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SUMMARY

Heavy metal pollution is one of the formidable dangers that confront human beings. Every day to day use articles contain contaminating levels of heavy metals. Even the tooth pastes we use daily contain heavy metal packing. Most of the heavy metals present in the environment enter into the human body through food chain resulting in deleterious effects on human beings. One would be astonished when they know about the ill effects that heavy metals can induce in living organisms. So amelioration of heavy metals from the environment would be an unreachable goal of researchers. In this context, an attempt to suggest ways and means to remediate these heavy metals from environment would be an interesting area that a researcher can choose. In this small piece of work, we have also made an attempt to solve the unending problem and the findings are discussed in the various sections.

1. In an attempt to isolate metal ameliorating bacteria, 17 metal tolerating strains were initially isolated from polluted soil and water by pour plating on medium containing graded levels of salt of CdCl₂, Pb(NO₃)₂, NiCl₂.
2. From these 17 metal tolerating strains three were selected as metal removing strains based on the metal accumulation studies conducted in synthetic metal solution.
3. The selected strains were identified as
   a. *Pseudomonas stutzeri* SBS17 for Cadmium
   b. *Bacillus subtilis* SBS9 for Lead
   c. *Pseudomonas fluorescens* SBS7 for Nickel
4. *Pseudomonas stutzeri* SBS17 could give a maximum cadmium removal of 75%, *Bacillus subtilis* SBS9 could remove about 80% of lead and *Pseudomonas fluorescens* SBS7 could remove 60% of Nickel at 50 µg/ml metal concentrations and were selected as the most efficient metal removing bacterial strains.

5. Minimum inhibitory studies showed that *Pseudomonas stutzeri* SBS17 was tolerant up to 300µg/ml of Cd while *Bacillus subtilis* SBS9 showed inhibition at 700µg/ml of lead. *Pseudomonas fluorescens* SBS7 was tolerant up to 400µg/ml of Ni.

6. The Mechanism of metal uptake in all cases was found to be adsorption which was analyzed by fitting the Freundlich and Langmuir adsorption models.

7. Factors like pH, contact time, substrate and biomass concentrations affected metal adsorption. Adsorption increased with increase in metal concentration but reached an optimum in all cases.

8. The optimum pH for cadmium was found to be 6 for Cd for all the species but the optimum pH for Pb and Ni was found to be 5.

9. Metal adsorption is very rapid in all cases and reached maximum within 30 minutes.

10. Metal adsorption increased with increase in biomass but reached an optimum.

11. They immobilized cells could also do well in metal medium and could remove metal by 60-70% within 24 hrs of incubation.

12. On applying the free cells of *Pseudomonas stutzeri* SBS17 for the treatment of industrial effluent, the Cd content was reduced by 89% within 30minutes and the COD was reduced by a maximum of 50% with 40 hours. Similarly *Bacillus subtilis* SBS9 could remove 92% of
lead from industrial effluent and could reduce COD to a maximum of 42%.

13. The immobilized cell of *Pseudomonas stutzeri* SBS17 could result in 84% of Cadmium removal after 24hrs of contact while immobilized cells of *Bacillus subtilis* SBS9 removed 89% of lead with the above time.

14. Metal tolerating cyanobacterial strains, *Plectonema, Anabaena* and *Lyngbya* were selected by screening using synthetic metal solution.

15. Metal induced growth inhibition studies showed the tolerance limit for Cd and Pb for these organisms.

16. The mechanism for metal uptake was studied using the Freundlich - Langmuir absorption models and was found to be mainly adsorption and the adsorption capacities were calculated.

17. The antioxidant systems of Plectonema under sublethal and lethal dose of Cd stress were studied. Antioxidant enzymes like catalase, SOD, GSH-Px, GST increased while GSH level reduced. This metal induced oxidative stress showed some intracellular uptake of metals but failed to isolate any metal binding proteins.

18. Lower hydrophytes were screened for their metal tolerating capacity and *Azolla pinnata* was selected for further studies.

19. Adsorption experiments were conducted to study the mechanism. It was found that the data obtained did not follow the Freundlich - Langmuir absorption models.

20. Bioaccumulation studies and biochemical analysis conducted revealed the intracellular mechanism of metal uptake.

21. Metal binding protein (phytochelatin) was purified by ion exchange chromatography and gel chromatography.

22. RP-HPLC isolated and detected the subtypes of Phytochelatin.
IMPORTANT FINDINGS

In the present study, three sets of organisms having the ability to ameliorate heavy metals but at different levels were selected, viz,

a) at a lower level—Microorganisms (Bacteria and Cyanobacteria)

b) at a higher level—Lower plants (Pteridophytes)

The metal bioremediation activities of the above organisms and the mechanisms by which these heavy metals are taken up by these organisms have been studied in detail. The bacteria and cyanobacteria were selected after screening from different sources and also on the basis of their ability to uptake metal from the contaminated environment. It was found that three species of bacteria - *Pseudomonas stutzeri* SBS17 (Cd), *Bacillus subtilis* SBS9 (Pb), *Pseudomonas fluorescens* SBS7 (Ni) and cyanobacteria namely *Plectonema* could take up these metals very effectively. The most potent bacterium was *Pseudomonas stutzeri* and the most tolerant cyanobacterium was *Plectonema*. The metal uptake by these organisms have also been studied at different concentration of metals and found that these organisms could take up metals even when very high concentration of heavy metals are present in the environment. *Pseudomonas stutzeri* was found to be the best remover of Cd from the polluted environment. No report seems to be available on bioremediation of Cd by *Pseudomonas stutzeri* and perhaps this is the first report on bioremediation of Cd by *Pseudomonas stutzeri*. Similarly *Plectonema* was found to have a high tolerance to Cd and this property of *Plectonema* has not been reported so far.

