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HEAVY METAL TOLIARENT SOIL MICROFLORA : A NATURAL RESOURCE OF BIOREMEDIATION

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ABSTRACT

Soil provides mineral material on surface of earth, considerably very suitable culture medium for growth of microorganism, but due to rapid industrialization, so many undesirable substances including heavy metals are being widely distributed in the environment including soil. Soil contaminated with heavy metals, produces unhealthy food that may enter to the food chain as residual elements and may become injurious to human society. Such findings reveal a new area of study to sustain the environment. So to determine the effect of heavy metals on soil, an attempt was done to isolate stress tolerant microbes from soil mixed with different concentration of salts of heavy metal (Hg, Zn, Cu, Cd & Pb). During present investigation three heavy metal salts were selected for assessment of heavy metal tolerance both in bacteria& fungi and such tolerant microbes were isolated and identified. It was noticed that heavy metals affect the total microbial population. Fungi were found to be more tolerant to heavy metals rather than Bacteria. So these microorganisms proved to be the powerful sources for bioremediation of metal contaminated soils.

Key words : *Soil, Microorganisms, Heavy metals, Soil pollution, Bioremediation.*

Heavy metal contamination in the environment is a major concern because of their toxicity and threat to human life and environment. Soil facilitates various biological processes that perform significant services to the ecosystem. But due to natural and anthropogenic activities so many undesirable substances including heavy metals are being widely distributed in the environment including soil. Heavy metals exhibit toxic effects on soil microflora (Pwlowska & Charvat, 2004). Rabia shraf and Tasneem Adam Ali in 2007 studied the effects of heavy metal pollution on natural microbial communities and mung beans seed germination, Ahamad *et al.*, 2005, observed the effect of heavy metal on survival of certain groups of indigenous soil microbial population, that have attracted increased attention. Ahmed *et al.*, 2001, Hayat *et al.*, 2002, observed that heavy metal tolerance via specific group of microorganism in artificial media supplemented with heavy metal showed high tolerance. According to Ali & Wainwright, 1995, The microorganisms can also be applied to remove toxic metals from contaminated areas because they have the ability to accumulate heavy metals. Rajendran et al., 2003, studied the role of microbes in heavy metal remediation. Main objective of our present investigations is to discuss the heavy metal tolerance soil micro flora and their potential in metal remediation.

Control standards of heavy metal of soils :

Heavy metals	Standards mg/kg
Hg	5
Zn	600
Pb	500
Cu	200
Cd	5

MATERIALS AND METHODS

Sample collection : The soil samples during month of September were collected from agronomic field. The field was under cultivation and has received industrial untreated waste water. Soil samples 200g each were collected in sterilized zipped polythene bags and stored at 28±2°C.

Physicochemical characterization of soil : Soil colour , pH & Moisture content all were determined using the methods described by Gupta, (2004).

Metals used in the study : Heavy metal salts i.e. $CuCl_2$ $PbCl_2$ $HgCl_2$, $ZnSo_4$ $CdCl_2$ were Selected for present Investigation. The Soil sample was field into pots (200g/pot) and pots were amended with different concentrations of these metal salts. Control was maintained without any metal amendment.

Isolation and identification technique applied for microorganisms : Isolation was done after 15 days of incubation. Sample were serially diluted and an amount of 0.1ml from the diluted sample was spread on respective culture media, These plates were incubated at ambient temperature- 24 h for bacteria and 4-6 for fungi , Colonies were counted and CFU/g were calculated for bacteria. Pure culture was maintained on respective media. After

Table-1 : Bacterial Morphological characteristics.

S. No.	Colour	Gram reaction	Size(mm)	Shape	Margins	Elevation	Opacity
1.	White	Gm –ve rods	2.53	Irregular	Irregular	Flat	Opeque
2.	Cream	Gm –ve rods	0.52	Circular	Entire	Elevated	Mucoid, Translucent
3.	Yellow	Gm +veCoccus	0.65	Circular	Entire	Elevated	Translucent
4.	Bluish green	Gm-ve rods	0.34	Irregular	Irregular	Elevated	Mucoid, Translucent

Table-2 : Biochemical characteristics of Bacterial isolates.

