ISOLATION OF HEAVY METAL TOLERANT ORGANISM AND TO ASSESS ITS EFFECT ON PLANT GROWTH.

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***Abstract*: Microremediation is a process in which micro-organism are used to degrade the heavy metal pollutants present in the environment. These heavy metal tolerant organisms were isolated from the soil taken from the metal cutting industry and garage. These heavy metal tolerant organisms were differentiated and characterized by various biochemical tests and the probable genus of these organisms was found to be *Paenibacillus, Streptococcus, Pseudomonas* and *Bacillus*. Some of the heavy metals used are Iron (Fe), Manganese(Mn), Molebdenum(Mo), Copper(Cu), Cobalt(Co), Chromium(Cr), Nickel(Ni). The test plant for this project were Corn(*Zea mays*) and Jowar (*Sorghum bicolor*). These plants were germinated with the solution comprising of heavy metal solution along with the organism that degraded that particular heavy metal. The organisms were selected based upon their MIC(Minimum Inhibitory Concentration) values. The germinated seeds were then added to the sterile soil and the growth of the plant was observed. The various physical and chemical parameters of the plant was checked. The growth of the Jowar plant was enhanced by the activity of organism when the solutions such as copper, chromium, manganese were used. The growth of corn plants were also enhanced when the solution of iron, nickel and molebdenum were used.**

Keywords*:Microremediation, heavy metal, degrade, soil.*

1. INTRODUCTION :

A heavy metal is of high density and it also has high atomic weight. Heavy metals are conventionally defined as elements with metallic properties and an atomic number more than twenty. There are a number of heavy metals that are present in the environment. Heavy metals like Fe (Iron), Cd(Cadmium), Co(Cobalt), Hg(Mercury), Mn(Manganese), Ni(Nickel), Cr(Chromium), Pb(Lead) are common heavy metal contaminants that are found at a polluted site. Elevated levels of heavy metals can result in decreased microbial community (*shodhganga.inflibnet.ac.in*). Metal pollutant has harmful effect on biological systems and does not undergo biodegradation. Heavy metals can be added in the soil through anthropogenic activities natural sources of heavy metals such as volcanoes emissions transport of continental dust and also by human activities by exploitation of mines and smelters, application of metal-based pesticidesand metal-enriched sewage sludge in agriculture, combustion of fossil fuels. The anthropogenic activities have been categorized into five groups: (1) metalliferous mining and smelting, (2) industry, (3) atmospheric deposition, (4) agriculture and (5) waste disposal and these activities result in soil pollution (Oves *et al*,2016).The release of untreated industrial waste into the environment has become a foremost concern in the developing countries and is viewed as one of the most important environmental issues. If heavy metal polluted soil is used for crop cultivation then the heavy metal ions deposited in the soil can get accumulated in the food crop in high concentrations creating severe human health problems related to the gastrointestinal system and the nervous system (Oves *et al*,2016). Toxic heavy metals entering into the ecosystem leads to geo-accumulation, bioaccumulation and bio-magnification processes which has intense ecological and public health implications (*shodhganga.inflibnet.ac.in*). It is therefore important to lower the amount of heavy metal concentration in the soil. There are a number of bacteria and fungi that can accumulate an abundant range of metal species. This is achieved by some heavy metal tolerant organisms that are found in the polluted environment. These organisms can interact firmly with metal ions in soil solution through their charged surfaces. Bacterial cells have a huge capacity to enable them to absorb and immobilize toxicions from soil solution(Oves *et al*,2016) The degradation of these heavy metals are important in the soil where cultivation is carried out so that the soil is free from most of the pollutants. This is done by the microorganism and the process is called as bioremediation. Bioremediation is process in which the microorganisms degrade the heavy metal pollutant present in the environment. Bacteria and other microorganisms exhibit a number of metabolism-dependent processes and carry out the uptake

and accumulation of heavy metals and other pollutants. The most important benefit of microbial biosorption over conventional treatment methods are low cost, minimization of chemicals and biological sludge, high efficiency, regeneration of biosorbent and possibility of metal recovery (*shodhganga.inflibnet.ac.in*). Our project is based upon the idea of lowering the amount of heavy metal present in the soil by using microorganisms and to grow plants in in heavy metal polluted soil by using these microorganisms.

