6. SUMMARY

Contaminated air, soil and water by the effluents from the paper mill effluent industry are associated with heavy disease burden. Untreated effluent are highly toxic to plant and human beings. This is of environmental concern if not taken care of and becomes a threat to biosphere. An appropriate control technology is needed to eliminate or reduce pollution arising from industrial process and operations. So in the present study, paper mill effluent was bioremediated and used to assess the plant growth of blackgram.

The use of microorganisms possessing multiple properties of metal resistance/ reduction and the ability to promote plant growth through different mechanisms in contaminated sites make them one of the most suitable choices for bioremediation studies. In this connection, various experiments have been conducted on the use of bacterial strains as consortium for bioremediation of paper mill effluent in accordance with the prescribed materials and methods and the findings are summarized here under.

Sample collection and analysis of physico-chemical parameters

- Sample was collected from the outlet of a paper mill industry in Vadamangalam at Pondicherry. The paper mill industry effluent was brown and had a pungent odour. The pH of the effluent was found to be in alkaline range.
The physical parameters was studied in the effluent namely total hardness, electrical conductivity, total suspended and dissolved solids were at higher level and Oil and grease is low level.

The chemical parameters namely chemical oxygen demand, biological oxygen demand, carbonate, bicarbonate, calcium, magnesium, chloride, sodium, potassium, fluoride, nitrate, nitrite, sulphate, phenolic compound, chromium, zinc copper, iron, lead and manganese were found to be much above the permissible limits prescribed by the Bureau of Indian Standards (2009).

**Gas Chromatography- Mass Spectrum analysis of raw paper mill effluent**

The Gas Chromatography Mass Spectrum (GC-MS) of paper mill effluent were analyzed. Showed three different compounds of raw paper mill effluent as listed below.

i. Dichlorvos

ii. 2,4-dichloro phenol,

iii. 1,2,4,5-tetrachloro benzene

**Isolation and identification of heavy metal tolerant bacterial isolates**

Nine strains were isolated and identified based on morphological and biochemical characteristics as *Alcaligenes faecalis, Bacillus cereus, Pseudomonas fluorescens, Bacillus subtilis, Lactobacillus* sp., *Bacillus megatherium, Micrococcus* sp., *Escherichia coli* and
*Pseudomonas aeruginosa.* The isolates were further screened. Among the nine bacterial isolates, only three strains *Pseudomonas fluorescens* (PMB-3), *Bacillus megatherium* (PMB-6) and *Pseudomonas aeruginosa* (PMB-9) were able to grow up to the highest level of Fe\(^{2+}\), Zn\(^{2+}\), Pb\(^{2+}\), Mn\(^{2+}\) and Cu\(^{2+}\) (100 mg/L).

- The three strains of (PMB-3, PMB-6 and PMB-9) able to decolourizes the paper mill effluent at higher level.
- The three bacterial isolates were subjected to growth behavior in minimal salt medium and nutrient medium at different concentrations of heavy metals.

- Screening of biosurfactant for bioremediation analysis of all the strains, *Pseudomonas aeruginosa* (PMB-9) showed the β- hemolysis, maximum zone production (22 mm) in oil spread method and emulsification index (68%) followed by *Pseudomonas fluorescens* (PMB-3) and *Bacillus megatherium* (PMB-6).

- The hydrocarbon overlay activity of the nine isolates towards kerosene, crude oil and diesel. PMB-9 and PMB-3 were founded to be more efficient of all hydrocarbons. The PMB-6 to hyrolyse crude oil and diesel.

- The biosurfactant on adsorption on heavy metals. The PMB-9 (*Pseudomonas aeruginosa*) is maximum adsorbed the heavy metals followed by PMB-3 (*Pseudomonas fluorescens*) and PMB-6 (*Bacillus megatherium*).
Based on screening, the best three efficient strains *viz.*, *Pseudomonas aeruginosa* (PMB-9), *Pseudomonas fluorescens* (PMB-3) and *Bacillus megaterium* (PMB-6) were further identified by 16S rRNA gene sequencing.

**Optimization process for heavy metal degradation**

- To find the optimum condition for the reduction of heavy metals by the bacterial strains, the effect of different carbon, nitrogen sources, pH, temperature, inoculums load and the incubation time were investigated.
- Among all the carbon and nitrogen sources sucrose and ammonium nitrate exhibited higher degradation of heavy metals. The pH and temperature was 8.0 and 30°C for all the three isolates. Inoculum level at 2.0 ml was taken for maintaining optimum condition. The optimum incubation time was found to be 30 hours. The same optimum conditions were observed for all the five heavy metals (*Fe*<sup>2+</sup>, *Zn*<sup>2+</sup>, *Pb*<sup>2+</sup>, *Mn*<sup>2+</sup> and *Cu*<sup>2+</sup>).

