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In situ Carica papaya stem matrix and *Fusarium oxysporum* (NCBT-156) mediated bioremediation of chromium

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Removal of heavy metal chromium was carried out using the fungus *Fusarium oxysporum* NCBT-156 strain isolated from soil of leather tanning effluent in *in situ* condition using potassium dichromate solution with 10 per cent Czapek-dox liquid medium. Biosorbent matrix was developed using *Carica papaya* plant dry stem to colonize the fungal strain to facilitate bioabsorption process. Bioabsorption of chromium was by metabolically mediated intracellular accumulation process. Maximum efficiency of chromium removal by biosorption upto 90 per cent was achieved at the end of 5th day of incubation (120 h of contact time) for 100 and 200 ppm concentration, upto 80 per cent for 300 and 400 ppm, and upto 65 per cent for 500 ppm to 1000 ppm concentrations with *p*H ranging from 5.8, 5.6, 5.5, 5.4 and 5.2, respectively for 100, 200, 300, 400, 500-1000 ppm concentration. SDS-PAGE protein profile showed significant difference in 34 kDa protein band after chromium absorption by the fungus. FTIR spectroscopic analysis revealed that the main functional groups involved in the uptake of chromium by *F. oxysporium* strain were carbonyl, carboxyl, amino and hydroxyl groups.

Keywords: Bioabsorption, Bioaccumulation, Bioremediation, Biosorption, Chromium, *Fusarium oxysporum*, FTIR, SDS-PAGE

Heavy metals are conventionally defined as elements with metallic properties like conductivity, stability as cations, ligand specificity and atomic number > 20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Ni. Heavy metals are known to be toxic to plants and most organisms when present in soils in excessive concentrations[1].

However contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment.[2,3] A sudden boost in the industrial activities has contributed quantitatively as well as qualitatively to the alarming increase in the discharge of metal pollutants into environmental sink, especially the aqueous environment. Dispersion of the metal ions in water bodies leads to their biomagnification through the food chain and results in increased toxicity.[4] Disposal of industrial and urban wastes into the soil and water bodies violating stringent regulation in many countries including India has led to disastrous consequences to the ecosystems. Due to the excess loading of these wastes beyond their self-cleaning capacities, these ecosystems have resulted in decreased availability of clean water to drink and normal soil for crop production. Compared to the organic wastes, inorganic wastes like heavy metals, pose a greater threat, as they cannot be completely removed or degraded from the ecosystem.[5]

Chromium is an important heavy metal widely used in the metallurgic, refractory, chemical and tannery industries. Deposition of metallic chromium imparts a refractory nature to materials rendering resistance to microbial attack and flexible over extended periods of time.[6] More than 1,70,000 tons of chromium wastes are discharged to the environment annually as a consequence of industrial and manufacturing activities.[7] Of the total chromium used in the processing of leather nearly 40% is retained in the sludge, disposal of which onto land and into water bodies has led to increased chromium levels.[8] Considerable attention has been focused on bio-remediation of heavy metals using bacteria and fungi in recent years due to public and scientific awareness regarding the release of such pollutants from the industries.[9] Biosorption or bioaccumulation of metals from aqueous solutions using varying species of fungi such as *Aspergillus niger*, *Rhizopus nigricans*, *Mucor rouxii* and *Trichoderma atroviride* have been studied.[10-14] The current paper describes a study that evaluated the ability of *Fusarium oxysporum* NCBT-

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156 strain in bioremediation of chromium element in potassium dichromate solution *in situ* condition.

Materials and Methods

Stock solution of chromium (10 g/l) was prepared by dissolution of potassium dichromate ($K_2Cr_2O_7$) in deionized water. From the stock solution various concentrations of test samples 100 ppm to 1000 ppm were prepared.

Fungal culture—The fungus used for the study was *Fusarium oxysporum* NCBT-156 strain isolated from the tannery effluent affected soils of Sempattu, Tiruchirappalli. The strain was deposited in the culture collection centre at the Microbiology Laboratory, Department of Botany, National College, Tiruchirappalli in polyurethane foam immobilization and stored for further experimental use.

