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Bioremediation of Chromium Contaminated Soil by a Brown-rot Fungus, *Gloeophyllum sepiarium*

[1]Varenyam Achal, [2]Deepika Kumari and [1]Xiangliang Pan
[1]Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China
[2]Crop Improvement Section, Directorate of Mushroom Research, Solan 173213, India

Corresponding Author: Dr. Varenyam Achal, Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830 011, China Tel: +86-15199137383 Fax: +86-991-7885300

ABSTRACT

The aim of present study was to remediate chromium contaminated soil by a brown rot fungus. Chromium has become an important soil contaminant at many sites throughout the world and facilitating reduction of toxic Cr (VI) to nontoxic Cr (III) using different microorganisms such as bacteria or fungi, is becoming an attractive remediation strategy. There is a need to find out different fungal species that can remove such toxic heavy metal ions from contaminated sites. The potency of a brown-rot fungus, *Gloeophyllum sepiarium* was evaluated to remediate chromium contaminated soil for the first time. The contaminated soil sample containing fungal biomass was analyzed by flame atomic absorption spectrophotometer and soil nutrient analysis was also performed. The results of the study indicated removal of 94% Cr (VI) by the fungal biomass determined by atomic absorption spectrophotometry after 6 months. Also, *G. sepiarium* increased the contents of organic matter, carbon, nitrogen and phosphorus present in the contaminated soil after its inoculation. This research showed that fungal biosorption by brown-rot fungi also have a potential to be used in the removal of heavy metal ions from soils. Further this study showed that bioremediation is a viable, environmental friendly technology for cleaning-up the chromium contaminated site.

Key words: *Gloeophyllum sepiarium*, chromium, bioremediation, soil nutrient, environmental friendly

INTRODUCTION

One of the major problems the industrialized world is facing today is the contamination of soil, groundwater, sediments, surface water and air with hazardous and toxic chemicals. Removal of excesses of heavy metal ions from wastewaters is essential due to their extreme toxicity towards aquatic life and humans (Kapoor *et al*., 1999).

The wide range of chromium applications has resulted in its occurrence as a common contaminant in soils (Xu *et al*., 2009). Pollution by chromium is of considerable concern as the metal has found widespread use in electroplating, leather tanning, metal finishing and chromate preparation. Chromium occurs in the aqueous system as both trivalent and hexavalent forms, the latter being of particular concern because of its greater toxicity (Sharma and Forester, 1993; Pellerin and Booker, 2000). The bioremediation strategy is to detoxify Cr (VI) in the soil to reduce

it to Cr (III), so that it gets immobilized in the soil matrix. Besides eliminating the toxicity of Cr(VI) by its reduction to Cr(III) the latter forms a particularly insoluble $Cr(OH)_3$ in the pH range of 6-9 severely restricting its ability to migrate to soil (Tokunaga *et al*., 2003).

The remediation of chromium contaminated sites poses a number of unique challenges. Many technologies are currently used to clean up heavy metal contaminated soils. The most commonly used ones are soil removal and land filling, stabilization/solidification, physico-chemical extraction, soil washing, flushing and phytoremediation. None of these techniques are completely accepted as best treatment option (Yamamoto *et al*., 1993) because either they offer a temporary solution, or simply immobilize the contaminant or costly when applied to large areas.

Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy-metal ions (Mullen *et al*., 1989; Gadd, 2010). Fungi in particular have demonstrated unique metal adsorption characteristics and are easy to cultivate (Gadd, 1987). A large number of studies for the removal of metal ions have been reported using strains of *Penicillium*, *Rhizopus* and *Aspergillus* (Kapoor *et al*., 1999; Say *et al*., 2004; Ahmad *et al*., 2005). Studies have shown that certain bacterial species on surfaces of geologic materials can detoxify the compounds by reducing them to relatively insoluble and hence significantly less harmful trivalent chromium compounds (Wang and Xiao, 1995). Thus, treatment of Cr(VI) containing wastes consists primarily of reducing toxic and mobile Cr(VI) to nontoxic and immobile Cr(III).

White-rot fungi like *Pleurotus* species have been known for their ability to degrade lignin, a non-repeating structural polymer found in woody plant and this ability enables them to degrade xenobiotic pollutants (Bumpus and Aust, 1987). Adenipekun and Fasidi (2005) reported the ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil. Despite the current interest in microbial detoxification of contaminants, relatively little work has been concerned with metal uptake by brown-rot fungi. There are no more reports on the bioremediation of soil contaminated sites using brown-rot fungi. In the present investigation, we are reporting the ability of a brown-rot fungus, *Gloeophyllum sepiarium* ATCC 12677, to degrade chromium contaminated soil for the first time.

MATERIALS AND METHODS

The study was partly conducted during 2009-10 at DMR, India and Laboratory of Environmental Pollution and Bioremediation, XIEG, Chinese Academy of Sciences, Urumqi, China.