2. MATERIALS AND METHODS:

Sample and Seeds collection:
Surface soil samples were collected from garage and metal cutting industry located in Mumbai. Area was selected on the basics of heavy metals dealing with those industries. After collection of samples they were placed in plastic bags. Seeds of jowar and corn were collected from local shop in Mumbai.

Isolation of heavy metal tolerant bacteria:
One gram of soil sample obtained from each site was suspended in 10ml of sterile saline and vortex for 2 min. Appropriate dilutions of each stock were spread on sterile LB agar + heavy metal plates by spread plate technique and incubated at room temperature for 24hrs (Gupta *et al*, 2015). Isolated colonies of heavy metal tolerant bacteria were selected based on their colony characteristics.

Characterization and identification of isolated bacteria:
Pure cultures of selected isolates were obtained on LB+heavy metal plate. The Gram nature, shape and arrangement of heavy metal isolates were determined (Chhaya *et al*, 2015). Organisms were isolated on selective and differential media such as Cetrimide agar, Salt mannitol agar, starch agar plate, Bile esculine agar and macConkey's agar plate.Various biochemical tests of selected heavy metal tolerant organisms were performed based on Gram nature of them (Gurave *et al*, 2015).
Based on result obtained from biochemical test genus level identification of organisms was identified by using Bergey's manual of systematic bacteriology (Gurave *et al*, 2015).

Determination of Minimum Inhibitory Concentration(MIC) and Minimum Lethal Concentration(MLC):
Minimum inhibitory concentration of each heavy metal for the isolate was determined by broth dilution method (Haroun *et al*, 2017). Different concentration (60-600µg/ml) for every heavy metal was prepared by using heavy metal salt and diluent used was sterile LB broth. On the basis of MIC result the MLC was carried out. The three tubes afterMIC not showing any turbidity were spot inoculated on LB plate for determination of MLC.

To check the effect of heavy metal tolerant bacteria on germination of seeds:
Germination of seeds was carried using filter paper method (Garg *et al*, 2009). Seeds were first soaked overnight in tap water and then surface sterilized. Three controls and one test were run for every metal in triplicates.Filter paper was first sterilized in autoclave then transfer into sterilized plate under aseptic condition. Solution for controls and tests were prepared and added into their respective plates.
Control 1: distilled water + heavy metal (concentration based on MIC value).
Control 2:distilled water + organisms of respective heavy metals.
Control 3:distilled water.
Test:distilled water + heavy metal (concentration based on MIC value) + organisms of respective heavy metal.
Sterilized seeds were transferred to control and test plates and incubated in dark condition for 3 days. After incubation the germination rate was calculated (Garg *et al*, 2009).

Formula for germination rate (Chhaya *et al*, 2015):
Germination = Total number of seeds germinated × 100 ÷ total number of seeds sown.

To check the effect of heavy metal tolerant bacteria on growth of plant :
Garden soil was taken and autoclaved for three days (once in a day). After autoclaving soil was measured to 150gm and was transferred to plastic cup. Solution for control and tests were prepared same as that prepared for germination concentrations based on MIC values and added into respective cups .Germinated seeds from plates were transferred to cups and cups were placed where enough sunlight was present. After 10 days of incubation the various plant parameters were checked. (Garg *et al*, 2009)

3. RESULTS:

Isolation of heavy metal tolerant bacteria :

The isolation of the organisms was carried out and various colonies were obtained on the LB+heavy metal plates. The colonies were selected based upon their colony characteristics, gram nature and the pigments they produced for metals such as Cr, Co, Cu

,Ni ,Mo, Mn ,Fe. 30 colonies were obtained and out of which further we proceeded with only 7 colonies one for each respective heavy metal.(Table.1.)

