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Chromium stress management on plant growth using biosorption and bioreduction

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Abstract
Cr-induced stress in plants that cause severe damage of production of pigments. Effect of Chromium reduction and its toxicity on plant growth was evaluated. Soil enriched chromium treated with Cr resistant *Pseudomonas fluorescence* and *Neurospora* sp isolated from the tannery effluent contaminated site. Efficiency of live bacterial and dead fungal mat was tested under in situ pot culture method. Chromium removal was noted by UV and effluent characters were tested. The results showed that tested bacteria and fungi greatly stimulated plant growth under chromium stress followed by bacteria and fungi alone. Plant growth was moderate by bacteria and fungi independently with 1442 and 951.5 VI with 3.8 and 3.2 mg/g chlorophyll content. The concentration of $Cr^{6+}$was reduced as Cr^{3+} and estimated as 140 mg by bacteria, 120 mg by fungi and 98 by dual culture. Though the concentration of Cr^{3+} is higher in bacterial the fungi absorption reduced the availability of concentration. The treated sample gave significant result on *V. radiata* seed germination indicates, that it's free from chromium toxicity as Cr(VI) is reduced. This study confirms that the isolated bacteria and fungi are potent hexavalent chromium stress remover in agriculture soil and enhance the plant growth.

Keywords: chromium, bioremidation, plant growth, vigor index, germination

1. Introduction

Discharge of Cr in soil or water uptake by plant tissues may provoke toxicity with several morpho-physiological and biochemical processes in plants. Tanneries are the main source of environmental pollution, huge quantities of chromiumare used in leather processing and itinhibit the growth of animal, plants and microbes [1, 2]. These compounds are toxic and persist longer in the environment, it cause adverse effects to flora and fauna. Exposure to polluted water may can causes fatal disease like cancer, neurological disordersdelayed nervous responses and mutagenic changesetc [3]. Many toxic heavy metals among chromium is considerable environmental concern as it is widely used in electroplating, leather tanning, metal finishing and chromate preparation [4]. Chromium occur in two form one is trivalent and other one is hexavalent forms. The concentration of chromium varies from 500 to 7000 ppm in tannery effluent. Chromium metal (Cr) occurs naturally in the environment Cr exists asCr (III) and Cr (VI) being the primary existing oxidation states in the environment and has both beneficial and potential human risks. Cr (III) is an essential nutrient for maintaining lipid, insulin, and glucose metabolism and its deficiency may lead to diabetes [5]. Hexavalent chromium is toxic and carcinogen but trivalent chromium is less soluble and less toxic.

Detoxification is termed as the ability of a microbesto survive at toxic effect whenit exposed to metal by means of a mechanism produced with direct response to the metal species concerned. Themicrobessurvivesat metal toxicity due to its intrinsic property called as tolerance. The microbes have potential to eliminateimpurities and absorb toxic metals during treatment. Bacteria that transform hexavalent chromium to trivalent chromium by the Enzymatic reduction widely reported in gram negative bacteria [6]. Fungialsotermed as biological material act as anabsorptive material and eradicate hexavalent chromium [7]. Bioremediation is a naturalapproach that involves the use microorganism or enzyme to degrade contaminants is less expensive and more sustainable. From the past few years microorganisms have broadly researched for their ability to detoxify tannery contaminants. Biosorption mechanism is occur through physical and chemical interaction between metal and functional group present on the surface of cell wall [8]. Two methods involved in biosorption mechanism such as metabolism dependent and non-metabolism dependent. Chromium get bound to the functional group and adsorbed into the cell wall. Fungal strains have significant potency to adsorb chromium. Microbes play avital role in the environment and act as a bio-degradation.

Microorganisms are very effective in pollution control especially in effluent treatment [9] and used for compost the solid part. The microbes present in the effluent sample can tolerate the adverse conditions such as pH, turbidity, high BOD, COD, etc. The microbial consortium has been isolated, identified and used for the treatment. *Pseudomonas aeruginosa*, *Penicilium*sp and *Aspergillus* spisolated from polluted sites fromtannery and shown to be resistant to hexavalent chromium which is highly toxic reported in many studies [10]. The application of consortium is used to eliminate chromium from polluted waste and contaminated soil [11]. Mixed population of microbes degrade very high when compare to single strains.Das *et al*. [12] have also statedthat TDS, BOD, COD, EC, salinity, alkalinity, hardness are high in tannery effluent. The present study was carried out with chromium (VI)and chromium (III) reduction ability of bacteria and fungi isolated and screened from tannery effluents.

