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Impact of heavy metals on micro flora of crop plants

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Abstract: Heavy metal pollution of soil is known to adversely affect microbial activities at elevated concentrations. In this context study was carried out to assess and determine the cumulative effects of heavy metal on reduction of microbial number in the soil sample where the crops i.e. ground nut, green gram, and turmeric were grown in pot culture experiments with three treatments in black soil. In present study heavy metals like Ni, Cd and Cr were added in soil under laboratory conditions with different concentrations (16ppm, 10ppm and 20ppm) in soil with sufficient moisture. The microorganisms were stored at 28± 1°C for 28 days. Viable count of fungal and bacterial species was determined using serial dilution method with three types. Type-I consist of control, Type-II control soil along with heavy metal and Type-III consist of spiked heavy metal soil with1% calcium hydroxide .Fungal species (A.terrus.Thom Aspergillus niger van Tieghan, Alternaria alternata .Nees, Colletrichum and Cladosporium) and Bacterial species (Gram negative + bacteria) population were more sensitive to metal groups like Ni, Cd and Cr in Type –II when compared to Type I and III. Loss of microbial diversity is evident as we move towards higher concentration of heavy metal in soil. Type-III which was treated with calcium hydroxide has worked as an inhibitor for heavy metals and no adverse effect was observed in microbial activities.

Keywords: Heavy metals, Crop plants, Microorganisms, Calcium hydroxide, treatment.

INTRODUCTION

The soil microbial population is under tremendous pressure due to contamination of soil by a variety of toxic substances such as pesticides, heavy metals and other organic pollutants of sewage sludge and waste water[1, 2]. Industrial inputs and the agronomic applications of fertilizers, pesticides and metal contaminated sewage continue to contribute the metal accumulation in the soil[3].Heavy metals come from a variety of sources but principally anthropogenic activities such as chemical manufacturing, electric power generation, coal and ore mining, smelting and metal refining, metal plating. When these heavy metals are retained in the soil by repeated and uncontrolled additions, they interfere with these key biochemical process. Heavy metal toxicity may affect all forms of life including microorganisms, plants and animals. Heavy metals at concentration levels affect the soil microbial population this have impact on soil fertility[4] , its toxicity affects on growth of microorganism results reduction in diversity, population size of microbial communities[5-10]. Heavy metal inhibits the development of bacteria, fungi and actinomycetes[11, 12] and also decrease the reproduction and biological activity in micro organisms[13]. A change of the diversity and abundance of microbial communities in soils therefore there is a huge impact on terrestrial ecosystems; in this view microorganisms are important and eco-friendly to the crop plants.

MATERIALS AND METHODS

Experimental Crop plants: *Arachis hypogea an* important oil seed yielding crop belongs to the family *Fabaceae*. The genus *Arachis* systematically placed into the division Magnoliophyta, class Magnoliopsida order Fabales and family Fabaceae. Mung bean or green gram is an important cultivated pulses crop has long been a food crop in Asia. It is commonly known as Green gram. The genus is *Vigna* systematically placed into the division Tracheophyta, class Magnoliopsida order fabales and family fabaceae .*Curcuma longa* a perennial herb belongs to family zinzgiberaceae (ginger) family; it is cultivated mostly in Asia, India, China and other tropical climate countries, and rhizome widely used in medicines.

Soil collection: The soil samples were collected from the crop plants grown in earthen pots at Greenhouse of Botanical Garden, Department of Botany, Osmania University, Hyderabad. Soil samples were collected randomly which were stored in plastic bags at 4°C in the laboratory till processed. A replicate of three samples were taken from each pot culture.

Preparation of soil: In pot culture experiments *Arachis hypogea*, *Vignaradiat* and *Curcuma longa* plants were grown in three types of black soil till the yield of crops. Type I. controls without any addition of heavy metals to the black soil, Type II. Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the black soil and Type III. 1 % of Calcium hydroxide was added along with heavy metals to the black soil, and the crops were grown up to the productivity levels.

