

Full Length Research Paper

Biosorption of fireworks pollutants by indigenous soil fungi from Sivakasi, India

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Sivakasi a notable industrial town, which is known for its fireworks industries that accounts 70% of the country's yield. Besides largest production, release of the wide range of deleterious chemicals increases the concern about environmental conservation. Fungi are the eukaryotic organism which has enormous metabolite profile. These distinct features of fungi made excessive attention towards the mycoremediation. The present study was focused on mycoremediation of soil samples from the fields of nearby fireworks industries. Physico-chemical properties and biological parameters were analysed within 24 h of sample collection. Total of 20 fungi were isolated from the collected samples. Chemical contaminant degrading efficiency of the fungal isolates was screened on the soil waste agar medium. Among all the fungal isolates, four were shown to have positive results. The selective fungal isolates were evaluated for their heavy metal utilization and other pollutants degradation potential using atomic absorption spectroscopy.

Key words: Mycoremediation, pollutants, heavy metals, atomic absorption spectroscopy.

INTRODUCTION

Excessive usage of toxic chemicals and metals in the production industries has resulted in the release of large quantities of contaminants to the environment (Bogdal et al., 2010). Contaminants released from fireworks, match works, printing and pesticide industries are destructive agent to ecosystem (Sukumar and Subramanian, 1992; Katoria et al., 2013). Management of releasing pollutants and development of treatment processes are challenging

areas in the fireworks industries. Fireworks are the major source of contaminants, which generates carbon monoxide, sulphur, aluminium powder, barium nitrate, potassium nitrate, sodium nitrate, strontium nitrate, charcoal, magnesium powder and boric acid (Jonsson et al., 1995). All of these chemicals are hazardous in nature because of their explosive properties (Chen et al., 2002). Sivakasi is the second largest fireworks producers in the

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world and capital fireworks in India. Metal xenobiotics released by fireworks leads to metal deposition, which result in explosion injuries, deep wounds, intra ocular foreign body retinal trauma, glaucoma, etc. Case study reports found that workers from Sivakasi fire industries had higher levels of heavy metals like Cr, Mn and higher incidence of nervous disorders (Rajathilagam and Azhagurajan, 2012).

The advancement of bioremediation technology focuses on accomplishing successful removal of these metal pollutants by increasing the effectiveness of microbes related to metal-binding fungi. Fungi are known to degrade or deteriorate a wide variety of materials and compounds, processes known as mycodegradation and mycodeterioration. Fungal enzymes utilize the heavy metals by incorporating them in their metabolic pathways. Biochemical and ecological potential was increased by fungi to degrade the risky metals and metalloids from environment (Harms et al., 2011). Fungi possess an elevated capability to immobilize toxic metals by either insoluble metal oxalate formation biosorption into their fruiting bodies or chelation (Adeyemi, 2009). Fungal mycelium has the key role in heavy metal adsorption and has higher metal binding capacity (Barr and Aust, 1994; Bennet et al., 2002). The present work focused on isolation and screening of the fungal species present in soil from the polluted area nearby fireworks industries and evaluate its bioremediation efficiency over the pollutants.

MATERIALS AND METHODS

Collection of sample

Random soil samples were collected in sterile bags from the fireworks industrial area of Sivakasi (9° 25' 13.61"N, 77° 50' 35.11"E). The collected soil samples were mixed in large containers and air-dried at room temperature, crushed and sieved to remove rocks and un-decomposed organic materials (Gaelene et al., 2002). Soil physical parameters were determined after mixing 1g of soil in 2.5 ml water (Jianying et al., 2016). The physico-chemical parameters of fireworks industry soil such as pH, electrical conductivity, total dissolved solids, chlorinity, salinity, calcium, magnesium, sulphate and nitrate content were determined as per the standard protocols of American Public Health Association (APHA, 2005; Adams, 1990).

Isolation and screening of fungi for mycoremediation

The initial isolation of fungal species from soil was done on potato dextrose agar (PDA) media with chloramphenicol (1 mg/ml) by serial dilution and pour plate method (Cappuccino and Sherman, 1996). The pure cultures were made on PDA plates and screened for mycoremediation. Soil waste agar medium was prepared from fireworks waste soil, supernatant obtained from (100 g) waste soil was boiled in 1 L of hot water (90°C) for 15 min. Fungal strains were inoculated on soil waste agar medium and the plates were incubated at 30°C for 10 days. The radial growth of fungus was measured on both PDA medium and waste agar medium on the 10th day after inoculation. Efficient fungi were screened and selected for further studies as referred by Parani et al. (2012).