2. Materials and methods

2.1 Collecting site

Tannery effluent sample was collected from KMM Tannery during March 2018 located at Airport, Tiruchirappalli in a sterile bottle and processed for microbial studies. Sterilized containers were used for collection and they were transported to the laboratory within 2-4 hours and stored at 40^0C for further analysis.

2.2 Isolation of Cr tolerant *Pseudomonas* sp [13]

For the enumeration of bacteria, samples were serially diluted and plated on Luria–Bertani (LB) agar (tryptone: 10 g l-1; yeast extract: 5 g l-1; Nacl: 10 g l-1; glucose: 0.1 g l-1) adjusted at normal pH value (7.0). The medium amended with 1000 ppm potassium di-chromate to isolate tolerant strain. Medium without chromium used as control.

2.3 Isolation of Cr tolerant *Neurospora* sp [14]

PDA medium with 1000 ppm of potassium di chromatewas added to the media to select resistant strain. The plates were incubated at 25±2º C for five days. The fungi were identified by morphological observations.Strains are identified by lactophenol cotton blue staining technique. Medium without chromium are used as control.

2.4 Dead fungal preparation

The fungal cells was grown at 28°C in an stirred and aerated liquid Sabourauds media containing ampicillin at a concentration of 0.1g/L (p/v). After five days of incubation, the cells were recovered by centrifugation (5000 rpm/10 min), and washed thrice with phosphate buffer and subsequently oven dried at 40°C/24 h. The biosorption of the metal by fungal dry cells was determined followed by autoclaving subsequently for 3 days.Electrical conductivity tested by EC meter and pH by digital pH meter.

2.5 Plant growth promotion studies

To investigate the effect on germination *Vigna radiata* L. Seeds were chosen for the test. Triplicate of five Seeds surface sterilized with 0.1% $HgCl_2$ and washed thrice to remove all the traces of unwanted particles. 5 g seeds were soaked in RO water for 3 h and then soaked in 1000 ppm chromium solution for 30 min. Seed germination and seedling growth test on filter paper pre coated with bacteria, fungi was carried out in glass petridishes (125 mm in diameter, whatman No.1). The petridishes were covered by lid and incubated at 28^0C in dark condition for 24 hrs. The seed germination percentage, were observed in 24 hrs.

% of Seed germination= Seed germinated/ Total number of seed tested X 100

2.6 Pot study

Soil sample enriched with chromium (1000 ppm/gm) and treated with dead fungi and bacteria as the following groups. All the group were kept under room temperature for 7 days. Sample was used for Cr^{3+} estimation by UV absorption and then germinated seeds planted on pot and irrigated with treated and untreated effluent for 7 days. The morphological parameters like plant height, no. of Branches and no. of Leaves were observed and recorded. The growth effect was checked as follows

Vigour index= (Mean root length+Mean shoot length) x % of seed germination

Pot 1- Soil with Cr^{6+}+ Dead fungi

Pot 2- Soil with Cr^{6+}+ Live Bacteria

Pot 3-Soil with Cr^{6+}+ Live Bacteria + Dead fungi (Combined treatment)

Pot 4- Soil with Cr^{6+} (Untreated soil)

Pot 5- Soil (Control)

Chlorophyll content

Pigment contents of 25 days old plants were extractedwere extracted with 80%acetone. The absorbance was measured at 645 nm and 663 nm for chl a,b and total chl

Total Chl(mg g-1 leaf fresh weight) =[20.2(OD645)-8.02(OD663)]×V/1000 × W.

3. Result and discussion

3.1 Isolation of chromium resistant bacteria

The bacterial and fungal colonies were isolated from tannery effluent and studied based on the colony morphological characteristics. Isolated strains were identified as *Pseudomonas fluorescens*, *Escherichia coli*, *Alcaligenes*sp, *Micrococcus* sp, *Bacillus methylotrophicus*, *Bacillus subtilis*, *E.faecalis*, *Pseudomonas putida*, *Streptococcus Anaerobius*, *Ruminococcusalbus*, *Bacillus licheniformis.* The fungal strain are identified as *Neurospora* sp, *A. fumigatus*, *A. terreus*, *Fusarium* sp, *A.nidulans*, *A.niger*, *Alternaria*sp, *Mucor*sp, *Penicillium*sp and *Curvularia*sp. Presence of chromium on tannery effluent gives adaptation ability to bacteria which makes it resistant in order to give a considerably high CFU/ml [15].