Enumeration of microbial population in soil: Serial dilutiontechnique[14, 15] was used to isolate gram negative bacteria and species of fungi like Aspergillus A.terrus.Thom, Aspergillus niger van Tieghan. Aspergillus sps, Alternaria alternata. Nees, Colletrichum, Cladosporium, Emericella nidulence, Fusarium, Pencillium and Rhizopus The composition (g/l) of media Potato- 200g, Dextrose -25g and Agar -20g for fungi was prepared by using Potato Dextrose Agar (PDA). The composition (g/l) of media for bacteria was prepared by using Nutreint Agar (NA) i.e. Nutrient Broth -13g in 1 liter distilled water at the pH is 7 and incubated at 27°C for 24 hours. All the above microbiological media was sterilized by autoclaving at 121°C for 15 min. UV light ON before 20 min inoculation. All the isolates were maintained at 4°C in equal volumes of nutrient broth and 30% glycerol.

Determination of survival of indigenous microbes and soil bacteria from soil amended with heavy metal: 1 gram of the soil samples amended with different concentration of heavy metals was serially diluted in sterile normal solution.0.1 ml of diluted sample and was spread over the surface of plate count agar of the respective medium at 0, 7, 14, 21 and 28 days after incubation. The total viable count of each group of population was noted. Decrease in viable count in the heavy metal amended soil over control sample indicates the effect of heavy metals on metal sensitive population and survival of indigenous microbes and soil bacteria.

Identification of fungi: On the basis of colony morphological characters the isolated fungi were identified up to genus level by following standard manuals[16] whereas gram stain (Crystal violet) was used for isolating bacteria.

Table-1: Fungal species in different soil types

S. No.	Fungal species	Type -1 (Normal soil)	Type-II (Soil+Heavymetal)	Type-III (Soil+Heavymetal)+ 1% $Ca(OH)_2$)
1.	*Aspergillus* sp	+	+	+
2.	*A. terrus*.Thom	+	-	+
3.	*Aspergillusniger* van Tieghan	+	-	+
4.	*Alternaria alternata* .Nees	+	-	+
5.	*Colletrichrum* sp	+	-	+
6.	*Cladosporium* sp	+	-	+
7.	*Emericella nidulence*	+	+	+
8.	*Fusarium*	+	+	+
9.	*Pencillium* sp	+	+	+
10.	*Rhizopus* sp	+	+	+

+=Present,-=Absent

Calculation: Isolated colonies were counted and (*c f u*) colony forming units per gram of soil was calculated by using

$$C\,f\,u \text{ or viable cells/ gm of dry soil} = \frac{\text{Mean plate count X Dilution factor}}{\text{Dry } \textit{Maintenance of isolates}}$$

RESULTS AND DISCUSSION

Heavy metals in ppm	Type of Organisms	Days after inoculation	Type -I		Type -II		Type -III	
			Dilution	*c f u* per gm of soil	Dilution	*c f u* per gm of soil	Dilution	*c f u* per gm of soil
Ni, Cd and Cr	Fungi	0^{th}	10^{-3}	$52X10^{-3}$	10^{-3}	$30X10^{-3}$	10^{-3}	$50X10^{-3}$
		7^{th}	10^{-4}	$64.2X10^{-4}$	10^{-4}	$45.7X10^{-4}$	10^{-4}	$60X10^{-4}$
		14^{th}	10^{-5}	$77X10^{-5}$	10^{-5}	$57.1X10^{-5}$	10^{-5}	$74X10^{-5}$
		21^{st}	10^{-6}	$88.5X10^{-6}$	10^{-6}	$71.4X10^{-6}$	10^{-6}	$90X10^{-6}$
		28^{th}	10^{-7}	$89.5X10^{-7}$	10^{-7}	$71.4X10^{-7}$	10^{-7}	$91X10^{-7}$
Ni, Cd and Cr	Bacteria Gram Negative (-)	0^{th}	10^{-3}	$50X10^{-3}$	10^{-3}	$28.5X10^{-3}$	10^{-3}	$52.X10^{-3}$
		7^{th}	10^{-4}	$61.4X10^{-4}$	10^{-4}	$42.8X10^{-4}$	10^{-4}	$60X10^{-4}$
		14^{th}	10^{-5}	$72X10^{-5}$	10^{-5}	$58.5X10^{-5}$	10^{-5}	$70X10^{-5}$
		21^{st}	10^{-6}	$85X10^{-6}$	10^{-6}	$64.2X10^{-6}$	10^{-6}	$84.2X10^{-6}$
		28^{th}	10^{-7}	$86.5X10^{-7}$	10^{-7}	$71.4X10^{-7}$	10^{-7}	$86X10^{-7}$