Preparation of inoculum—From the immobilized culture, the fungus *F. oxysporum* (NCBT-156) was cultured in Czapek-Dox-Agar liquid medium for 48 h[15]. Then the test samples of potassium dichromate solution was added individually for each concentration in conical flask with 10 percent Czapek-Dox liquid medium (90 ml potassium dichromate solution + 10 ml Czapek-Dox liquid medium) and the cultures were incubated at 28° ± 1°C and observed for biosorption of metal chromium.

Biosorption matrix—Dried stem material of *Carica papaya* was prepared as biosorbent matrix for *F. oxysporum* (NCBT-156) to reduce chromium at different concentrations. One-year old plant stem of *C. papaya* was cut into small pieces, dried in shade, sterilized and then used as biosorption matrix. The fungal strain was inoculated on the matrix and allowed for 48 h for its establishment. The matrix along with fungal strain was then introduced into potassium dichromate solution + 10 ml Czapek Dox liquid medium to facilitate bioremediation process.

Colorimetric analysis—Colorimetric analysis was employed for monitoring chromium decolourization by the fungal strain. Prior to this, the absorbance maxima of chromium incorporated broth was determined. The absorption maxima was at 520 nm and hence, decolourization was assayed at this absorption maxima. In order to avoid the interference of fungal mycelia in the absorbance value, the culture broth was centrifuged at 4000 rpm for 5 min and the supernatant was analysed colorimetrically (520 nm). Aliquots were withdrawn from the culture broth at 24 h interval for a period of 10 days and the extent of decolourization was monitored colorimetrically. The *p*H of each sample was measured by using *p*H meter.

Decolourization assay—Decolourization was calculated in terms of percentage, using the following formula.[16]

$$\text{Percentage of decolourization} = \frac{\text{Initial absoebance value-Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

The fungal isolates were tested for their capacity to utilize the chromium as mineral source. Two sets of mineral salt medium of 100 ml capacity were prepared. To one set, 1% glucose as carbon source and chromium at ten different concentrations of 100 to 1000 ppm as mineral source were added.[17] To another set ammonium nitrate (0.03 gm/100 ml) as nitrogen source and the chromium incorporated at ten different concentrations of 100 to 1000 ppm as mineral source were added. Both sets were inoculated with *F. oxysporum* (NCBT 156) culture and incubated at 28° ± 1° C for 10 days. At regular interval of the inoculation periods, the culture broth was assayed colorimetrically for chromium decolourization.

SDS-PAGE protein profile - SDS-polyacrylamide Gel Electrophoresis (SDS-PAGE) protein profile was performed for *F. oxysporum* (NCBT-156) before and after chromium absorption.[18]

Fourier transform infrared (FTIR) spectroscopy analysis—FTIR spectroscopy was used to detect vibration frequency changes in *F. oxysporum* (NCBT 156) strain biomass before and after chromium uptake. The spectra were collected by Perkin Elmer spectrometer with the range 4000-400 cm^{-1} using chloroform as mulling agent. The background obtained from the scan of pure chloroform was automatically subtracted from the sample spectra.

Results and Discussion

Biosorption of chromium—Biosorption of chromium by *F. oxysporum* (NCBT-156) strain is presented in Table 1. It is very effective upto 500 ppm concentrations in both normal and matrix mediated process (Fig. 2a-e and Fig. 3d) and then showed a steady status of biosorption upto 1000 ppm (Fig. 2f) under experimental conditions. Bioabsorption of chromium occurred in the mycelial internal structure of the fungus and the mycelial cell protoplasm changed from normal colourless nature to orange colour (Fig. 1d-g and Fig. 3b). The chromium mediated biosorption resulted in non-sporulation condition *F. oxysporum* (Fig. 1a-c).

