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DEGRADATION OF HEAVY METALS BY *SERRATIAMARCESCENS*

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ABSTRACT

The water and soil contaminated with heavy metal pollutants pose a major environmental threat and human health hazards which need effective and affordable technological solutions.Metals are common in our environment and diet, and are actually necessary for good health, but large amount of any of them may cause acute or chronic toxicity. Heavy metal toxicity can result in damaged or reduced mental and central nervous functions low energy levels and damaged to blood composition, lungs, kidneys, liver and other vital organs.Copper, Lead. Zinc, Nickel, Mercury are some of the important common pollutants, that are to be considered. In order to degrade such pollutants, the present study is carried on using *Serratiamarcescens*as the biological agent.*Serratiamarcescens* were isolated from heavy metal contaminated aquatic environment and was characterized. It was found to show a maximum tolerance against lead of concentration 50000 ppm. Also showed similar degree of tolerance against copper and zinc where its tolerating concentration was 1000ppm. *Serratiamarcescens* is known to accumulate high concentrations of metal. Hence, it can used for biological treatment of effluents rich in heavy metals.

Key Words: Heavy metals, Degradation, *Serratiamarcescens*

INTRODUCTION

A pollutant is a misplaced resource, more truely in context of heavy metals. Heavy metals are chemical elements with a specific gravity that is at five times the specific gravity of water (Lide 1992). There are 35 metals that concern us because of environmental or residential exposure. 23 of these are heavy metals: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, gallium, gold, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc (Glanze, 1996).Interestingly, small amount of these metals are common in our environment and diet, and are actually necessary for good health, but large amount of any of them may cause acute or chronic toxicity. Heavy metal toxicity can result in damaged or reduced mental and central nervous functions low energy levels and damaged to blood composition, lungs, kidneys, liver and other vital organs. Long term exposure may result in slowly progressing physical, muscular and neurological degenerative process that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are not uncommon and repeated long term contact with some metals or their compounds may even cause cancer (International Occupational Safety and Health information centre, 1999).Heavy metals are wide spread pollutants of great environmental concern as they are non degradable and thus persistent (Stratton,1987).

Increasingly higher quantities of heavy metals are being released into the environment by anthropogenicial activities, primarily associated with industrial processes, manufacturing and disposal of industrial processes, manufacturing and disposal of industrial and domestic refuse and waste material (Ross,1994).Copper is the 29^{th} element and the first group IB of the periodic table, displays four oxidation states; metallic copper Cu, cuprous ion Cu^+, cupric Cu^{2+} and trivalent copper Cu^{3+}. Copper also forms organic metallic compounds (Demayo and Taylor, 1981).Lead can exist in several valence state:Pb (0) - metal, Pb (I), Pb (II) and Pb (IV), all of which, with possible exception of Pb(I),are of environmental importance (Bowen,1979). In natural waters, lead is generally present in low concentration of Pb range from .0003 to .003ppm in natural fresh water's while Forstner reported the likely levels of .0002 ppm. Typical natural levels of lead in some representative groups of animals, humans and plants are .001 to 130ppm, up to 170 ppm and 0.32 to 8000 ppm respectively (Bowen, 1979).

Lead causes increase in R.B.C numbers, decrease in RBC volume and decrease in RBC cellular iron content (Hodson et al, 1978).The symptoms of acute lead poisoning include tiredness, abdominal discomfort, irritability and anaemia.Zinc is an essential and beneficial element for human bodies. However, concentrations above 5ppm cause bitter taste and opalescence in alkaline waters. In drinking water, the Zinc concentration range from 0.06 to 7.0ppm. Zinc enters the domestic water supply from the deterioration of galvanized Fe and dezincification of brass besides industrial wastes.Thus the water and soil contaminated with heavy metal pollutants pose a major environmental threat and human health hazards which need effective and affordable technological solutions. The commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation (Rich *et al*, 1987). The search for new technologies involving removal of toxic metals from waste water has directed attention to biosorption based on metal binding capacities of various biological methods. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from waste water through metabologically mediated or physico-chemical pathways of uptake (Fourest and roux, 1992).

Biosorption is the passive sequestration of metals and radionuclide's by interaction with live or dead biological matter and is at present the most practical and widely used approach for bioremediation of metals and radionuclide's. Biosorption is an effective treatment of waste water (

Schiewers*et al*,2000). Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents (Volesky,1986). The major advantages of biosorption over conventional method include (Kratochvil*et al*, 1998). The *Serratia* species are found in water, soil and on leaves, shrub, fruits vegetables, herbs, mushrooms, mosses and insects (AnuradhaGiri*et al*,2004). *Serratia* species are gram negative bacteria, classified in the family of Enterobacteriaceae. *Serratia*spcan be distinguished from other genera by its production of three special enzymes DNase, lipase and gelatinase..*Serratiaplymuthica* is found in soil(Kable*et a*l,1996) and has been isolated from different types of food (Lopez *et a*l,1996;Lyhs*et al*,1998).Most of *Serratiaplymuthica* strain have been isolated from fresh water and fish suggesting that they may be a potential opportunistic pathogen for animals and human (Nieto *et al*,1990;Rodriguez *et al*, 1990). The present study was carried out using *Serratiamarcescens* as the biological agent to absorb and degrade the heavy metals copper, zinc and lead because of its potential environmental problem and high toxicity.

