Prevalence of Metal Resistance in Bacterial Population Isolated from Metal Contaminated Soils

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ABSTRACT

The aim of this study was to analyze the soil contaminated by different heavy metals. The Soil sample was analyzed by AAS. The soil samples were subjected to enrichment technique which allowed the growth of metal tolerating microorganisms. The microorganisms in the contaminated soil was then characterized morphologically and biochemically. A study on MIC was carried out to detect the maximal metal tolerating species. A study on the microbial diversiy may represent an important tool to expand our knowledge on the role of various microbial communities in metal contaminated soil.

***Key words*:** heavy metals, metal resistance, MIC, soil microbes, *Pseudomonas sp.,*

INTRODUCTION

Heavy metals are discharged from various industries such as electroplating, metallurgical processes, textile, storage batteries, pigment, fertilizers, plastic manufacturing, mining, ceramic and glass making. Chromium, copper and nickel are released into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc., and the concentration levels of them in the environment widely varies. These three metals are of major concern because of their larger usages in developing countries and their non-degradability nature [1]. Since the conventional physicochemical methods are expensive recently, microbial systems like fungus, bacteria and algae have been successfully used as adsorbing agents for removal of heavy metals. Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant [2]. There are so many metal-based industries located in various districts of Tamil Nadu in an unorganized manner. From these industries, enormous amount of solid wastes containing heavy metals has been indiscriminately disposed and left unattended by the Municipal Corporation of Coimbatore. Electroplating industry in Avarampalayam area of Coimbatore district is acting as the main source of heavy metal (nickel, copper and chromium) contamination [3]. Hence an attempt was made to isolate microbes from metal polluted soil and to study its metal tolerating ability.

MATERIALS AND METHOD

Sample collection

In the present study, it was preferred to explore the electroplating industrial areas of Avarampalayam, Coimbatore, Tamil Nadu, India for sample collection since the waste from the electroplating industry acts as rich source of chromium, copper, nickel and various other heavy metals.

Heavy metal analysis in soil sample

One gram of soil was preserved by mixing it with 3 ml of concentrated hydrochloric acid. It was then transferred to a beaker containing 5 ml of concentrated nitric acid. The sample was heated on a hot plate for complete digestion, till a light coloured, clear solution was obtained and the solution was filtered through whatman filter paper [4]. The sediment was discarded and the filtrate was diluted by taking 0.2ml and made upto 7ml. This diluted sample was used for the determination of various metals, using a SHIMADZU double beam Atomic Absorption Spectrophotometer (Model No: 63008).

Enrichment method:

One gram of soil was added to 100ml of nutrient broth and kept in shaker for 3 days at 120 rpm. From that 1 ml of sample was transferred to another 100ml of nutrient broth containing 10 ppm concentration of heavy metals namely chromium, copper, lead, iron, zinc, nickel and kept in shaker for 3 days at 120 rpm and this procedure was repeated twice. Then a loopful of culture was taken and streaked over the nutrient agar plate containing 10 ppm concentration of heavy metal. The plates were incubated at 37 ^{0}C for 24 hours. By using this method the heavy metal resistant organism has been isolated in the primary isolation plate [5].

Isolation of micro organism

The heavy metal resistant microorganism thus obtained on the primary isolation plate was observed for the different colony morphology and the individual isolates were picked up separately and purified by quadrant streaking in Nutrient agar plates and single streak in Sabouraud dextrose agar plates for the isolation of individual bacterial and fungal colonies respectively [6]. The bacterial isolates were incubated at 37 ^{0}C for 24 hours and fungal isolates were incubated at temperature of 24^0 C for 3 days [7].

Minimal Inhibitory Concentration test (MIC)

A stock solution with concentration of 1000 ppm was prepared for heavy metals namely chromium, nickel, lead, copper, mercury and iron. The bacterial isolates were inoculated on PYG (Peptone-Yeast extract-Glucose) medium containing different concentration of heavy metals namely chromium, copper, nickel, lead, iron and mercury ranging in concentration of 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm upto 1300 ppm. The inoculated plates were incubated at 37 ^{0}C for 24hrs. Following which, based on the presence of excellent or good growth at higher metal concentration, the maximal metal tolerating isolate was taken for further experimentation [8] .

RESULTS AND DISCUSSION

Heavy metal analysis in soil sample

Soil sample was analyzed through Atomic Absorption Spectroscopy(AAS) which showed the presence of chromium, copper, nickel, lead, iron and mercury metals and its concentration in parts per million i.e ppm was tabulated (Table 1).

Table 1 : Heavy metal analysis in soil (AAS)

HEAVY METALS	CONCENTRATION IN PPM
Chromium	1.8828
Copper	9.0056
Lead	0.3678
Iron	11.5828
Mercury	0.5490
Nickel	0.1144

Heavy metal analysis done by Atomic Absorption Spectroscopy (AAS) showed the presence of various heavy metals in the soil sample. Our study revealed the presence of six different heavy metals in soil sample. Similarly, heavy metals like chromium, lead, zinc and copper was detected in water sample using AAS [9]. The long term reception of untreated industrial effluents is the main reason for the high heavy metal content in the soil sediments [10]. This heavy metal analysis showed that the soil was contaminated with heavy metal and there may be a possibility for the existence of metal resistant organism.