Soil sample: The soil samples were collected from seven different locations of the contaminated site located at Bokaro, Jharkhand, India in radiation sterilized polypropylene bottles in 2009 and was stored at -20°C. The site was highly contaminated with Cr (VI). An amount of 150 g of soil is taken, mixed well and dried in the oven at 60°C for 24 h, crushed the sample using a crusher to remove the boulders. A representative sample of the soil was characterized in the laboratory as per standard procedure (BIS, 1989). The soil characteristics thus determined are given in Table 1.

Biomass production: The fungus used in the present study was *Gloeophyllum sepiarium* ATCC 12677. This culture was transferred to the potato dextrose agar (PDA) plates. These plates were incubated for 10 days at 28°C and growth was observed after an interval of two days. The cultures were routinely transferred onto fresh PDA plates by streaking. Before *G. sepiarium* cultures were used for inoculation of liquid growth medium, the fungus was subjected to three transfers on PDA

Table 1: The physico-chemical characteristics of soil

Parameter	Value
pH	7.2-7.6
Soil organic matter	6.1%
Cr(VI)	3.4 mg g^{-1} of soil
Total chromium	8.1 mg g^{-1} of soil
Uniformity coefficient	6.5
Coefficient of curvature	1.1
Sand	48%
Silt	33%
Clay	8%

agar plates. Fungal biomass was cultivated in liquid medium using the shake flask method. The mycelium from the PDA spread plate cultures were transferred to 250 mL Erlenmeyer asks containing 100 mL growth medium. This growth medium had the following composition (g L^{-1}): dextrose, 15; peptone, 8; NaCl, 0.4; $CaCl_2.2H_2O$, 0.1; KCl, 0.1; $MgSO_4$, 0.25; K_2HPO_4, 0.5; $Fe(SO_4)_2.7H_2O$, 0.01; $NaHCO_3$, 0.05. The pH of the growth medium was adjusted to 5.0 using 1 N HCl before autoclaving. Once inoculated, flasks were shaken on a rotary shaker at 150 rpm for one week at 28°C. Harvesting of the biomass was done by filtering the cultured medium in the shake flask. Once harvested, the biomass was washed with deionized water. This washed biomass was used to bioremediate the chromium contaminated soil.

Biomass inoculation: One hundred gram of sterilized contaminated soil, moistened with sterile milliQ water was weighed into wide mouth jam bottles. Twenty five gram of rice husk was also mixed with the soil and autoclaved for 20 min each bottle was inoculated with five gram of fungal biomass. The contents of bottles were mixed well and incubated at 28°C for 1-6 months. The control experiment was also prepared in similar way where fungal biomass was not inoculated.

At an interval of one month, bottles were collected in triplicates. The mycelial biomass was separated from soil and soils were analyzed for estimation of Cr(VI).

Extraction and analysis of Cr(VI) and total chromium: For the extraction of Cr(VI) and total chromium from soil (before and after fungal biomass treatment), an alkaline nitric acid digestion method as per standard methods APHA, AWWA and WEF (1999) were used. Briefly, 50 mL of sterilized milliQ water was added to the soil and mixed with concentrated HNO_3 (5 mL) and boiling chips. The content was boiled and evaporated to 15 mL on a hot plate. Concentrated HCl (5 mL) was added and boiled again. The solution was boiled until the sample became clear and brownish fumes were evident. Finally, it was cooled and diluted up to 50 mL with distilled water. An aliquot of this solution was used for determination of the concentration of total chromium with the help of a flame atomic absorption spectrophotometer.

Soil nutrients analysis: The soil was analyzed for different nutrient contents. The pH, organic matter, carbon, nitrogen and phosphorus contents were determined according to standard methods (BIS, 1989; AOAC, 2005).

Statistical analysis: All the experiments were performed in triplicates. Error bars on graphs show the standard deviation. The data were analyzed by Analysis of Variance (ANOVA) and the means were compared by Tukey's test using GraphPad Prism software (version 5.0.3).

RESULTS AND DISCUSSION

Bioremediation of Cr (VI) was studied using biomass obtained from *G. sepiarium* with an initial Cr (VI) concentration of 3.4 mg g^{-1} of soil and the results are shown in Fig. 1. It was observed. Even though the Cr (VI) reduction rate increased with an increase in incubation time period, 94% reduction of Cr (VI) was observed at the end of 6 months (Fig. 2). Reduction of Cr (VI) to Cr (III) is a microbially mediated process and provision of a suitable electron donor to contaminated soils shall greatly speed up this reaction, thereby decreasing Cr (VI) toxicity and mobility (Jeyasingh and Philip, 2005). The biotransformed Cr (VI) remained in the soil as Cr (III).

