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Comparative Study of Detoxification of Heavy Metals using Microbes and Plant *Cicer arietinum* (Chick pea)

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In the present work chromium, arsenic and lead were selected as a model for heavy metal contamination. These metals were selected based on the fact that metal effluents are discharged from industries and they pollute the environment. The isolation of heavy metal tolerant bacteria was done from industrial wastewater, metal scraps and soil samples from the MIDC area of Nagpur. Four heavy metal tolerant isolates were screened and pure cultures were obtained. The detoxification study was done for different concentrations of Pb. The detoxification of Pb was carried out using three approaches. In first approach only heavy metal tolerant isolates were used for detoxification of heavy metals. In second approach, detoxification was done only by plant i.e. *Cicer arietinum* (chick pea) and finally in third approach, a combined detoxification was by both microbes and plants were performed. The metal analysis was done using Inductive coupled Plasma (ICP). This study concluded that the detoxification done by plants is more effective than that by microbes but the combination of both plants and microbial detoxification was the most effective. Thus this analysis has opened a new venture in the field of bioremediation strategies.

Key words: Heavy metals, Wastewater, Metal resistance, Bioremediation, Detoxification.

In this 21st century, human beings have achieved a peek in industrialization and modernization. Nature has been the major victim of its harsh effects. Industries manufacturing machinery, automobiles, oil refineries, etc. releases toxic metals like chromium, arsenic, lead, cadmium in the environment rendering it useless. These metals not only effect the environment but also are toxic to the flora and fauna[1]. A growing awareness among society and concern about environmental pollutants, many techniques has been researched to overcome this pollution. Of the technologies that have been investigated, bioremediation and phytoremediation have emerged as the most desirable and ecofriendly approach for cleaning up many environmental heavy metal pollutants. A group of metals whose atomic density is greater than 5g/cm are referred as heavy metals. They are toxic or poisonous even at the lowest concentration[2].

Bacteria are among the most abundant organism that occur every where on earth. Heavy metals are increasingly found in microbial habitats due to several natural and anthropogenic processes; therefore, microbes have evolved mechanisms to tolerate the presence of heavy metals by either by efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration[3]. Most mechanisms studied involved the efflux of metal ions outside the cell, and genes for tolerance mechanisms have been

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found on both chromosomes and plasmids. Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions[2]. Considering the importance of these tolerance mechanisms, in the present study organisms were isolated from contaminated sites of the automobile workshop and welding workshop were presence of heavy metals is abundant.

These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity[4]. Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA[4].

Presence of different type of metal in the soil poses potential environmental problems and heavy metal contamination of soil is wide spread[5]. So it is necessary to reduce or oxidize heavy metal to non–harmful form. For this reduction/oxidation, biological remediation is one of the best methods which are cost effective, easy and eco-friendly for treatment of heavy metal contaminated soil. Our objective is to test the detoxification potentials of microorganisms, plant along with their combination.

Toxic metals in the environment (Cd, Cr, As, Pb) have been reported to cause pollution in environment. This project studies the degradation of Pb metal. Thus this biological detoxification strategies ensures us a better green environment.

MATERIAL AND METHODS

Isolation of heavy metal tolerant bacteria

For the preliminary isolation of heavy metal tolerant bacteria, soil samples were selected from M.I. D. C. (Industrial area), soil sample from welding work shop and metal scrap (NEERI Campus).

Identification of metal tolerate microbes

The identification and morphological characterization was done by biochemical tests and microscopic study of the pure isolates.

To study detoxification of heavy metals by obtained isolates

For detoxification study 200 ml autoclaved nutrient media was taken in 250ml flask. The culture having high growth was inoculated into media. Different concentrations of Pb metal were made such as 100, 200 and 1000 ppm to produce heavy metallic environment. These flasks were kept spinning on shaker for 72 hours and then kept at static condition for 24 to 48 hrs. 5kg garden soil was taken into each pot. Soil was mixed with media having high growth of tolerant microorganisms with heavy metals concentrations and added to the pots. Soil samples were taken from pots after 5 days interval for ICP analysis.

