6. SUMMARY

Determining the microbial diversity in extreme environments is one of the outstanding tasks for microbiology. Coal mine ecosystem is one of the ecosystems, which abounds in microbial diversity. Traditional microbiological techniques have limitations for the isolation and identification of most microorganisms. In this present research, the bacterial species were identified by metagenomics and fungi, actinomycetes were isolated and identified by conventional microbial techniques. The mine effluent contained unacceptable amounts of soluble salts and heavy metals (Pb, Cd, Cr, Ni, Cu, Fe & Zn) which enter the soil through irrigation. The microbes found in heavy metal contaminated environments possess the capacity to degrade heavy metals. Here, heavy metal resistant bacteria were isolated and used to remove the heavy metals from the mine affected soil and effluent. The treated soil and effluent were investigated for the utilization of paddy cultivation. The findings obtained in the present study are summarized below.

- In the present investigation, samples of lignite mine soil, lignite mine effluent and soils of agricultural fields irrigated by the mine effluent were collected at different sites of Neyveli lignite mines, Neyveli, Cuddalore District, Tamil Nadu. The samples were homogenized and analysed for their physico-chemical parameters, levels of heavy metals and for their microbial diversity.

- The effluent was brownish orange in color. The pH of the effluent was found to be in alkaline range. The physical parameters namely, electrical conductivity, total suspended solids were at higher level.
The chemical parameters namely chemical oxygen demand, biological oxygen demand, calcium, magnesium, chloride, sodium, potassium, sulphate were found to be much above the permissible limits prescribed by the Tamil Nadu Pollution Control Board (TNPCB). The heavy metals viz. chromium, nickel and mercury in effluent were found to be high above the permissible limits prescribed by WHO (1989) and FAO (1985).

- The soil samples collected from agricultural field irrigated with lignite mine effluent were highly polluted with heavy metals, especially, chromium and nickel above the normal level.

- The study of microbial diversity of lignite mine ecosystem revealed that the presence of 33 bacterial species of five different phyla viz., Proteobacteria, Nitrospira, Actinobacteria, Firmicutes and Crenarcheota and identified by metagenomics.

- Fungi and actinomycetes were isolated from lignite mine soil sample using conventional microbiological techniques. Ten strains of fungi were isolated and identified based on morphological characteristics as *Trichoderma reesei*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., *Fusarium* sp., *Rhizopus* sp. and *Chaetomium* sp.

- Eight strains of actinomycetes were isolated from lignite mine soil sample and identified based on phenotypic and biochemical characterization. The identified actinomycetes isolates *Streptomyces vastus*, *Streptomyces mirabilis*, *Actinomycetes longiporus*, *Actinomycetes*
*aureocirculatus*, *Streptomyces roseole*, *Streptomyces platensis*, *Actinomycetes janthinus*, and *Actinomycetes malachitorectus*.

- The heavy metal resistant bacteria *viz.*, HMB1-5 and four different fungal species (HMF1-4) were isolated. The bacterial and fungal isolates were further tested for their efficiency of higher concentrations of heavy metal tolerance. Bacterial strains were having more tolerance to heavy metals (Hg, Cr and Ni) than fungal strains and the bacterial strains were selected for further studies.

- The five heavy metal tolerant bacterial isolates were also having resistant to other heavy metals like manganese, lead, copper, zinc and cadmium.

- All the five bacterial strains were identified based on morphological characters and 16S rRNA sequencing. They were identified as *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas putzeri*, *Pseudomonas alcaligenes* and *Serratia marcescens*.

- Based on the screening results, all the five bacterial isolates (HMB1, HMB2, HMB3, HMB4 and HMB5) were used for the bioremediation of heavy metals as live cells, dead cells and immobilized cells.

- All the live bacterial cells (Bioaccumulation) had the capacity to adsorb the heavy metals. Among the isolates, *Bacillus subtilis* (HMB2) adsorbed maximum levels of the heavy metal Hg (39.1 mg/L), Cr (51.8 mg/L) and Ni (52.2 mg/L) followed by HMB3.

- The adsorption of heavy metals by dead cells (Biosorption) was more pronounced in HMB2. The isolate HMB2 (*Bacillus subtilis*) adsorbed
maximum levels of the heavy metal Hg (52.3 mg/L), Cr (62.1 mg/L) and Ni (63.1 mg/L).

- Immobilized microbial cells showed the maximum adsorption capability for heavy metals. Similarly among the isolates, *Bacillus subtilis* adsorbed heavy metals at higher level (69.5 for Hg, 74.2 for Cr and 75.0 for nickel).

- Among the various methods (Bioaccumulation, Biosorption and Immobilization) used for removing heavy metals from polluted water, immobilization was found to be better than other processes. Thus, the three best isolates from immobilization study viz., *Bacillus subtilis* (HMB2), *Pseudomonas stutzeri* (HMB3) and *Pseudomonas alcaligenes* (HMB4), were further considered for bioremediation of lignite mine effluent.

- In the same ways, different forms of bacterial isolates (living cells, dead cells and immobilized cells) were tested to remove the heavy metals from polluted soil. Among the three forms, the living cells showed better results in removing heavy metals from soil.

- Among the five isolates the living cells of *Bacillus subtilis* (HMB2) removed more amounts of heavy metals from the soil.

- To find the optimum conditions required for the removal of heavy metals by the bacterial strains, the effect of different pH, temperature, inoculum load, and the incubation time were investigated.

- The optimum pH and temperature was 7.0 and 30°C respectively for all the three isolates. 3% of inoculum level removed more amounts of
heavy metals. The optimum incubation time was found to be 72 hours. The same optimum conditions were observed for the removal of all the three heavy metals (Hg, Cr and Ni).

- The consortium of three bacterial species (*Bacillus subtilis*, *Pseudomonas stutzeri* and *Pseudomonas alcaligenes*) showed higher removal of heavy metals. So it is selected to remediate the polluted lignite mine effluent with optimum conditions.

- In the same way in the polluted soil, the maximum metal removal was found at the pH of 7, 4% inoculum load and at 30% moisture content using the consortium of three strains and so they were selected for the bioremediation of heavy metals from lignite mine effluent irrigated soil.

- The bioremediation of effluent with the consortium of *Bacillus subtilis*, *Pseudomonas stutzeri* and *Pseudomonas alcaligenes* showed a drastic reduction in the levels of BOD, COD, total suspended solids, heavy metals after three weeks of treatment.

- The consortium was also efficient in bioremediating the polluted soil when it is used as living cells. The heavy metals level in the soil after treatment with optimized conditions were reduced from 22.40 mg/kg of mercury, 101.3 mg/kg of chromium and 89.07 mg/kg of nickel to below detection level after 120 days of treatment, except lead (Pb), which was found after 120 days at the level of 0.85 mg/kg.

- The studies on the influence of bioremediated soil and mine effluent on the growth and yield of paddy revealed that, the growth and yield parameters such as plant height total leaf area index, grain yield and
weight were observed to be higher in the bioremediated soil than in normal soil and untreated soil.

- The results of the present study concluded that the Neyveli lignite mine ecosystem possess a wide variety of microbial diversity. The bacterial consortium (*Bacillus subtilis* + *Pseudomonas stutzeri* + *Pseudomonas alcaligenes*) played a significant role in the bioremediation of heavy metal polluted lignite mine effluent and soil and made them more suitable for the cultivation of paddy.