Effect of fungal growth on physico-chemical parameters

The selected fungi were inoculated separately into 250 ml of soil waste broth medium. Seven days old cultures of the four fungal isolates was used as inoculum and were incubated at 30°C for 10 days (Parani and Eyini, 2012). Culture filtrate was taken for the analysis of various physico-chemical parameters.

Heavy metal utilization potential of the fungal isolates

Heavy metals like Nickel, Zinc, Copper, and Chromium quantity were determined by digesting 200 mg of soil in a mixture of concentrated HCl/HNO₃ (4:1, v/v). Metal concentrations in the acid digest solution was analysed by atomic absorption spectrometry (AAS). The selected fungi were inoculated separately into 20 ml of solid waste broth medium. Heavy metal studies were carried out from day one to 3rd, 6th and 9th day (Ajaz Haja Mohideen et al., 2010). Mycelial extract was prepared by grinding 2 g of mycelium using mortar and pestle and digested with nitric acid (HNO₃). Culture filtrate and mycelial extract were used for the analysis of heavy metals.

RESULTS AND DISCUSSION

Isolation and screening of potential heavy metal utilizing fungus

Total of 20 fungal isolates were obtained from heavy metal polluted soil by serial dilution. All fungal isolates were obtained as pure cultures in PDA medium. Fungal isolates were screened in soil waste water agar medium. The growth rate and concurrent appearance of fungal isolates reveals that these fungal strains are much adapted to heavy metal polluted sites. The radial growth of the fungi in waste agar medium demonstrates that fungal strains are able to resist and absorb the heavy metal present in soil. Based on the growth rate in soil waste water agar medium, four fungal strains such as *Curvularia* sp. DMTMME01, *Aspergillus* sp. DMTMME02, *Fusarium* sp. DMTMME03, *Penicillium* sp. DMTMME04 were morphologically identified and selected for the further studies on bioremediation (Figure 1).

Influence of fungal growth in physico-chemical parameters

Polluted soil pH ranged from 9.1 to 9.2, which indicate the alkaline nature. Alkalinity of the soil content is high due to the load of calcium, magnesium, sulphate and nitrate from fireworks industries. Alkalinity of the soil reaches considerable decrease due to fungal growth. The amount of calcium, magnesium, sulphate, nitrate content were decreased significantly and the physico-chemical parameters reach permissible standard level due to fungal growth (Table 1). Substantial reduction of sulphate content (420 mg/L) was observed in culture filtrate. Fungal growth greatly influences the physico-chemical parameter change (Sasek and Cajthami, 2005).

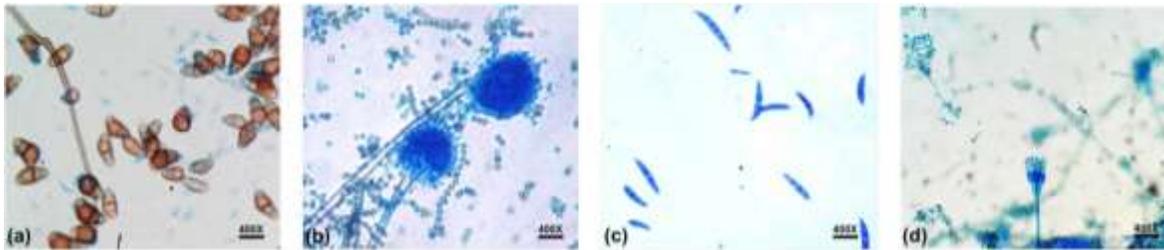


Figure 1. Morphological features of (a) *Curvularia* sp. DMTMME01 (b) *Aspergillus* sp. DMTMME02 (c) *Fusarium* sp. DMTMME03 and (d) *Penicillium* sp. DMTMME04.

Table 1. Physico-chemical parameters of fireworks industries waste soil sample before and after treatment with fungal isolates.