MATERIALS AND METHODS

SAMPLE COLLECTION

The soil sample was collected in a sterile container from Thoothukudi seaport, situated in Tamilnadu, which is known to be a metal contaminated area. The sample was transported to the lab under refrigerated condition.

ISOLATION OF BACTERIA FROM THE SAMPLE

The soil sample collected was serially diluted and it was plated (spread) on nutrient agar plates. All the plates were then incubated at 37C for 24 hours.

Nutrient Agar Medium	
Beef Extract	0.3 g
Peptone	0.5 g
Yeast Extract	0.2 g
Sodium Chloride	0.8 g
Agar	2.0 g
Distilled Water	100 ml

GRAM STAINING

Using sterile technique, bacterial smear was prepared on a slide. The smear was flooded with crystal violet for one minute. It was then washed with tap water to remove excess of primary stain. The slide was flooded with Gram's iodine and left for one minute, and it was decolorized with alcohol. The slide was then counterstained with safranin and left for 45 seconds. The preparation was washed, air dried and viewed under oil immersion of the microscope.

MAINTENANCE OF ISOLATED *Serratiamarcescens*

The isolated strains were maintained by serially sub culturing on to nutrient agar plates and were preserved in refrigerated condition until further use.

METAL TOLERANCE OF *Serratiamarcescens*

Muller Hinton agar plates were prepared. The ppm solutions of metals: zinc, copper and lead at different concentrations were prepared using double distilled water. A sterile cotton swab was dipped into a cell suspension and entire agar surface was inoculated, and allowed to dry for 5 minutes. Using a gel punch, wells were made on the agar surface at well spaced intervals. The metal solutions of different ppm concentrations were added to the wells using a micropipette. All the plates were incubated at 37^0C for 24hours. Following incubation, zone of inhibition was measured and the results were noted.

METAL ACCUMULATION

The heavy metal (Zn, Cu, and Pb) solutions of different ppm concentrations (100ppm, 200ppm, 500ppm) were prepared and added into the packed column. The column was incubated at room temperature for a period of 4-6 hours. Following incubation, the metal solutions were eluted out from the column and the results were determined using the atomic absorption spectrophotometer of model AA6300, SHIMADZU, KYATOJAPAN.

ANTIBIOGRAM OF ISOLATED *Serratiamarcescens*

Muller Hinton agar plates were prepared. A sterile cotton swab was dipped into an organism suspension and was swabbed onto the entire agar surface. The inoculated media was allowed to dry for 5 minutes. Antibiotic discs were taken using sterile forceps and kept on the agar surface at well spaced intervals. The discs were pressed gently with a sterile forceps to ensure firm contact with agar surface. All the plates were incubated at 37C° for 24hours in an inverted position. The zone of inhibition was measured and compared with standard chart and the results were noted.

RESULTS

ISOLATION OF BACTERIA FROM THE SAMPLE

The dominant microbial florafrom the soil sample were isolated. From this, the mucoid, slightly raised, pink colored colonies were selected for further studies.

GRAM STAINING

The cells observed were found to be gram negative rods.

The cells were observed as gram negative and a large, mucoid, slightly raised, pink colored colonies on Nutrient agar plates.. The pigmentation was observed more on Muller Hinton agar plates.

METAL TOLERANCE

Serratiamarcescens was found to show maximum tolerance against lead of concentration 100ppm. It was found that the degree of tolerance against copper and zinc was at a concentration of 200ppm.(Table. 1, 2 and 3).

ANTIBIOGRAM

*Serratiamarcescens*was found to be sensitive to Netromycin, Cefaperazone, Gentamycin, Piperacillintazobactum and Imepenem. It showed an intermediate sensitivity against the antibiotic Trimethropin and also observed to be resistant to Cefazolin, Cephaloridine, Norflox and Ciprofloacin. (Table. 4)

Table-1. Showing the Degradation of Zinc by *S.marcescens*

SAMPLE ID	TRUE VALUE (ppm)	Absorbance.
STD	0.2	0.17
STD	0.4	0.29
Control	100	1.19
Sample	100	1.12
Control	200	1.24
Sample	200	1.06
Control	500	1.32
Sample	500	1.27

Table: 2. Degradation of Copper by *S.marcescens*

SAMPLE ID	TRUE VALUE (ppm)	Absorbance.
STD	0.2	0.02
STD	0.4	0.06
Control	100	1.52
Sample	100	1.44
Control	200	2.10
Sample	200	1.06
Control	500	2.14
Sample	500	2.06