**Bioremediation of heavy metals**

- Based on the optimization, the three efficient isolates, *Pseudomonas fluorescens* (PMB-3), *Bacillus megaterium* (PMB-6) and *Pseudomonas aeruginosa* (PMB-9) were subjected to bioremediation (Bioaccumulation, Biosorption and Immobilization) of heavy metals like *Fe*<sup>2+</sup>, *Zn*<sup>2+</sup>, *Pb*<sup>2+</sup>, *Mn*<sup>2+</sup> and *Cu*<sup>2+</sup> in paper mill effluent by using live cultures, inactivated cells and immobilized beads.
All the live bacterial isolates (Bioaccumulation) had the capacity to degrade the heavy metals. Among the three isolates PMB-9 (*Pseudomonas aeruginosa*) exhibits maximum level of the efficiency percentage in Fe²⁺ (55.6%), Zn²⁺(52.6%), Pb²⁺(50.6), Mn²⁺ (49.2%) and Cu²⁺ (48.3%).

The adsorption of heavy metals by dead or inactivated cells (Biosorption) was more pronounced in PMB-9 (*Pseudomonas aeruginosa*) followed by PMB-3 (*Pseudomonas fluorescens*) and PMb-6 (*Bacillus megatherium*). The removal capacity of PMB-9 was 58.9%, 57.2%, 56.9%, 56.1% and 55.3 mg/L for Fe²⁺, Zn²⁺, Pb²⁺, Mn²⁺ and Cu²⁺.

Immobilized microbial cells showed the maximum reduction capability for heavy metals. Similarly among the isolates PMB-9 (*Pseudomonas aeruginosa*) adsorbed heavy metals at higher level. The efficiency percentage was 78.8 % for Fe²⁺, 77.5% for Zn²⁺, 76.3% for Pb²⁺, 75.2 % for Mn²⁺ and 74.1% for Cu²⁺ followed by PMB-3 and PMB-6.

**Bioremediation of effluent**

The physico-chemical properties of the raw effluent were found to be with a high BOD (602 mg/L), COD (1488 mg/L), total hardness (687 mg/L), total suspended solids (381 mg/L), total dissolved solids (765 mg/L), heavy metals (Chromium 0.02 mg/L, Zinc 4.64 mg/L, Iron 8.01mg/L, Cadmium 12.8mg/L, Lead 5.92 mg/L and Manganese 12.2 mg/L) and also other micro nutrients. The PMB3, PMB6 and
PMB9 strains used for bioremediating the effluent showed a drastic reduction in the levels of BOD (310 mg/L), COD (580 mg/L), total hardness (65 mg/L), total suspended solids (93 mg/L), total dissolved solids (105 mg/L), heavy metals (Chromium 0.0 mg/L, Zinc 0.19 mg/L, Iron 1.0 mg/L, Cadmium 0.5 mg/L, Lead 0.09 mg/L and Manganese 0.01 mg/L) and other chemicals also decreased.

Influence of bioremediated effluent on blackgram

Germination studies

- The effect of untreated and bioremediated paper mill effluent on seed germination, seedling length, vigour index, total chlorophyll, carbohydrate and protein content, root length, root nodules, fresh weight, dry weight, starch content, total free aminoacid, enzyme activity of blackgram under in vitro condition were studied.

- The lower concentration of 20% paper mill effluent (untreated) favoured the seedling growth of blackgram. Then the germination decreased with increase in the concentration of untreated effluent, whereas in bioremediated effluent all the parameters were gradually increased over control upto 60% concentration. The bioremediated effluent favoured the seedling growth of blackgram even at higher concentrations.
**Pot and field study**

- In pot and field experiments, the growth parameters was found to be maximum in blackgram treated with 40% concentration and increased over control upto 60% concentration of the bioremediated effluent in pot. It was slightly decreased with higher concentration of the bioremediated effluent but was minimal compared with raw effluent. Whereas 20% recorded maximum in untreated effluent in pot.

- The various morphological, biochemical and yield parameters and mineral elements studied showed maximum responses at 60 per cent concentration of bioremediated effluent. At 60 per cent concentration, slight increase in mineral contents was also observed that supported the plant growth. It was conducted from the present investigation, that 60 per cent concentration is ideal for the better growth and maximum yield of blackgram under field condition.

- The accumulation of heavy metal from soil to plant part did not follow any particular pattern and varied with respect to metals and plant parts. The concentration of metals especially Fe$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Mn$^{2+}$ and Cu$^{2+}$ was much higher in plants cultivated in raw effluent and exceeded the average normal concentration reported and also beyond consumption level. Whereas the bioremediated effluent contained the metals in less concentration.