The present study was carried out to isolate chromium resistant fungi and bacteria from tannery effluent and to evaluate the bioremediation potential. Chromium tolerant isolates was assessed by growingon Luria–Bertani (LB) agar containing concentration of chromium at 1000 ppm. Among the isolates, tolerance limit was 1000 ppm recorded among *Pseudomonas fluorescens* and *Neurospora* sp. Table 1 reveals the pH, electrical conductivity and concentration of chromium reduction. Th pH of soil 8 reduced to netral pH by bacteria and fungal treatment. The electrical conductivity was high in untrated chromium soil also reduced by dual treatment due to reduction and absorption of chromium. The UV spectrum of untreated soil (control) shows maximum of 1200 mg / g of chromium and 640 mg Cr^{3+}. The spectrum of treated sample it was estimated that reduction of hexavalant chromium was estimated as 86 mg/ g of soil in dual treatment whereas 120 mg/g by fungi alone. Bacteria alone shows 530 mg/g of hexavalent chromium. Similarly trivalent chromium was estimated as 140≥120≥98 and 60 mg/L respectively for bacterial, fungal, combined and untreated soil (table 2). Presence of Chromium reductase in bacteria mediates resistant to chromium, which catalyse the reduction reaction of Cr (VI) to Cr (III) [16, 17]. Though various biological techniques used to reduce toxic substances such as live microbes may transform the chromium metal or remove Cr (VI) as Cr III [18] and later it was absorbed by Fungi effectively removes chromium. The combined treatment shows a better Cr reduction. Bacterial reduction shows moderate and fungi alone show good reduction. The result indicates that untreated soil contain high concentration of chromium gave 10% of seed germination and its vigour index was 43%. The soil were enriched with chromium (VI) and treated with live bacteria tested in pot 1 were found to be 70% germination and 1442 VI. In Pot 2 treated with dead fungi have given 951 VI with germination index of 55%. Pot 3 reveals dual treatment

impact on germination index was found to be 100% and enhances the plant growth with vigour index 2410.all the pot 1 to pot 4 compared with plant growth without chromium in pot 5 which shows mean of root growth 2.6 cm and stem is 12.8 cm with 70% GI and 1078 VI. Data shows high concentration of Cr6+ retard plant growth whereas as dual culture remediation promote plant growth by reducing the toxicity. The total leaf chlorophyll content in fresh leaves (fig 1) was estimated using spectroscopy and found to be increased among plants grown under biologically treated soil than untreated and normal. Maximum 4.2 mg/g was recorded in dual culture treatment and minumum3.2 mg/g in bacterial treated. Fungi treated pot plants shoed 3.8 mg/g. Untreated showed 1.2 mg/g much lesser than normal plant grown without chromium (1.8 mg/g). Similarly Barton et al. [19] reported that Cr addition inhibited shoot growth and chlorophyll content. The inhibition of chlorophyll biosynthesis under Cr stress in many plants were explored the inhibition of δ-aminolevulinic acid dehydratase (ALAD) by chromium [20]

Table 1: Concentration of Chromium after 7 days biological treatment

Treatment	Cr $^{6+}$ mg/g	Cr^{3+} mg/g	pH	Electrical conductivity
Bacterial treatment	530	140	7.8	2.6
Dead fungi	120	120	5.8	1.2
Combined	86	98	6.2	0.8
Untreated	1200	60	8	22

Table 2: Mean value of root and shoot length of *Vigna radiata* L. Treated with raw effluent

Experimental group	Mean value of stem	Mean value of root	Percentage of Germination index(GI)	Percentage of Vigour index (VI)
Pot 1 bact	18.1±0.20	2.5±0.02	70%	1442
Pot 2 fungi	15.5±0.21	1.8±0.21	55%	951.5
Pot 3 dual	20.5±0.21	3.6±0.01	100%	2410
Pot 4 unt	3.3±0.21	1.0±0.21	10%	43
Pot 5 control	12.8±0.24	2.76±0.22	70%	1078

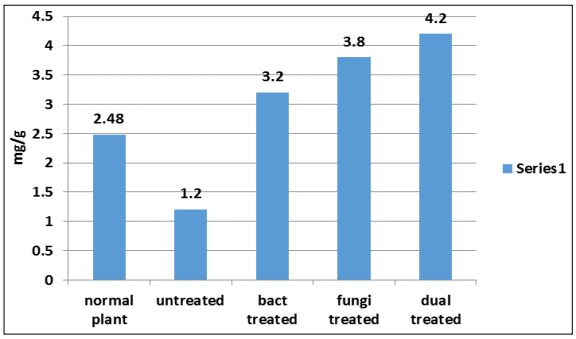

Fig 1: concentration of chlorophyll

4. Conclusion

Plant growth was maximized under chromium stress by dual culture treatment. Isolate Pseudomonas fluorescence and Neurospora sp found to be effective stress management among plant growth under heavymetal contaminant.

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