To study detoxification of heavy metals by *Cicer arietinum*

200 ml autoclaved nutrient media were taken into sterile 250ml flask. Different concentrations of Pb metal were made such as 100, 200 and 1000 ppm to produce heavy metallic environment. 5 kg garden soil was taken in to each pot. Soil was mixed with media having heavy metals concentrations and sown with *Cicer arietinum* seeds into the pots. Soil samples were taken from pots after 5 days interval for ICP analysis.

To study detoxification of heavy metals by obtained isolates and *Cicer arietinum*

For detoxification study 200 ml autoclaved nutrient media were taken into sterile 250ml flask. The culture having high growth was inoculated into media. Different concentrations of Pb metal were made such as 100, 200 and 1000 ppm to produce heavy metallic environment. These flasks were kept spinning on shaker for 72 hours and after that incubate it for 24 to 48 hrs. 5kg garden soil was taken into each pot. . Soil was mixed with media having high growth of tolerate isolates with heavy metals concentrations and sown with *Cicer arietinum* seeds in to the pots. Soil samples were taken from pots after 5 days interval for ICP analysis.

Preparation for ICP

Beaker, watch glass, funnel and 100 ml heavy metal in Nessles tubes were leached with 10% nitric acid solution for 4 to 5 hrs, washed with distilled water and dried. Soil sample was taken from each 0, 5th and 10th day pot. It was sun dried and sieved. 1 gram of sieved soil was taken into beaker and acid was added to the mixture of 20 ml having 1:2 ratio (percolic acid: nitric acid) in a beaker. The mixture was digested on hot plate till white precipitation was formed. Then it was filtered with distilled water using Whatmann filter paper

no. 42 and make up the volume up to 100 ml in heavy metal tubes. The sample was prepared for Inductive Coupled Plasma analysis (ICP-AES Jobin Yvon).

RESULTS

1. Isolation of heavy metal tolerant bacteria was successfully done. On the basis of morphological and biochemical characterization the isolates were identified as *Actinomyces* spp., *Rizobium* spp., *Staphylococcus* spp. and *Pseudomonas* spp.
2. The detoxification study of heavy metal concentration was carried out by obtained isolates, *Cicer arietinum*, and both. The results obtained were as shown in following Table & Figures.

Metal	Pb conc on 0 day	Pb conc on 5th day	Pb conc on 10th day	Pb conc on 0 day	Pb conc on 5th day	Pb conc on 10th day	Pb conc on 0 day	Pb conc on 5th day	Pb conc on 10th day
	(100 ppm)			(200 ppm)			(1000 ppm)		
Microbes	3.72	3.54	2.69	5.56	5.25	5.13	20.69	19.45	18.13
Plant	3.7	3.4	2.2	5.56	5.03	4.5	21	19.31	17.15
Both	3.74	3.22	1.97	5.48	4.83	4.1	21.58	18.22	16.7

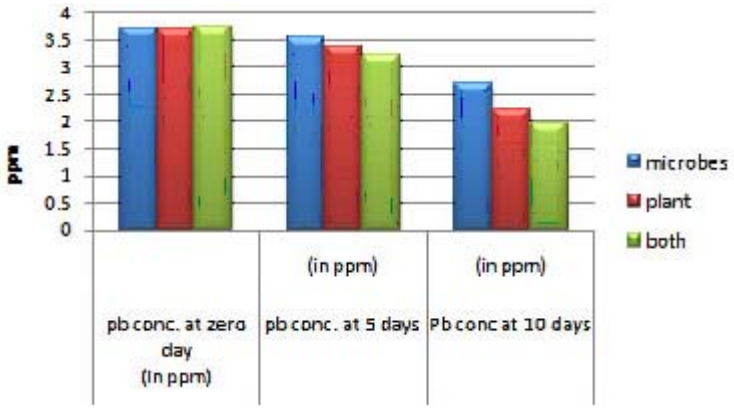

Fig. 1. Concentration (100 ppm) of lead at zero days and after 5 & 10 days of Incubation