Table 1—Efficiency of chromium decolourization by *F. oxysporum* NCBT-156 strain
[Values are mean ± SD of 3 replications]

Chromium	Mean percentage of decolourization by bioaccumulation in 10 days									
conc. ppm	1	2	3	4	5	6	7	8	9	10
100	40 ± 0.8124	60 ± 0.8164	70 ± 0.9914	85 ± 0.9430	90 ± 0.4778	90 ± 0.8164	90 ± 0.4778	90 ± 0.4778	90 ± 0.8164	90 ± 0.4778
200	40 ± 0.8124	60 ± 0.8164	70 ± 0.9914	85 ± 0.9430	90 ± 0.8164	90 ± 0.4778	90 ± 0.8164	90 ± 0.8164	90 ± 0.4778	90 ± 0.8164
300	35 ± 0.9427	50 ± 0.4759	60 ± 0.9427	70 ± 0.8164	80 ± 0.8164	80 ± 0.9914	80 ± 0.9430	80 ± 0.8164	80 ± 0.8164	80 ± 0.9914
400	35 ± 0.9427	50 ± 0.4759	60 ± 0.9427	70 ± 0.8164	80 ± 0.9430	80 ± 0.8164	80 ± 0.8164	80 ± 0.9914	80 ± 0.9430	80 ± 0.8164
500	30 ± 0.4759	45 ± 0.8124	60 ± 0.9427	65 ± 0.8164	65 ± 0.4759	65 ± 0.8164	65 ± 0.9914	65 ± 0.4759	65 ± 0.8164	65 ± 0.9914
600	30 ± 0.4759	45 ± 0.8124	50 ± 0.8164	65 ± 0.4759	65 ± 0.4759	65 ± 0.9914	65 ± 0.4759	65 ± 0.8164	65 ± 0.4759	65 ± 0.4759
700	30 ± 0.4759	45 ± 0.8124	60 ± 0.9427	65 ± 0.8164	65 ± 0.8164	65 ± 0.8164	65 ± 0.4759	65 ± 0.4759	65 ± 0.8164	65 ± 0.8164
800	30 ± 0.4759	45 ± 0.8124	60 ± 0.9427	65 ± 0.9914	65 ± 0.4759	65 ± 0.8164	65 ± 0.8164	65 ± 0.8164	65 ± 0.8164	65 ± 0.8164
900	30 ± 0.8164	45 ± 0.4759	60 ± 0.8164	65 ± 0.8164	65 ± 0.8164	65 ± 0.4759	65 ± 0.4759	65 ± 0.4759	65 ± 0.8164	65 ± 0.4759
1000	30 ± 0.8164	45 ± 0.4759	60 ± 0.9427	65 ± 0.8164	65 ± 0.4759	65 ± 0.4759	65 ± 0.4759	65 ± 0.8164	65 ± 0.4759	65 ± 0.4759