TESTS	B1	B2	B3	B4
Catalase	+	-	+	+
Glucose	A	AG	AG	A
Lactose	-	AG	A	-
n547Mannitol	A	AG	AG	A
Indole	-	-	-	-
MR	+	-	-	-
VP	+	+	+	+
Citrate	-	+	-	+
Starch	-	-	-	+

Table-3 : Enumeration of viable microorganisms in heavy metal abended soil:

Heavy metals	Number of Heterotrophs cfu/g for:		
Types	Control	25mM	50mM
$HgCl_2$	$6.543x10^7$	$2.025x10^7$	$1.201x10^7$
$Znso_4$	$6.543x10^7$	$7.533x10^7$	$8.672x10^7$
$PbCl_2$	$6.543x10^7$	$3.653x10^7$	$2.853x10^7$
$CuCl_2$	$6.543x10^7$	$6.622x10^7$	$5.750x10^7$
$CdCl_2$	$6.543x10^7$	$4.154x10^7$	$3.252x10^7$

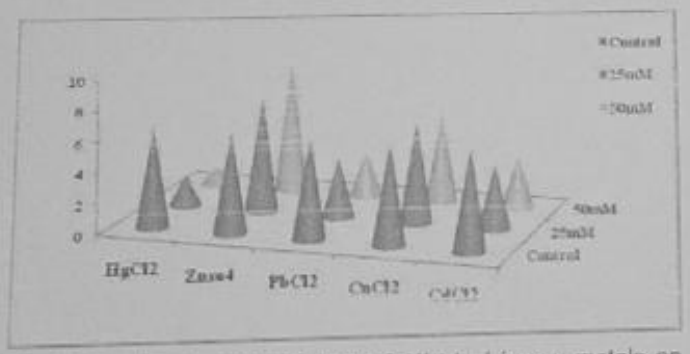

Fig.-1: Graphical representation of effect of heavy metals on growth of Bacteria.

obtaining pure culture by streak plate method, isolated bacterial samples were identified via morphological characteristics & biochemical tests. Fungal strains were stained with lactophenol cotton blue stain and were identified by keys given by Alexopolus and mims.

RESULTS AND DISCUSSION

Physicochemical analysis : The soil collected was dark brown porous with small aggregates and moisture content was of 2.15% (w/w), pH of the soil sample was observed as 7.3

Microbial analysis : Via applying biochemical tests four bacterial strains were identified *i.e. Staphylococcus sp., Pseudomonas sp., Bacillus sp., Klebsiella sp.* Simultaniously by propoer technique five fungal sp. i. e. *Fusarium solani, Aspergillus niger, Alternaria sp., Penicillium sp. and Cladosporium sp.* were identified. It was noticed that increasing concentration of $Hgcl_2$ in the soil resulted decreased growth of microorganisms. The overall decrease in the growth of microorganisms was observed, due to increased concentration of heavy metal contamination. (Kikovic,1997 and Smejkalova *et al*, 2003). However in present study marked increase in the number of Bacteria were found in Zinc amended soil, and zinc was found to have favourable effects on the growth. The overall scenario was *i.e.* Hg > Pb > Cd > Cu > Zn. Most of the fungi and bacteria were found to be resistant to change in pH, they could grow in a wide range of pH. Gram –ve bacteria were more resistant then Gram+ve. Fungi were found to be more resistant to heavy metals.

CONCLUSION

The presence of toxic metals in soil leads to the decreased value of land with reference to its productivity and ultimately affects the human health in the form of poisoning. The bacteria and fungi isolated and identified prove to be potential candidate in the bioremediation of metal contaminated soils and industrial effluents. Due to its low cost, high efficiency and ecofriendly nature.

Further experiment is needed to understand the genetic diversity of metal tolerant microbial population and metal microbe interaction in soil.

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