Table-2: Survival of soil bacteria and fungi (CFU/gm) in soil amended with heavy metals.

A **B** **C**

A) Normal Soil Microbial Plate **B)** Heavy Metal Soil Microbial Plate **C)** 1% Ca $(OH)_2$ Soil Microbial Plate

Figure-1: Isolation of fungi in Type-I, Type-II and Type-III soils

Bacteria: The predominance of gram negative bacteria in Type-I and Type-III and the plate viable count in Type–I (control soil without heavy metals) in the range of Gram negative(-) bacteria $86.5X10^{-7}$ *c f u* per gm of soil and Type-III(control soil without heavy metals + $Ca(OH)_2$) range of Gram negative(-) bacteria $91X10^{-7}$ *c f u* per gm of soil .In Type-I and Type-III there was no significant inhibition in the viable count of fungal organisms up to 28th day of incubation **(Table-2)**. On the other hand treatment of Type-II (with heavy metals Ni, Cd and Cr) in the range Gram negative(-) bacteria $71.4X10^{-7}$ *c f u* per gm of soil there was significant decline in the viable count or complete inhibition of fungal organisms up to 28th day of incubation .

To sustain the agriculture good quality of soil is important for fertility .The microbial community plays an important role in decomposing organic matter and is involved in important biogeochemical cycles, soil fertility, primary production through organic matter decomposition and nutrient cycling. The contamination of soils by heavy metals is significant problem, which leads to negative influence on microbial activity and slow down the speed of growth and reproduction of microorganisms. In this the heavy metals like Cd, Cr and Ni, decrease the microbial diversit which is evident. Microorganisms in the soil are responsible for nitrogen fixation, assimilation and degradation of organic residues to release nutrients[17, 18]. The relational ship of microorganisms to heavy metal soil pollution is complex and contradictory in case of sewage sludge application to the land[19]. Pollution by the heavy metals adversely affect on microbial activities. Microbial systems for regulating trace metal uptake can be important factors in competition with other microbes when the metal ions are either limiting[20] or present at toxic levels[4]. The medicinal plant treated with heavy metal and remediation soil microflora from rhizosphere of the plant[17].The understanding of microbial adaptation to the presence of metal in the soil is critical in determining the management and potential long term effect of land receiving heavy metal contamination. In the above 3 soil types 10 fungal types are isolated. Among them 3 speciesare Aspergillus 1 species of Alternaria alternata.Nees, Colletrichum, Cladosporium, Emericella nidulence, Fusarium, Pencillium & Rhizopus. The species of Aspergillus A.errus.Thom and Aspergillus niger van Tieghan. In Normal and $Ca(OH)_2$ soil types Aspergillus sps, Alternaria alternata .Nees, Colletrichum, Cladosporium, Emericella nidulence, Fusarium, Pencillium and Rhizopus are present whereas in heavy metal soil type Aspergillus, Emericella nidulence, Fusarium, Pencillium and Rhizopus are present which is shown in **Table1**.

CONCLUSION

In the present investigation toxicity of the heavy metal concentration is time dependent for each group of soil organisms Fungal and bacterial species populations were sensitive to metal group like Cd, Cr and Ni in the Type-II soil, when compared with Type-I and Type-III Soil which are control and treated with calcium hydroxide. Similar study using soil of different level of metal pollution and control should be studied to draw a long- term impact of heavy metal pollution on genetic diversity of soil microbial populations to explore the possible Metal-Microbe interaction and their possible impact on soil health.

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