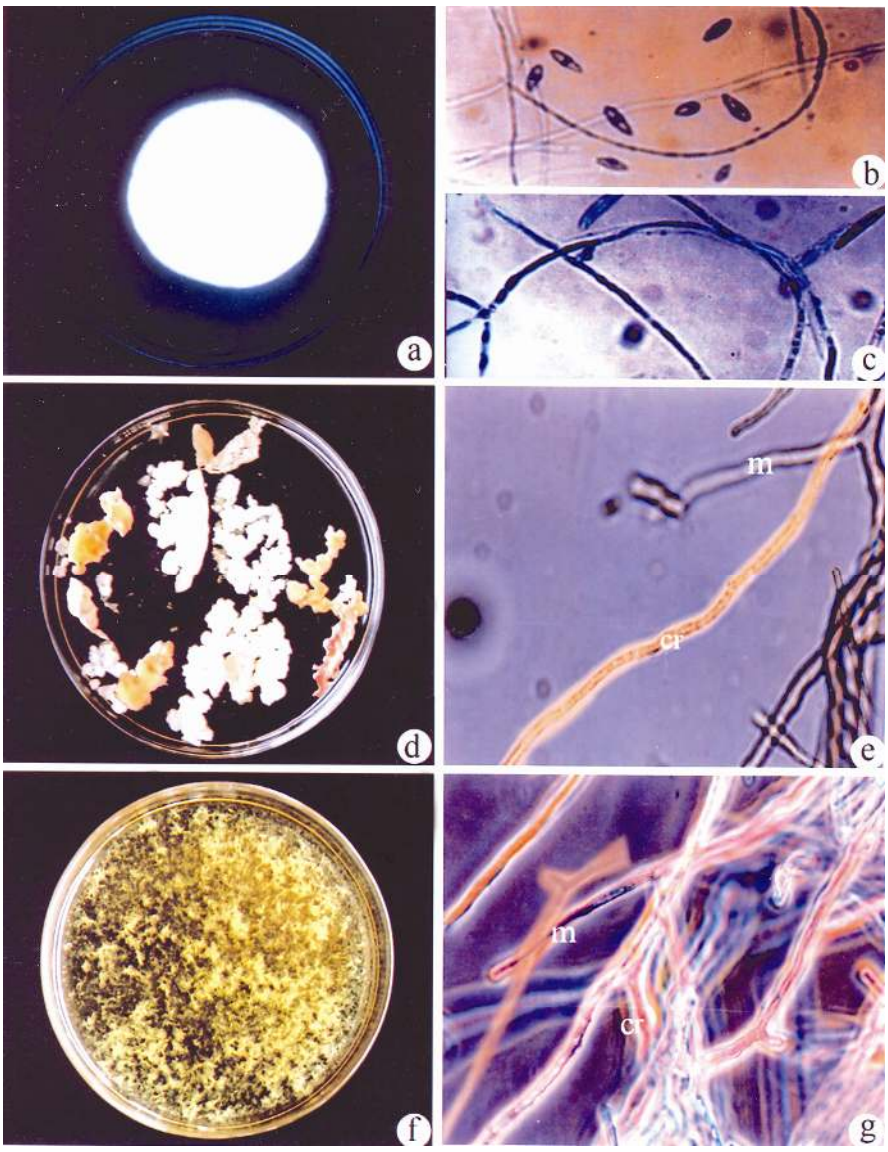

Fig. 1—Bioabsorption of chromium by *Fusarium oxysporum* (NCBT - 156). [(a)-*Fusarium oxysporum* (control); (b)-Microconidia; (c)-Macroconidia;(d & f)-Bioabsorption of chromium by *F. oxysporum* (NCBT-156); and (e & g)-Bioabsorption of chromium by *F. oxysporum* (NCBT-156) mycelia. m-Mycelium; cr-Chromium bioabsorption].

