Wei W, Chen J, Zeng G, Liu C, Wan Y and He Y. Bioremediation Of Heavy Metals By Growing Hyperaccumulaor Endophytic Bacterium Bacillus Sp. L14. Bioresour Technol. 2010;101(22):8599-605.
29. Vinod Nagashetti, Mahadevaraju.G.K, T.S.Muralidhar, Aamir Javed, Dwijendra Trivedi and Khum Prasad Bhusal. Biosorption of Heavy Metals from Soil by Pseudomonas Aeruginosa, International Journal of Innovative Technology and Exploring Engineering. 2013;2(6):22-24.
30. Bogdanova ES, Bass IA, Minakhin LS, Petrova MA, Mindin SZ, Volodin AA, Kalyaeva ES, Tiedje G M, Hobman JL, Brown NL and Nikiforov VG. Transfer of mer operons amongest environmental isolates of gram-positive bacteria. Microbiology. 1998;144(3):609-620.
31. Silver S and Misra TK. Plasmid mediated metals resistance. Annual Review of Microbiology. 1988; 42:717-743.
32. Puranik P, Modak J and Paknikar K. A comparative study of the mass transfer kinetics of metal biosorption by microbial biomass. Hydrometallurgy. 2005;52:189-197.
33. Volesky B. Biosorbent materials. Biotechnol Bioeng Symp. 2003;16:121-126.
34. Kang SY, Lee JU and Kim KW. Biosorption of Cr(III) and Cr(VI) onto the cell surface of Pseudomonas aeruginosa. Biochem Eng. 36:54-68.
35. Ji G and Silver S. Bacterial resistance mechanisms for heavy metals of environmental concern. Journal of Industrial Microbiology. 1995;14(2):61-75.
36. Ahmed N, Nawaz A and Badar U. Screening of copper tolerant bacterial species and their potential to remove copper from the environment. Bulletin of Environmental and Contamination Toxicology. 2005;74:219-226. -
37. Khan Md. Anik Ashfaq, Sutradhar Pijush, Islam Mohammad Majharul, Ojha Ravi Kant and Biswas Gokul Chandra. Screening of antibiotic resistant gram negative bacteria and Plasmid profiling of multi-drug resistant isolates present in sewage associated with health care centers. International Journal of Medical Research & Health Sciences. 2013;2(4):923.