Parameter	Soil sample	DMTMME01	DMTMME02	DMTMME03	DMTMME04
pH	9.2 ± 0.1	8.1 ± 0.2	8.2 ± 2	8.7 ± 0.1	8.8 ± 0.2
Electrical conductivity (S/m)	41.2 ± 0.3	25.3 ± 0.3	27.8 ± 0.2	32.6 ± 0.3	38.4 ± 0.3
Total Dissolved Solids (mg/L)	650 ± 0.4	398 ± 0.2	428 ± 0.9	602 ± 0.4	580 ± 0.8
Chlorinity (mg/L)	335 ± 0.5	235 ± 0.8	235 ± 3	290 ± 0.12	320 ± 0.2
Salinity (mg/L)	4.25 ± 0.25	1.85 ± 0.3	1.85 ± 0.4	2.25 ± 0.25	2.8 ± 0.6
Inorganic phosphorus (mg/L)	195 ± 0.2	128 ± 0.65	135 ± 0.88	168 ± 0.2	180 ± 0.1
Calcium (mg/L)	410 ± 0.3	260 ± 0.2	320 ± 0.32	332 ± 0.65	350 ± 0.68
Magnesium (mg/L)	480 ± 0.7	180 ± 0.3	256 ± 0.2	398 ± 0.82	415 ± 0.7
Sulphate (mg/L)	980 ± 0.6	420 ± 0.7	446 ± 0.42	670 ± 0.12	760 ± 0.2
Nitrate (mg/L)	475 ± 0.8	268 ± 0.4	298 ± 0.2	365 ± 0.62	429 ± 0.5

Values are represented as mean ± SD of triplicate.

Heavy metal reduction by fungi

Reduction of heavy metal concentration in the culture filtrate at different time intervals were observed and shown in Figure 2. Initial heavy metal concentration of fungal growth medium consist Nickel (43.16 mg/L), Zinc (58.1 mg/L), Copper (52.1 mg/L) and chromium (41.3 mg/L). From the time interval observation, 3rd day results showed high rate of reduction rather than 6th and 9th day. So that the 3rd day of fungal growth and their heavy metal reduction were high in culture filtrate. Among all the four isolates, *Penicillium* sp. DMTMME04 showed the significant removal or reduction of all four heavy metals Ni (2.3 mg/L), Zn (4.98 mg/L), Cu (6.1 mg/L) and Cr (4.2 mg/L). Heavy metal concentration was gradually reduced in culture filtrate after the treatment. Noticeable amount of Ni and Cu concentration were also reduced due to the growth of *Fusarium* sp. DMTMME03.

Biosorption of heavy metals also examined using 3rd day mycelium of all selected fungi. *Curvularia* sp. DMTMME01 showed higher biosorption efficacy and refers its high metal binding ability of the fungus (Figure 3). *Aspergillus* sp. DMTMME02 results the high adsorption of copper heavy metal similar to the *Curvularia* sp. DMTMME01. These results were revealed that the bioremediation capability of heavy metal can be

done with all four fungal isolates. But, for the efficient removal of heavy metals *Curvularia* sp. DMTMME01 and *Penicillium* sp. DMTMME04 will be recommended for the further large scale field studies to verify results of *in situ*.

Conclusion

This study reveals that the fungal strains of *Curvularia* sp. DMTMME01, *Aspergillus* sp. DMTMME02, *Fusarium* sp. DMTMME03 and *Penicillium* sp. DMTMME04 were isolated from the heavy metal contaminated site having the great potential to survive and remove the contaminants. Among the isolates, *Curvularia* sp. DMTMME01 proves that it has potential to the remove heavy metals from fireworks industries. These fungal isolates can be used as bio-remediating agent *in situ*. The risk of heavy metals can be reduced by the mycoremediation. This clearly holds a promising economical and eco-friendly metal bioremediation technology to develop a pollution free environment.

Conflict of interest

The authors have not declared any conflict of interest.