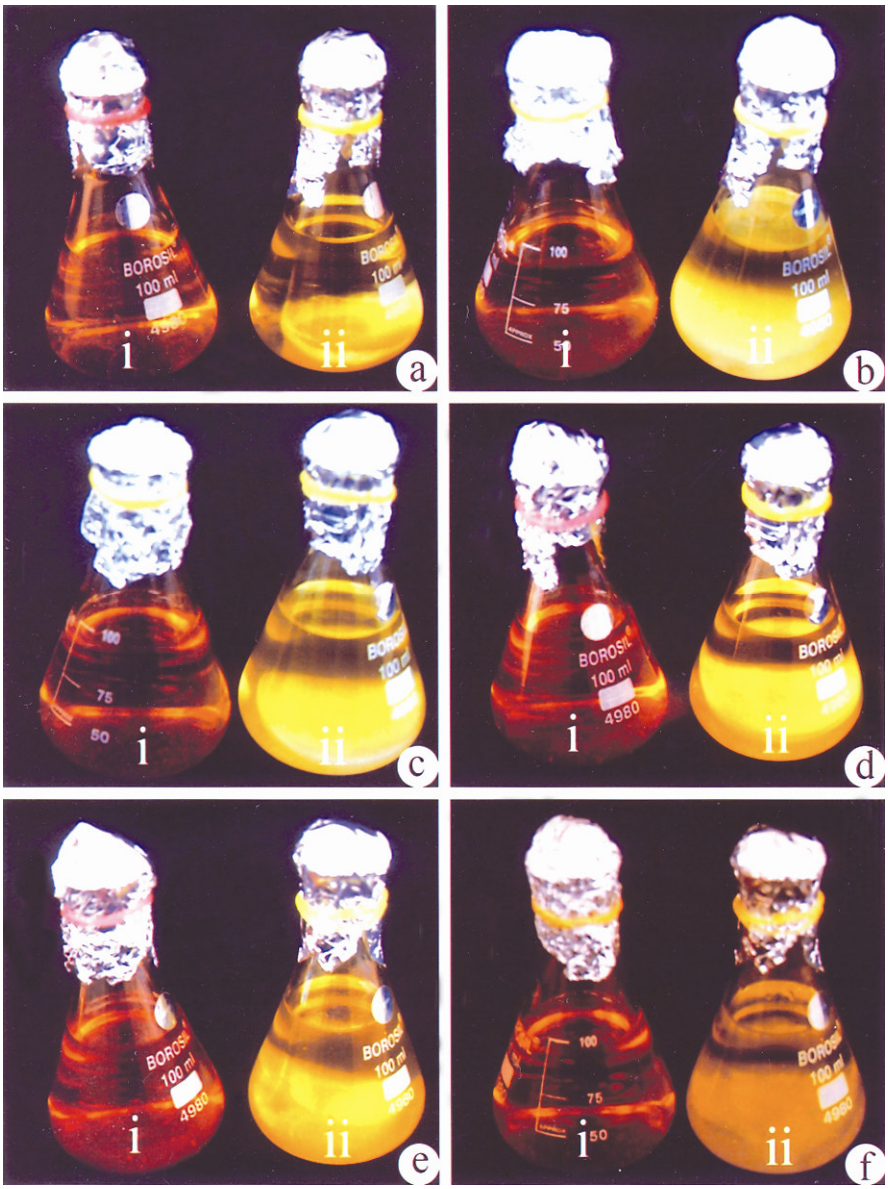

Fig. 2—Efficiency of chromium decolourization by *Fusarium oxysporum* (NCBT-156). [(a)-100 ppm - 90% decolourization; (b)-200 ppm - 90% decolourization; (c)-300 ppm - 80% decolourization; (d)-400 ppm - 80% decolourization; (e)-500 ppm - 65% decolourization; and (f)-1000 ppm - 65% decolourization. (i)-Before; and (ii)-after decolourization].

Percentage of decolourization—The percentage of decolorization of chromium was maximum (90%) at the end of 5th day of incubation (120 h of contact time) in 100 and 200 ppm concentrations with *p*H 5.8 and 5.6, 80% in 300 and 400 ppm concentrations with *p*H 5.5 and 5.4 and 65% in 500 to 1000 ppm concentrations with *p*H 5.2 and the *p*H was static at the end of 10th day of reaction (Fig. 2a-f). Effect at different *p*H [1st day (24 h), 5th day (120 h) and at the end of 10th day (240 h) contact time] on decolourization has been presented in Table 2. The metal removal from a solution is achieved through enzymatic system of fungus.[19] Heavy metals cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another.[20] Such transformation mechanisms might have occurred in chromium decolourization by *F. oxysporum* strain.

Metal remediation strategies using microorganisms can minimize the bioavailability and biotoxicity of heavy metals.[21] Microbial approach for metal detoxification affords the potential for selective removal of toxic metals, operation flexibility and easy adaptability for *in situ* and *ex situ* application.[22] The

F. oxysporum (NCBT-156) in *Carica papaya* stem matrix (Fig. 3a, c and d) used in this study could serve as bioremediating agent for application in a range of bioreactor configurations to reduce the pollutants.

Biosorption mechanism—Mechanism by which metal ions bind to the cell surface include electrostatic interactions, Van der Waals forces, covalent bonding, redox interactions and extracellular precipitation or combination of these processes. Negatively charged groups (carboxyl, hydroxyl and phosphoryl) of the fungal cell wall absorb metal cations, which are then retained by mineral nucleation. The microbial metal resistance as well as remediation might be due to the presence of plasmids encoded specific metal conferring resistance to a variety of metals including chromium. The biosorption occurred in *F. oxysporum* (NCBT-156) in chromium medium might have similar mechanism for their resistance and bioabsorption phenomenon.[23,24]