Table-3. Degradation of Lead by *S.marcescens*

SAMPLE ID	TRUE VALUE (ppm)	Absorbance.
STD	0.2	0.00
STD	0.4	0.01
Control	100	1.57
Sample	100	0.18
Control	200	0.72
Sample	200	0.13
Control	500	0.47
Sample	500	0.12

Table-4. ANTIBIOGRAM OF *Serratiamarcescens*

S.No	Antibiotics	Sensitivity	Intermediate	Resistant
1.	Cefazolin	-	-	R
2.	Cephaloridine	-	-	R
3.	Norflox	-	-	R
4.	Netromycin	S	-	-
5.	Trimethoprim	-	I	-
6.	Cefaperazone	S	-	-
7.	Gentamycin		-	-
8.	Piperacillintazobactum	S	-	-
9.	Ciprofloxacin	-	-	R
10.	Imepenem	S	-	-

S – Sensitive, HS – Highly Sensitive, R – Resistant

DISCUSSION

The discharge of heavy metal into aquatic ecosystem has become a matter of concern in India over last few decades. The pollutants are introduced into aquatic systems significantly as a result of various industrial operations. The pollutant of concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel. These toxic materials may be derived from mining operations, refining ores, sludge disposal, fly ash from incinerators, the process of radioactive materials, metals plating or manufacture of electrical equipment, paints, alloys, batteries, pesticide or preservatives.Norton *et al*(2003),studied and compared the adsorption capacities of zinc using biosolids and seaweed *Durvielleaportatorum*. The use of biosolids for zinc biosorpion with adsorption rate of 0.564 Mm/g was favorable when compared to the bioadsorption rate of 0.299 Mm/g by seaweed *Durvillaepotatorum*.The Serratia species are found in water, soil and on leaves, shrub, fruits vegetables, herbs, mushrooms, mosses and insects (AnuradhaGiri*et al*,2004).*Serratiaplymuthica* is found in soil(Kable*et a*l,1996) and has been isolated from different types of food (Lopez *et a*l,1996;Lyhs*et al*,1998).Most of *Serratiaplymuthica* strain have been isolated from fresh water and fish suggesting that they may be a potential opportunistic pathogen for animals and human (Nieto *et al*,1990;Rodriguez *et al*, 1990).Kesisnkan*et al*, 2003 studied adsorption characteristics of copper, zinc and lead on submerged aquatic plant *Myriophyllumspicatum* and were found to be 46.69mg/g for lead, 15.5mg/g for zinc and 10.37mg/g for copper.

It have an important role in several biotechnological applications, including the fields of biomineralization , bioremediation , bioleaching and microbial corrosion and have gained growing attention in recent years(TanjaKlausjeorger*et al* ,2001).Microbial interactions with small quantities of metals or metalloids do not exert a major impact on metal or metalloid distribution in the environment, where as interaction with larger quantities are required in energy metabolism to have a noticeable impact (Ehrlich ,1997).

METAL TOLERANCE AND RESISTANCE

The resistances of bacteria have been shown to increase proportionally along industrial contamination gradients (Vaun McArthur *et al*, 2000). Resistance to antibiotics is acquired by a change in the genetic makeup of a bacterium, which can occur by either a genetic mutation or by the transfer of antibiotic resistance genes between bacteria in the environment (American Academy of Microbiology, 2000).Noton*et al*, 2003 used dewatered waste activated sludge from a sewage treatment plant for the zinc from aqueous solutions. The use of biosolids for zinc absorption was favorable compared to the bioadsorption rate of 0.0299Mm/g by the seaweed *Durivilleapotatorum*. Keskinkian*et al*, 2003 studied the adsorption characteristics of copper, zinc and lead on submerged aquatic plant *Myriophyllumspicatum*. The adsorption capacity was 46.69mg/g for lead, 15.59mg/g for zinc and 10.37mg/g for copper.Pagnanelli*et al*, 2002 have carried out preliminary study on the use of olive mill residues as heavy metal sorbent material. The result revealed that copper was maximally adsorbed in the range of 5.0 to 13.5mg/g under different operating conditions.The simultaneous biosorption capacity of copper, cadmium and zinc on dried activated sludge (Hammaini*et al*, 2003) were 0.32Mm/g for Cd-Zn. The results showed that biomass has a net preference for copper followed by cadmium and zinc.Whistler and Daniel (1985) reported that heat treatment could cause a loss of amino acid functional groups on the fungal surface through the non enzymatic browning reaction. Amino functional groups in the polysaccharide contribute to the binding of heavy metals (Loaec*et a*l, 1997).Galun*et al*, 1987 reported that Penicillium biomass pretreatment at 100C for 5 minutes increased the bioadsorption of lead, cadmium, nickel and zinc, the increase was attributed to the exposure of latent binding sites after pretreatment.

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