Table.1.Biochemical chart:

Test	Cr	Co	Ni	Mo	Mn	Fe	Cu
Sugar fermentation test							
Glucose	+	-	+	+	+	+	+
Sucrose	+	-	-	-	+	+	-
Lactose	+	+	+	+	+	+	+
Maltose	-	-	+	+	+	-	+
Mannitol	+	-	-	-	-	+	+
Xylose	+	+	+	+	-	+	+
Gram nature	Gram positive	Gram negative	Gram negative	Gram negative	Gram positive	Gram positive	Gram positive
Catalase	+	-	-	-	+	+	+
Motility	-	-	+	-	+	+	-
IMVIC							
Indole	**NA**	**No results obtained**	**No results obtained**	**Noresults obtained**	**NA**	**NA**	**NA**
Methyl red							
Vogus proskaeur							
Citrate							
TSI							
Alkaline production	-	-	+	+	-	+	-
Acid production	-	-	+	+	-	+	-
Gas production	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-
Urease	**NA**	-	-	-	**NA**	**NA**	**NA**
Nitrate	+	+	+	+	+	-	+
Salt mannitol agar	+	**NA**	**NA**	**NA**	-	+	+
Mac Conkey	**NA**	-	+	+	**NA**	**NA**	**NA**
Cetrimide	**NA**	+	+	+	**NA**	**NA**	**NA**
Bile esculin	**NA**	-	-	-	**NA**	**NA**	**NA**
Starch agar plate	-	-	-	-	-	+	+

Keywords: (+): Growth was observed. NA: Not Applicable (-): Growth was not observed.

Figure.1 Mac Conkey plate

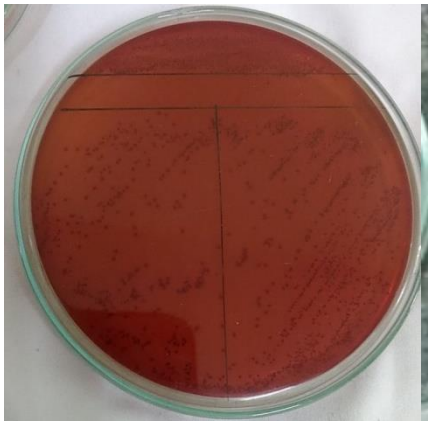


Figure.2 Cetrimide plate

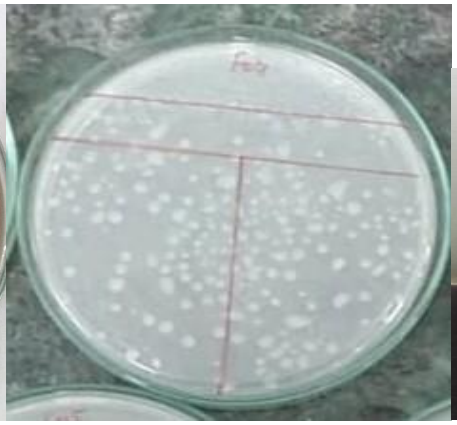


Figure.3 Nitrate broth test

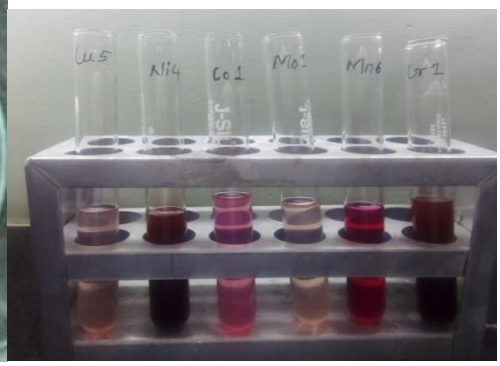
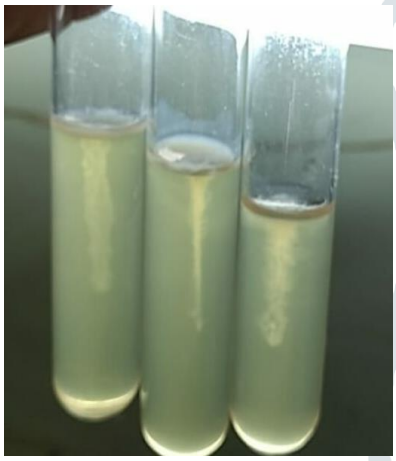


Figure.4 Motility test



Determination of Minimum Inhibitory Concentration and minimum lethal concentration :

The MIC for the various heavy metal tolerant organism was carried out and against the specific range of (60-600µg/ml) respective heavy metal. The MLC values for some of the heavy metal tolerant organism was not known as this range was exceeding to more than 600 µg/ml.(Table.2.)

