11. FINDING AND CONCLUSION
Summary of the findings:

a. A total of five bacterial strains have been isolated, and identified. They have been designated as DSGPM1, DSGPM2, DSGPM3, DSGPM4 and DSGPM5. All of them have shown strong resistance towards Pb, Ni and Cu metal ions.

b. The isolated species DSGPM3 was found as *Bacillus* sp. After 16S rRNA sequencing, the BLAST result showed it is having 99% similarity with other *bacillus* sp. in NCBI. The phylogenetic analysis revealed that this DSGPMP3 is having very close relation with *Bacillus anthracis* and *firmicutes*.

c. The DSGPM4 was found as Gram –ve bacillus sp. Values of 16S rRNA gene sequence similarity of this strain was found having 99 to 100% similarity with families *pseudomonadacea* (γ-proteobacteria) and the species was found as *Pseudomonas aeruginosa*.

d. The DSGPM5 was also found as Gram –ve bacillus sp. Values of 16S rRNA gene sequence similarity revealed that it is having very close relationship with γ-proteobacteria and families *pseudomonadacea*. This strain was found as *Pseudomonas putida*.

e. Isolation of multiple antibiotics and metals resistant organisms indicated the presence of higher concentration of antibiotics and heavy metals in effluent that may act as reservoir for resistant bacteria.

f. Studying metal ion resistance gives us important insights into environmental processes and provides an understanding of basic living processes.
g. *Pseudomonas aeruginosa* (DSGPM4) found to be predominant over other isolates in terms of antibiotic and metal resistance.

h. In isolates, concentration dependent mechanisms operate in different organisms for the resistance of same metal.

i. All plasmid bearing isolates showed production of an additional protein in presence of metal salt which was missing in cured derivatives.

j. A range of heterotrophic culturable bacteria were obtained from the metal contaminated site, mostly belonging to γ-Proteobacteria, Bacilli, Actinobacteria and β-Proteobacteria.

k. Unusually tolerant bacterial strains, resistant to high levels of Cd, Ni and Cu, were isolated from metal contaminated sediments, with different taxa recovered in the absence of metals.

l. Several environmental factors such as pH of the media, initial metal concentration, biomass of the organisms, temperature of the media shows effect on Pb, Ni and Cu removal from aquous system.

m. The selective pressure over a period of time enabled a bacterial community to survive and thrive in such a contaminated ecosystem.

n. The selective pressure over a period of time has enabled an ecological community not only to survive, but also to actively grow in such an ecosystem.

o. Criteria used in metal-resistance genes’ primer design would be the conservation of homologous sequences. Bacteria with known metal resistance genes could also be used as sources for probes.
p. Plasmid gene transfer of DSGPM4 and express in an unrelated host such as Gram-negative *E.coli* DH5α, give us an idea about the rapid dissemination of antibiotic resistance among different unrelated bacterial species. This plasmid can be used as a potential vector in molecular biology.

q. In presence of 1% sucrose as carbon source and 4% glycerol as nitrogen source strain DSGPM4 produces maximum amount of exopolymer (EPS).

r. Exopolymer has much more potential power to biosorb heavy metals (Pb, Ni and Cu) than dry cells of bacteria. So, our results could establish a basis for evaluating the role of bacterial extracellular polymeric substances in the search for an environmentally friendly approach to decontaminate toxic metals present in water.

s. Proteins are also responsible to give metal resistance power in organisms.

t. Live or dead bacterial biomass has also been used for biosorption applications.

**Conclusions:**

**A.** The DSGPM4 is found as Gram –ve bacillus sp (*Pseudomonas aeruginosa*) and one of the best metal (Pb, Ni and Cu) removing bacteria from the aqueous system.

**B.** *Pseudomonas aeruginosa* (DSGPM4) found to be predominant over other isolates in terms of antibiotic and metal resistance.

**C.** Plasmid gene transfer of DSGPM4 and express in an unrelated host such as Gram-negative *E.coli* DH5α, give us an idea about the
rapid dissemination of antibiotic resistant among different unrelated bacterial species.

**D.** Exopolymer has much more potential power to biosorb heavy metals (Pb, Ni and Cu) than dry cells of bacteria.

**E.** Plasmid bearing isolates showed higher protein content when grown in presence of metal salts in media.

**F.** Quantitative analysis of protein showed presence of additional protein band in presence of plasmid, where as no additional band was observed in cured derivatives when grown in subsequently metal salts containing media.

**G.** Variations in molecular weight of protein suggested there were some possibility of metallotolerant protein(s) which might be adopted version of some pre-existing proteins.

Finally we can conclude to say that, this study helps us to elucidate the role of metal microbe interaction in metal polluted environment and microbes can be used as one of the potent metal bioremediating agents.