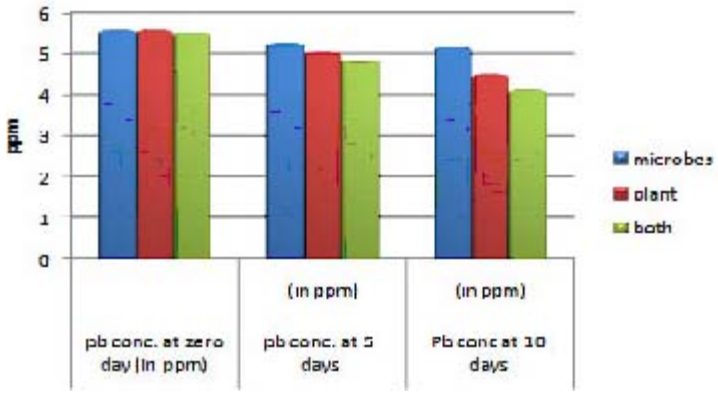

Fig. 2. Concentration (200 ppm) of lead at zero days and after 5 & 10 days of Incubation

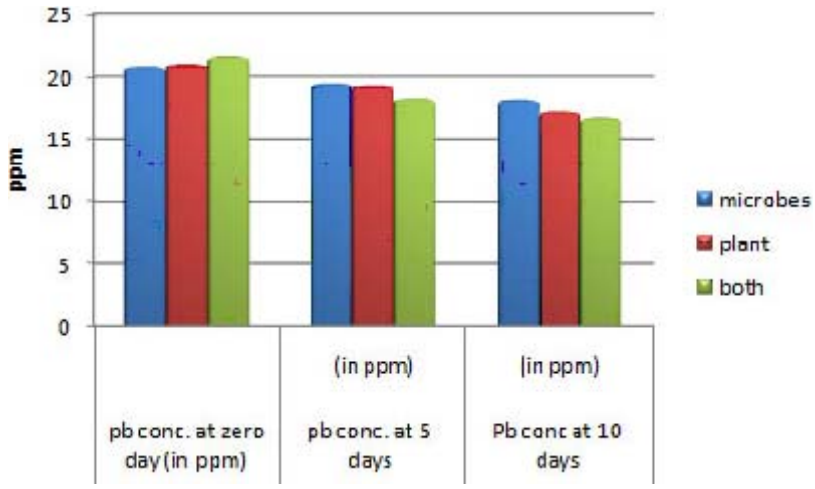

Fig. 3. Concentration (1000 ppm) of lead at zero days and after 5 & 10 days of Incubation

DISSCUSSION

Biological detoxification strategies like bioremediation, phytoremediation and rhizoremediation have emerged as innovative and ecofriendly techniques to cope with the problems like soil pollution and heavy metal contamination. This technique has been studied by many scientists and has found satisfactory results. Based on the literature survey it was found that heavy metal like Cr, As, and Pb cause hazardous effect on the environment[6]. Lead metal has been known to affect the human health severly. Many plants (*Brassica juncea, Cicer arietinum, Pisum sativm*) have been reported to enhance the process of bioremediation. Thus after a detailed survey, *Cicer arietinum* was selected for studying the detoxification of heavy metal Pb.

In the present investigation, initially, the isolation of microbes was performed from the metal contaminated sites (wielding workshop, industrial area & metal scrap). It was found that growth of metal tolerant bacteria was abundant in the media having 100ppm concentration of Pb. These tolerant bacteria were further enriched in media with metal concentration increasing up to 1000ppm. The growth was found to be abundant, tolerating the metals. Thus the potentiality of the bacteria was high.

In the detoxification process, the metal concentration of the soil decreased due to the microbial-metal interaction, plant-metal interaction and combination of both. This trend was observed in the ICP investigation. This test was done using metal concentrations of 100ppm, 200ppm and 1000ppm of Pb. In the study of Pb it was found that the degradation rate was highest in the sample having both plants and microbes. This was followed by degradation by only plant and finally only by microbes.

Thus this investigation opens up a new dimension in the field of biological detoxification of heavy metals.

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