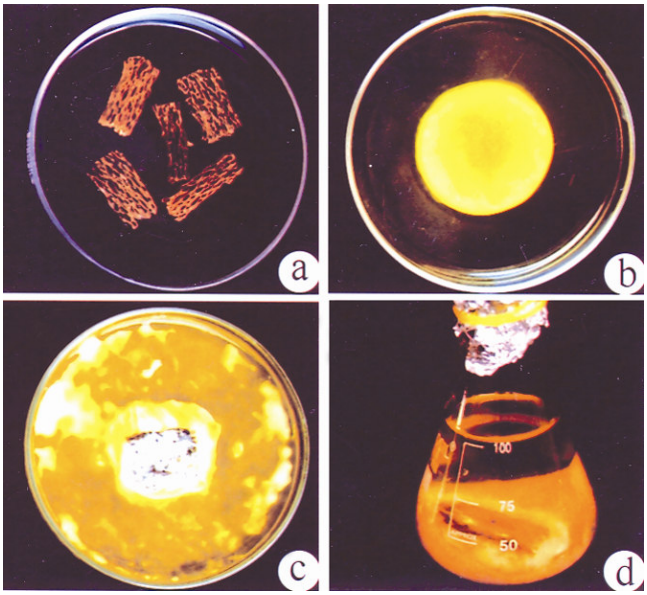

Fig. 3—Efficiency of chromium decolourization by *Fusarium oxysporum* (NCBT - 156). [(a)-Biosorption matrix - *Carica papaya* stem; (b)-Bioabsorption of chromium by *Fusarium oxysporum* (NCBT - 156); (c)-Growth characters of *Fusarium oxysporum* (NCBT - 156) on biosorption matrix; and (d)-Biosorption matrix mediated Bioasorption of chromium by *Fusarium oxysporum* (NCBT - 156)].

Table 2—Role of *p*H in chromium decolourization by *F. oxysporum* NCBT-156 strain

Chromium concentration (ppm)	*p*H values		
	1st day (contact time 24 h)	5th day (contact time 120 h)	10th day (contact time 240 h)
100	6.8	5.8	5.8
200	6.8	5.6	5.6
300	6.8	5.5	5.5
400	6.7	5.4	5.4
500	6.7	5.2	5.2
600	6.7	5.2	5.2
700	6.5	5.2	5.2
800	6.5	5.2	5.2
900	6.5	5.2	5.2
1000	6.5	5.2	5.2

Non-sporulation mechanism—Non-sporulation of *F. oxysporum* (NCBT-156) mycelium may be due to heavy metal stress using different defense systems such as exclusion, compartmentalization, formation of complexes and synthesis of binding proteins like metallothioneins (MTs) and phytochelatins (PCs). The toxicity mechanism of metal ions may be (i) blocking the essential biological functional groups of biomolecules especially proteins and enzymes, (ii) displacing the essential metal ion in biomolecules, and (iii) modifying the active conformation of biomolecules resulting in the loss of specific activity.[25] Such toxicity mechnisms might have resulted in *F. oxysporum* (NCBT-156) unable to produce the micro or macro-conidia under heavy chromium metal stress environment in the experimental conditions.

SDS-PAGE protein profile—SDS-PAGE analysis replicated thrice (L_1, L_2 and L_3) showed similar results for *F. oxysporum* (NCBT-156) before and after chromium absorption. The protein profile showed a significant difference in 34 K Da protein band after chromium absorption (Fig. 4a, b) and seems to be genetically improved strain.[26]

FTIR functional group analysis—The functional groups involved in absorption of chromium by *F. oxysporum* (NCBT-156) biomass were determined using FTIR spectroscopic analysis. The main functional groups involved in absorption process were found to be carbonyl, carboxyl, amino and hydroxyl groups (Fig. 5). Involvement of these functional groups in metal absorption process could be judged from change in frequency of absorbing groups (Fig. 5a, b). The absorbance of the peaks in chromium absorbed mycelium and control (unabsorbed) shows changes in the peaks.[27]

The present phenomena of biosorption of chromium by *F. oxysporum* (NCBT-156) strains could be an economically feasible and technically efficient technology for convenient metal ion