Table.2.The Minimum Inhibitory Concentration(MIC) and Minimum Lethal Concentration(MLC) of heavy metal tolerant organisms for various metals

Metals	MIC Concentration	MLC Concentration
Copper (Cu)	120µg/ml	300µg/ml
Cobalt (Co)	300µg/ml	>600µg/ml
Molebdenum (Mo)	480µg/ml	>600µg/ml
Nickel (Ni)	240µg/ml	>600µg/ml
Ferrous (Fe)	420µg/ml	480µg/ml
Manganese (Mn)	540µg/ml	>600µg/ml
Chromium (Cr)	120µg/ml	240µg/ml

Effect of heavy metal tolerant organism on jowar and corn plant :

The plant growth was checked by its physical characteristics and that are length of root, length of shoot, number of lateral root hairs, length of plant, number of leaves and colour of the leaves for jowar and corn plant. The various parameters were measured in terms of centimeters. The parameters of jowar control plant are given in the below table.(Table.3).The parameters of corn

control plant are given in the below table.(Table.4). The parameters for the test plant which consisted of both heavy metal and the respective heavy metal tolerant organism for jowar and corn are given in the table (Table.5.)

Control 1 : Distilled water + heavy metal

Control 2 : Distilled water + heavy metal tolerant organism

Control 3 : Distilled water

Test : Heavy metal + heavy metal tolerant organism + distilled water

Table.3.: Effect of heavy metal and heavy metal tolerant organism as controls on jowar plant

JOWAR	Control 1					CONTROL 2					Control 3				
	RL	SL	PL	NOL	NOLRH	RL	SL	PL	NOL	NOLRH	RL	SL	PL	NOL	NOLRH
CU	2	28	30	3	4	4	17.2	21.2	3	2	5.5	26	31.5	3	5
CR	5	5	10	3	1	3	19.3	22.3	3	4					
CO	2	34	36	3	4	2	17	19	3	4					
FE	3	28	31	3	4	3	10.5	13.5	3	3					
MO	1	13	14	3	4	3	19	22	3	4					
MN	3	29	32	3	7	2	15	17	3	2					
NI	4	30	34	3	5	2	16	18	3	2					

Table.4.: Effect of heavy metal and heavy metal tolerant organism as controls on corn plant

CORN	Control 1					CONTROL 2					Control 3				
	RL	SL	PL	NOL	NOLRH	RL	SL	PL	NOL	NOLRH	RL	SL	PL	NOL	NOLRH
CU	2	26	28	2	10	2	39	41	3	8	4	43	47	3	4
CR	2	17	19	3	5	2.5	20.5	23	3	8					
CO	4.5	12	16.5	3	3	2	16	18	3	7					
FE	4	37.5	41.5	3	4	2	26	28	3	7					
MO	2	14	16	2	6	1	14	15	3	5					
MN	6	34	40	3	6	7	32	49	3	5					
NI	3	30	33	3	6	3	25	28	3	4					

Table.5.: Effect of heavy metal tolerant organism in presence of heavy metal in jowar and corn plant.

	JOWAR TEST					CORN TEST				
	RL	SL	PL	NOL	NOLRH	RL	SL	PL	NOL	NOLRH
Cu	6	31	37	3	10	3	31	34	3	3
Cr	1.5	14	15.5	3	4	2	12	14	3	4
Co	2.5	26	28.5	3	6	2	19	21	3	3
Fe	1	24	25	3	3	3	36	39	3	6
Mo	1	11	12	3	4	2	22	24	3	2
Mn	5	32.5	27.5	3	5	5	38	43	3	3
Ni	3	32	35	3	7	3	35	38	3	4