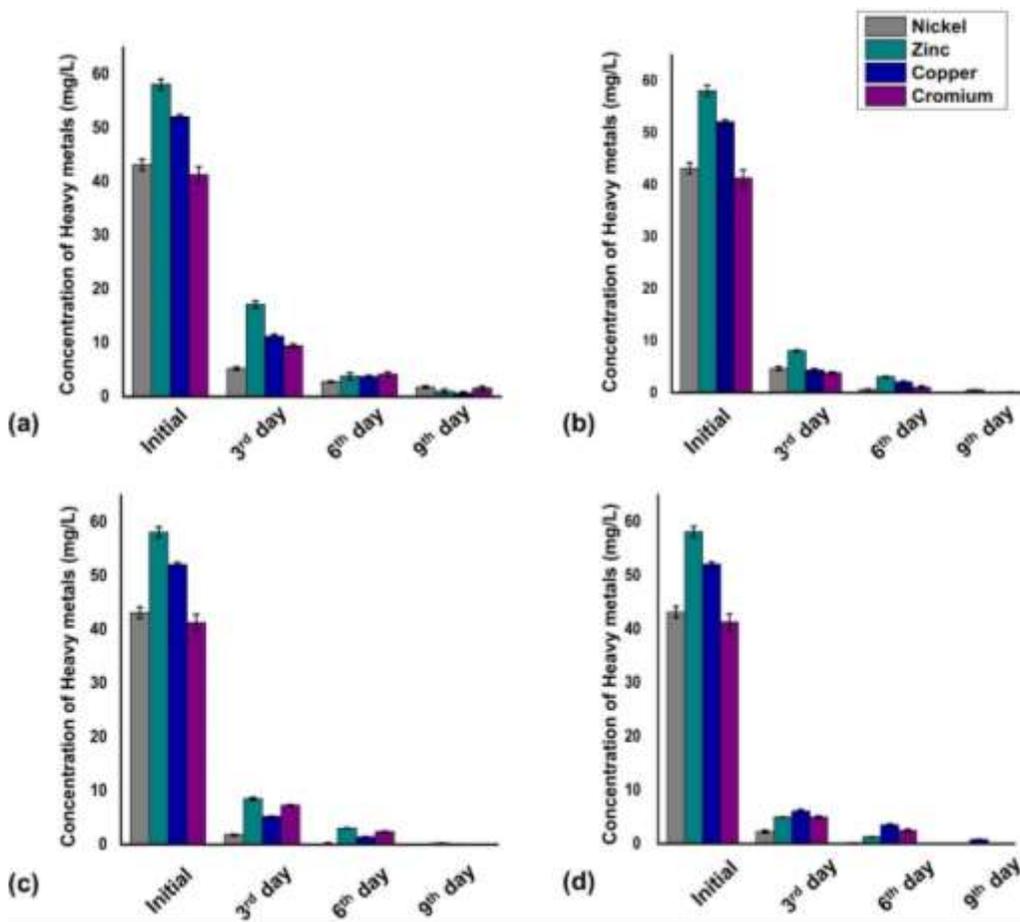


Figure 2. Heavy metals reduction in culture filtrate by (a) *Curvularia* sp. DMTMME01, (b) *Aspergillus* sp. DMTMME02, (c) *Fusarium* sp. DMTMME03 and (d) *Penicillium* sp. DMTMME04 at different time interval.

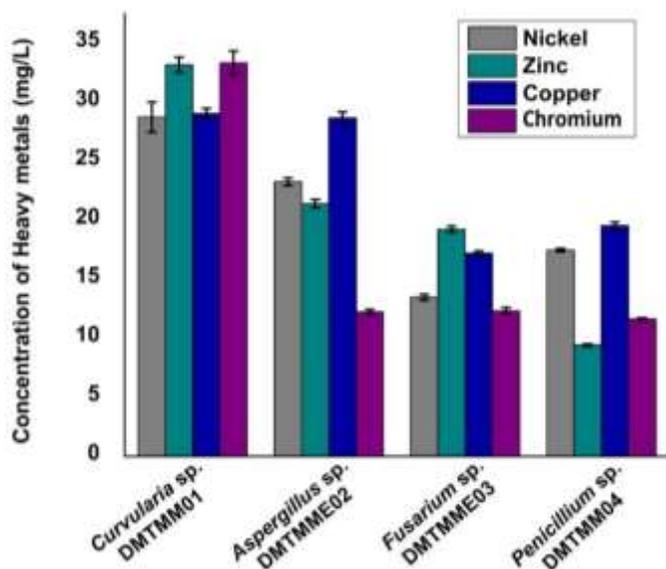


Figure 3. Biosorption of Heavy metals (Ni, Zn, Cu, and Cr) by fungal isolates.

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Abbreviations

AAS, Atomic absorption spectroscopy; **Cr**, chromium; **Cu**, copper; **EC**, electrical conductivity; **HNO₃**, nitric acid; **Mn**, manganese; **Ni**, nickel; **PDA**, potato dextrose agar; **TDS**, total dissolved solids.

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