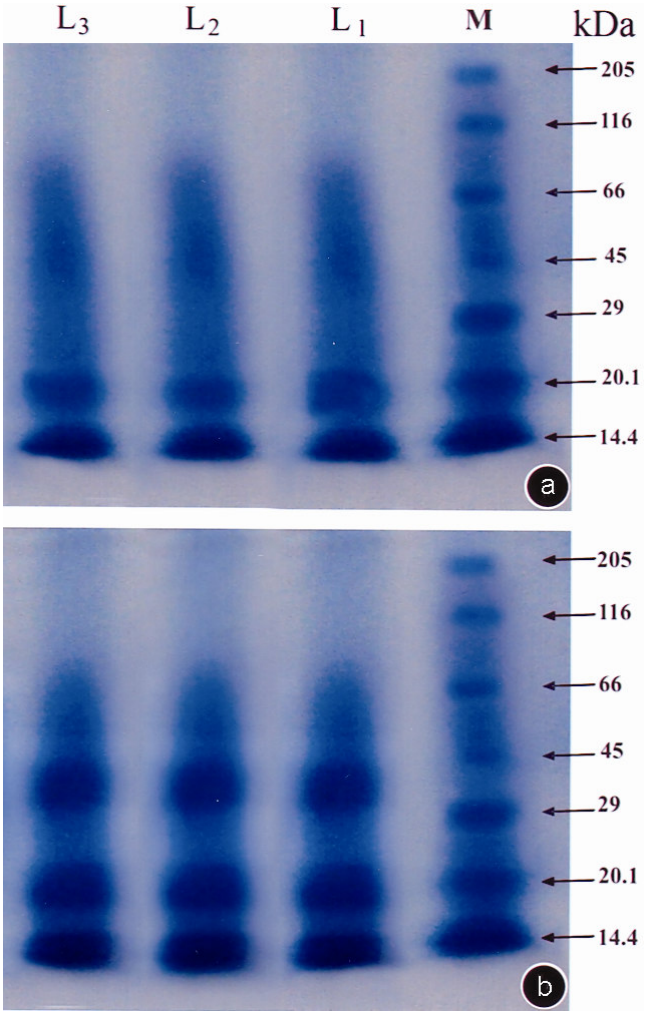

Fig. 4—SDS-PAGE protein profile *Fusarium oxysporum* (NCBT - 156). [(a) Before; and (b)-After chromium absorption].

removal, and could comfortably fit into the metal treatment processes. Further, it could be an eco-friendly approach as no further waste was generated into the environment. The industries generating metal polluted wastewater to follow genetically improved *F. oxysporum* NCBT-156, *Carica papaya* stem biosorbent matrix as clean up technologies before discharging their liquid effluents into the water bodies to create healthy environment could be recommended.

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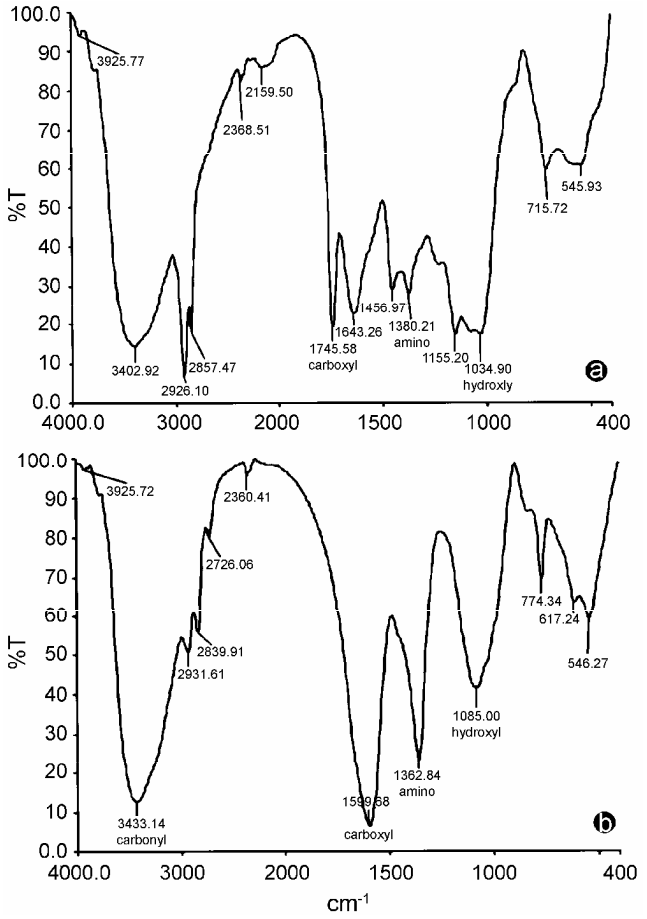

Fig. 5—FTIR spectra of *Fusarium oxysporum* (NCBT - 156). [(a) Before; and (b)-After chromium abosorption].

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