Keywords : RL : Root Length

SL : Shoot Length

PL : Plant Length

NOL : Number of Leaves

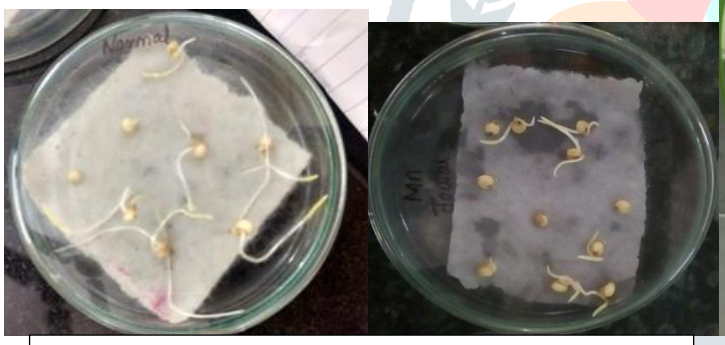
NOLRH : Number of Lateral Root Hairs

Figure.6 Germinated seeds of jowar

A B

Figure 7 Adult plant of jowar

A B



C D

C D

A- CONTROL: distilled water + heavy metal.

B- TEST : distilled water + heavy metal + metal tolerant organisms.

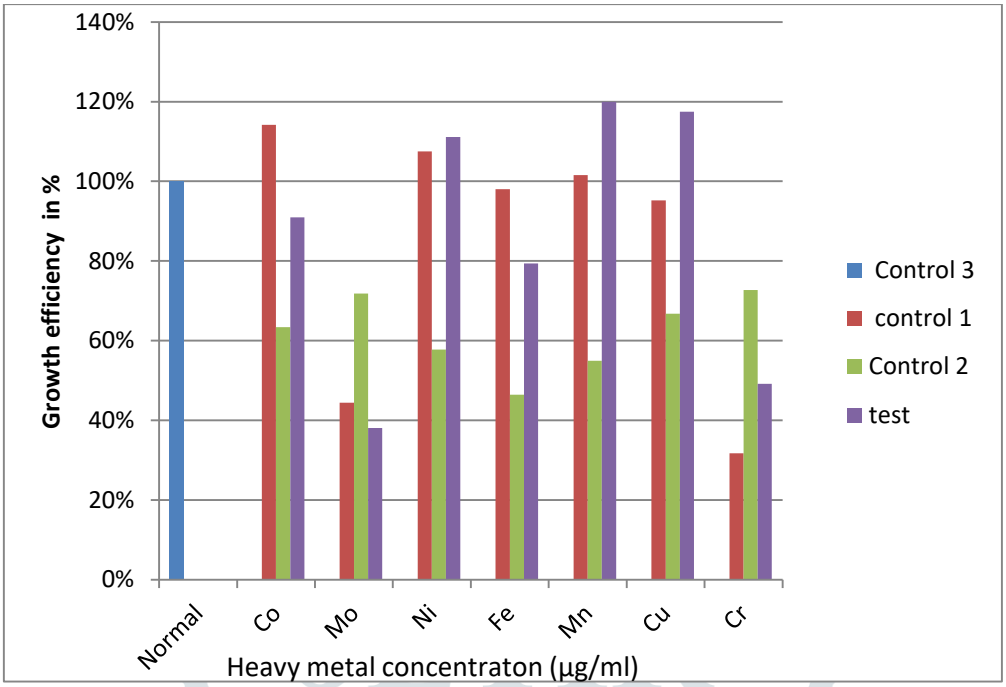
C-Control : only distilled water.

D-Control : distilled water + organism.

Graphical representation of the effect of Heavy metal tolerant bacteria on the growth of plant.