Lead was also taken up by *Bacillus subtilis* very effectively when compared to other organisms. About 70-80% of lead was found to be removed by this bacterium from the polluted environment and proved to be
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the best remover of lead. The percentage of metal removal depends upon many factors. *Plectonema* could also remove lead but it is not as good as *Bacillus subtilis*. As mentioned earlier this metal is also taken up by physical adsorption as evidenced from Freundlich- Langmuir isotherms.

*Pseudomonas fluorescens* was found to be the best organism for removal of Ni whereas *Plectonema* could not tolerate Ni even at very low concentration. From this it can be concluded that the organism is specific for a particular metal and a synergistic action of various organisms are required for the clear up of heavy metals from polluted industrial effluents containing various metals.

Lower plant selected for the study was *Azolla pinnata*. *Azolla* was found to be the best remover of Cd and Pb but the extent of removal was not comparable with that of bacteria and cyanobacteria. It was found to be very tolerant to Cd and Pb.

As there are three mechanisms for metal removal viz., biosorption, biotransformation and bioaccumulation, the microorganisms (bacteria and cyanobacteria) remove heavy metals through physical adsorption. The Freundlich and Langmuir adsorption isotherm studies have shown that bacteria and cyanobacteria could remove heavy metals by physical adsorption very effectively. It was found that 60-80% of the contaminated heavy metals could be taken up by these organisms by physical adsorption. The metal induced oxidative stress indicted some sort of intracellular metal uptake in cyanobacteria but failed to isolate any metal binding proteins. The mechanism of metal uptake by *Azolla* was through absorption. The metal is transported internally into different cells where it is converted chemically to phytochelatin. Phytochelatins are cystine rich peptides containing repeating $\gamma$Glu-Cys units with glycines i.e. ($\gamma$-Glu-Cys)$_n$-Gly
This type of metal removal is usually seen in plants but not in lower organisms like bacteria and cyanobacteria. Therefore phytochelatin was purified from *Azolla* by Sephadex gel chromatography and ion exchange chromatography. It was found that this phytochelatin isolated from *Azolla* contains three subunits PC1, PC2 and PC3 on the basis of extend of polymerization of the subunits $\gamma$-Glu-Cys) $n$-Gly where $n$ is different in the proteins. This had been established by using HPLC studies.

From the above we recommend that specific species of bacteria and cyanobacteria have to be selected depending upon the metals present in the industrial effluent/waste water. As *Plectonema* was found to be a tolerant of Cd but not the best remover the immobilization study was not conducted. The immobilization of bacteria for removal of Cadmium from effluent/waste water gave promising results. Just like free cells, immobilized cells could remove Cd very effectively. Maximum removal could be achieved with increase in contact time. So this method of removal of Cd from polluted environment, as it being the major heavy metal pollutant in our state would be recommended. In future technologies microbial systems might be the potential tools to deal with environmental problems.

The present study deals with the bioremediation of heavy metals by selected organisms and the mechanism of metal uptake. The organisms for effective removal of Cd, Pb, Ni were selected based on the metal accumulation studies conducted in synthetic metal solution. The selected bacterial strains were identified as *Pseudomonas stutzeri* SBS17 for Cadmium *Bacillus subtilis* SBS9 for Lead and *Pseudomonas fluorescens* SBS7 for Nickel. Among cyanobacteria *Plectonema* was found to be the best organism for the removal of Cd and Pb. The lower hydrophyte *Azolla*
also showed high tolerance to Cd. Minimum inhibitory studies showed the tolerance limit of the organisms for the metal tested. The different conditions optimized for maximum metal accumulation included pH, contact time, substrate and biomass concentration. Mechanism of metal uptake in bacteria and cyanobacteria was found to be adsorption which was analyzed by fitting the Freundlich and Langmuir adsorption models. The increase of antioxidant enzymes like SOD, catalase, GST and GSH-Px indicated some sort of intracellular metal uptake in cyanobacteria. The mechanism of metal uptake by Azolla was through absorption. The metal is transported internally into different cells where it is converted chemically to metal binding protein - phytochelatin. This metal binding protein was purified by ion exchange chromatography and sephadex gel chromatography. The isolated protein contains three subunits PC1, PC2 and PC3 which was established by HPLC. The immobilization of bacteria for removal of Cadmium from effluent/waste water gave promising results. Just like free cells, immobilized cells could remove Cd very effectively. Maximum removal could be achieved with increase in contact time. So this method of removal of Cd from polluted environment, as it being the major heavy metal pollutant in our state would be recommended.