Enrichment method :

This method allowed the growth of metal tolerant organism. In the present study, enrichment of soil sample was done which could allow the growth of all possible metal resistant organisms. Five bacterial isolates and seven fungal isolates were obtained from soil sample taken for heavy metal resistant studies. Among this only bacterial isolates were taken for further studies since they are rapid growers [11].

Isolation of microorganisms :

Morphologically different isolates were observed in the primary isolation plate. Five different metal tolerating bacterial isolates and seven different heavy metal tolerating fungal isolates were selected and

pure culture was obtained by repeated quardrant streaking.

Identification of microorganisms obtained in primary isolation plate

***Bacterial Identification*:** Through Gram's staining three Gram negative rods, one gram positive cocci and one Gram positive rod was identified. Through biochemical tests, five different bacterial isolates namely *Staphylococcus sp., Pseudomonas sp., Klebsiella sp., Proteus sp.,* and *Bacillus sp.,* were characterized.

***Fungal Identification*:** Seven fungal species namely *Diplosporium sp., Aspergillus sp., Cladosporium sp., Mucor sp., Monotospora sp., Fusarium sp., Penicillium sp.,* were identified based on microscopic, cultural characteristics, growth rate and pigmentation. Fungal species were also actively exploited for the removal of metal contamination from polluted soils. Filamentous fungi resistant to heavy metal was used for the metal removal from the contaminated sites by Ezzouhri *et al.,* (2009) [10]. Since the fungi are not rapid growers they are not exploited for the present studies.

***Minimal Inhibitory Concentration Test*:** The bacterial isolates were not able to tolerate the increase in the metal concentration of mercury, iron and lead. Through MIC it was found that *Pseudomonas sp.,* alone was able to tolerate upto 1300 ppm of chromium, 1200 ppm of copper and 1000 ppm of nickel, whereas other bacterial species were not able to tolerate high metal concentration and their tolerance limit was tabulated (Table 2, 3 and 4).

Minimal Inhibitory concentration (MIC) revealed that among the various metals used chromium exhibited the greatest effect on cell growth. Chromium was the most toxic and mutagenic metal ion that acts on the biological systems. *Pseudomonas sp.,* was able to tolerate chromium concentration of 1300 ppm. When the chromium concentration in the growth medium reached 1320 ppm the growth of *P. aeruginosa* PA01 was inhibited. This result is in accordance with Hassen

Table 2 : MIC of various isolates to Chromium

Isolates	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm	700 ppm	800 ppm	900 ppm	1000 ppm	1100 ppm	1200 ppm	1300 ppm
1	++	++	++	++	++	++	++	+	+	+	+	-	-	-
2	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++
3	++	++	++	++	++	+	+	+	+	+	-	-	-	-
4	++	++	++	++	++	++	++	+	+	+	+	+	-	-
5	++	++	++	++	++	++	++	++	++	+	+	+	+	-

Table 3 : MIC of various isolates to Copper

Isolates	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm	700 ppm	800 ppm	900 ppm	1000 ppm	1100 ppm	1200 ppm	1300 ppm
1	++	++	++	++	++	++	++	+	+	+	+	-	-	-
2	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	-
3	++	++	++	++	++	+	+	+	+	-	-	-	-	-
4	++	++	++	++	++	++	++	+	+	+	+	-	-	-
5	++	++	++	++	++	++	++	++	++	+	+	+	-	-

Table 4 : MIC of various isolates to Nickel

Isolates	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm	700 ppm	800 ppm	900 ppm	1000 ppm	1100 ppm	1200 ppm	1300 ppm
1	++	++	++	++	++	++	++	+	+	+	+	-		
2	+++	+++	+++	+++	+++	+++	++	++	++	++	++	-		
3	++	++	++	++	+	+	+	+	-	-	-	-		
4	++	++	++	++	++	++	+	+	+	+	-	-		
5	++	++	++	++	++	++	+	+	+	+	-	-		

Isolate : 1 - *Staphylococcus sp.,* 2 - *Pseudomonas sp.,* 3 - *Klebsiella sp.,* 4 - *Proteus sp.,* 5 - *Bacillus sp.,*
+++ — Excellent growth; ++ — Good growth; + — Moderate growth; - — absence of growth.

et al., 2005 [12], whereas the presence of copper and nickel had less effect than chromium on bacterial growth. Copper and nickel arrested the growth of *P. aeruginosa* PA01 when their concentration reached 1300 and 1100 ppm in the plates respectively. The MIC of *Pseudomonas aeruginosa* PA01 for copper and nickel was less than chromium. Thus these heavy metals have an antagonistic effect on the growth of microbial cells.

The growth order of metal resistant strain based on MIC was found to be in the order of chromium > copper > nickel as described by Hassen *et al.,* 2005 [12]. Whereas other isolates namely *Staphylococcus sp., Proteus* sp., *Klebsiella sp.,* and *Bacillus sp.,* tested were not able to tolerate high concentration of heavy metal. This results would suggest us that there is a prevalence of high metal tolerance among the *Pseudomonas sp.,*

CONCLUSION

It was found that the metal pollution results in the development of metal tolerant organisms among the bacterial population. The bacterial resistance may be due to the uptake of metals from the contaminated sites. This study provides a possible scope that the metal tolerating organisms could be exploited for the further study in the field of bioremediation of metal contaminated soils and in the cycling of toxic metals in the biosphere.

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