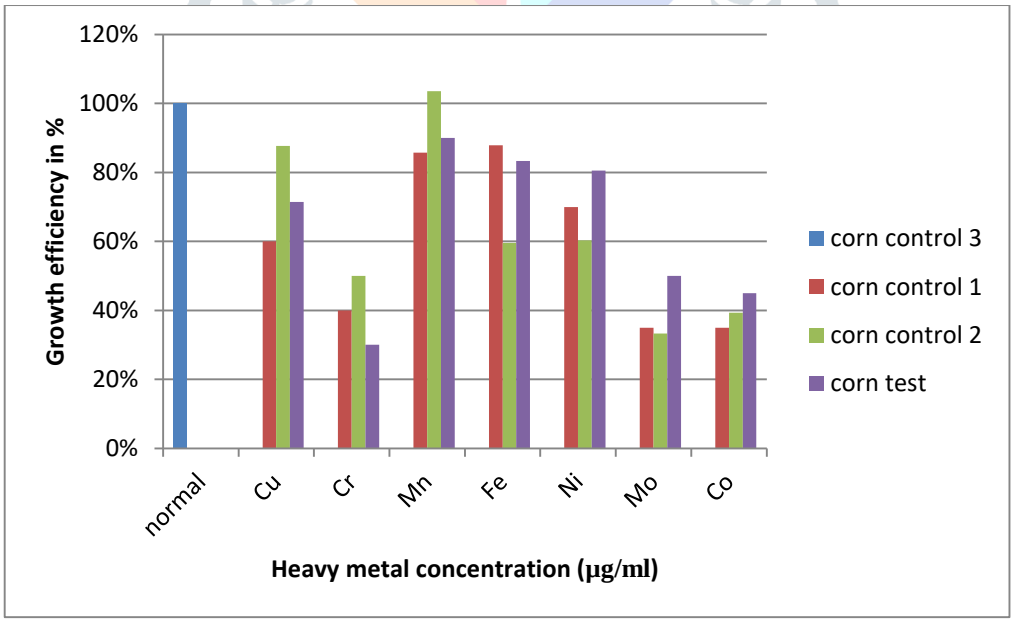
The parameters like length of root, length of shoot, number of leaves, number of lateral root hairs and plant length along a with the germination of seeds was calculated as percentage growth efficiency and is shown in the below graph. The average of each plants was taken by observing and calculating 10plants for each control plant and test plant for each respective heavy metals. The effect of heavy metal tolerant bacteria on the growth of jowar(Graph.1) . The effect of heavy metal tolerant bacteria on the growth of Corn plant(Graph.2).

Graph.1. Effect of heavy metal tolerant bacteria on the growth of jowar plant



Heavy metal	Cu	Co	Mo	Ni	Fe	Mn	Cr
Concentration(µg/ml)	60	240	420	180	300	480	60

Graph.2.Effect of heavy metal tolerant bacteria on the growth of corn plant.



Heavy metal	Cu	Co	Mo	Ni	Fe	Mn	Cr
Concentration(µg/ml)	60	240	420	180	300	480	60

4. Conclusion:

The heavy metal present in the soil can make the soil infertile and reduces the seed germination and radical growth in plants. Heavy metal tolerant organism isolated from soil sample shows its effects on *S.bicolor* and *Z. mays* From the above result obtained it can be that the heavy metal tolerant organism is helping the test plants i.e corn(*Zea mays*) and jowar(*Sorghum bicolor*)

to grow to a certain limit. As the plant soil contained both the metal solution and the organism that degraded that particular heavy metal. For jowar plant good growth was observed for copper, chromium and manganese as the heavy metal tolerant organism for copper, chromium and manganese degraded the heavy metals present in the soil and enhanced the growth of the plant similar to that of the normal plant which consisted of only water. For Corn plant good growth was observed for Molebdenum, Iron and Nickel. As the heavy metal tolerant organism for Molebdenum, Iron and Nickel degraded the heavy metal present in the soil and enhanced the growth of the plant similar to that of a normal plant.From the research paper we had selected as the reference the sample collection was done from a paper mill effluent industry, pharmaceutical drains and we had collected the samples from a metal cutting industry and garage.The probable organisms that we had obtained were belonging to the genus of *Paenibacillus*, *Bacillus*, *Pseudomonas*, *Streptococcus*, *Staphylococcus* and *Hafnia*. The specific strains of heavy metal tolerant organism were selected and characterised.The strains were selected based upon the MIC values and used for further bioremediation purposes.s

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