A continuous increase in nutrient content was also observed with chromium contaminated soil inoculated with fungal biomass for different time periods compared to control. A significantly higher soil nutrient distribution of 3.7% organic matter, 2% carbon, 0.25% nitrogen and 10.3% phosphorus was observed in soil contaminated with chromium after six months of incubation with fungal biomass compared to control (Table 2). The nutrient content in the control contaminated soil was least (2.1% organic matter, 1.3% carbons, 0.21% nitrogen and 7.9% phosphorus). Similar kind of

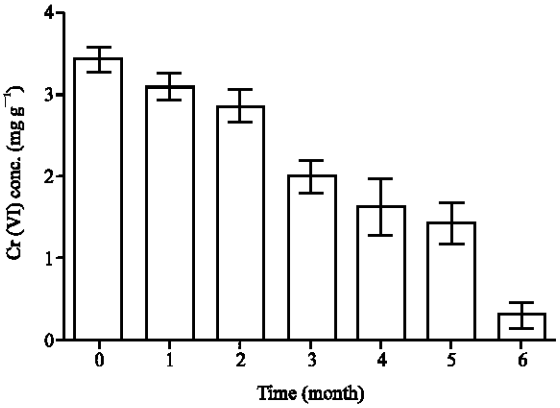

Fig. 1: Cr(VI) reductions in the soil for different time periods by *G. sepiarium*

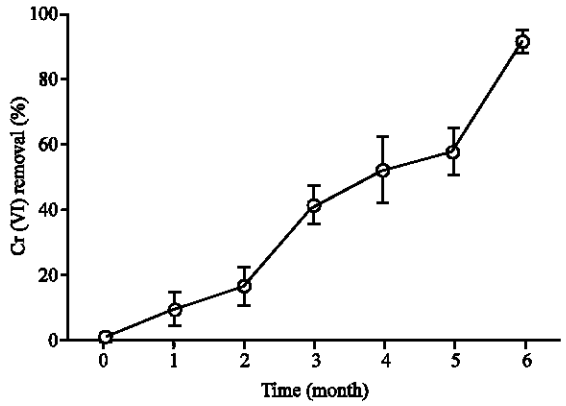

Fig. 2: Percent removal of Cr(VI) for different time periods by *G. sepiarium*

Table 2: The physico-chemical characteristics of *G. sepiarium* remediated chromium contaminated soil incubated at different time interval

	Biomass inoculation time (in month)						
Parameters	0	1	2	3	4	5	6
pH	7.4±0.1^{ab}	7.4±0.2^{ab}	7.5±0.4^{a}	7.2±0.2^{b}	7.2±0.1^{b}	7.1±0.1^{c}	7.0±0.1^{c}
Organic matter	2.1±0.2^{d}	2.1±0.1^{d}	2.4±0.3^{c}	2.8±0.4^{bc}	3.0±0.3^{b}	3.0±0.2^{b}	3.7±0.3^{a}
Carbon	1.3±0.3^{ef}	1.6±0.2^{e}	1.7±0.2^{e}	1.9±0.2^{d}	2.2±0.4^{c}	2.9±0.4^{b}	4.0±0.1^{a}
Nitrogen	0.21±0.1^{c}	0.21±0.3^{c}	0.22±0.4^{b}	0.23±0.2^{b}	0.23±0.2^{b}	0.25±0.5^{a}	0.25±0.2^{a}
Phosphorus	7.9±0.2^{ef}	8.0±0.3^{ef}	8.2±0.5^{e}	8.6±0.4^{d}	9.2±0.4^{c}	9.5±0.3^{b}	10.3±0.7^{a}

Values bearing different superscript letter(s) in the same row are significant at $p<0.05$. Values are Mean±SD (n = 3)

results was also observed by Adenipekun and Fasidi (2005). It was also noted that nutrient contents in soil increased with respect to biomass incubation time. This is also an indication of biodegradation of heavy metal present in the soils.

The pHs of soil did not differed significantly with respect to biomass incubation time. It was in the range of 7.0-7.4, although minimum pH was recorded at the end of 6 months. The results indicated the preferred pH of bioremediation by *G. sepiarium* around 7. (Verstrate *et al*., 1975) found optimum activity for microbial degradation at pH 7.4 but considerable inhibition at pH 4.5 and 8.5. Most other Cr (VI) reduction studies also were carried out at neutral pH (Fukuda *et al*., 2008; Kirk *et al*., 2008). Reduced chromium forms insoluble chromium hydroxide at a neutral pH (Faisal and Hasnain, 2006). The chromium removal abilities of *G. sepiarium* are equal or better than those of other reported strains, for example, *Candida maltose* (Ramirez-Ramirez *et al*., 2004). In particular, this strain was also has the capacity for efficient chromium reduction under neutral to alkaline conditions.

CONCLUSION

In the present study for the first time role of a brown-rot fungus has been evaluated to bioremediate chromium contaminates soil. The results suggest that *Gloeophyllum sepiarium*, a brown-rot fungus, is capable of remediating high concentration of Cr(VI) in contaminated soil. The 94% reduction of Cr(VI) was achieved within six months using fungal biomass. This fungus also increased the nutrient content of soil. Further it is concluded that the present method provides a viable and environmental friendly metal remediation technique.

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