Microbial resistance to zinc metal and their biosorption mechanism

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Abstract : In the present study with the intention of screening for heavy metal tolerance, bacteria were isolated and characterized for their resistance to metals. These samples were collected from an effluent and analyzed to assess their heavy metal contamination. The samples were isolated and characterized by the Gram staining, motility tests and biochemical tests. Metal tolerance in all bacteria showed higher sensitivity to Zinc metal which was observed for 48hrs, 72hrs, 96hrs, 120hrs and 148hrs. Results indicate the applicability of the isolated bacteria for the removal of Nickel, Zinc and Copper from the industrial effluent. The bacteria which survived for higher concentrations of heavy metal are Enterococcus species.
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Introduction

Microorganisms are vital for the efficient functioning of any ecosystem, hence factors that affect their metabolism, composition and abundance are of great concern. Monitoring microbial responses has been recommended as an early warning indicator ofecosystem stress as microbes respond promptly to environmental perturbations [Griffiths, R. P. (1983)]

Heavy metal ions react to form toxic compounds in cells in high concentrations, (Nies, 1999). To have a toxic effect, however, heavy metal ions must first enter the cell. Because some heavy metals are necessary for enzymatic functions and bacterial growth, uptake mechanisms exist that allow for the entrance of metal ions into the cell. There are two general uptake systems — one is quick and unspecific, driven by a chemiosmotic gradient across the cell membrane and thus requiring no ATP, and the other is slower and more substrate-specific, driven by energy from ATP hydrolysis. While the first mechanism is more energy efficient, it results in an influx of a wider variety of heavy metals, and when these metals are present in high concentrations, they are more likely to have toxic effects once inside the cell (Nies and Silver, 1995).

Many metals and metalloids (e.g., Zn, Cu, Mn) are essential in the functioning of living organisms as micronutrients serving as structural proteins and pigment, used in the redox processes, regulation of the osmotic pressure, maintaining the ionic balance and enzyme component of the cells (Kosolapov et al., 2004). Although some heavy metals such as Zn are essential trace elements for bacterial growth, at high concentrations of Zn, most bacteria are inhibited. Community diversity is severely reduced by high levels of Zn and only a very limited number of resistant bacteria can survive Goulder et al. (1980) and Kelly et al. (2003). This is mainly due to the fact that heavy metals alter the conformational structures of nucleic acids and proteins, and consequently form complexes with protein molecules, which render them inactive, (e.g., inactivation of enzymes, slow growth and destruction cell membrane integrity). Heavy metals are both naturally and artificially present in ecosystems and have a high ecological significance due to their toxicity and accumulative behavior.

There are several researches carried out on the removal of heavy metals from industrial wastes. Soltan in 2001, in Egypt isolated 240 Pseudomonas aeruginosa strains which were resistant to Pb, Cd, Hg, Zn, Ag and Cu. He found that some strains could accumulate high concentration of these metals. Hifelie et al., (1984) used one Pseudomonas stutzeri isolated from silver mine which could accumulate 2 mg/g biomass of Ag. Similarly, Wood and Wang (1985) used an strain of Peudomonas putida capable of biosorption of 6.5% of Cu. Cooksey and Azad (1992) used a strain of Pseudomonas syngeri which could remove 115 mg/g biomass of Cu. The aim of this study is to find the bacterial resistance to zinc metals.

Materials and methods

The samples were collected using pre-cleaned polythene bags. The effluent collected was diluted with Distillery water up to 10^{-9} dilutions. The samples were collected from BHEL effluent,Trichy.

Identification of microorganisms

The isolated bacterium was subjected to identification by staining and biochemical tests. Gram staining procedure was carried out to identify the gram reaction of the organism. The motility test was performed by hanging drop method to identify motility of the organism. The biochemical tests such as indole, Methyl red, Voges Proskauver, citrate, Urease test, starch hydrolysis, catalase test, mannitol fermentation etc. were performed to identify the organisms (Edmund, 1977).

Heavy metal resistance

The bacterial strains which are isolated from the effluent were grown in nutrient agar medium. The bacterial strains used were Pseudomonas, Enterococcus, Enterobacter and Bacillus species. The heavy metal zinc resistance to these bacterial strains was identified by colorimetric method .The metals were subjected to 30uM and 50uM concentrations and the growth of the bacteria were analysed. The experiments were done for 48, 72, 96,120 and 148 hours.

Results and Discussion

The aim of this study is to find out the microbial resistance to various metals. The bacterial strains collected from effluents showed high resistance to heavy metals. Among the following strains Bacillus showed high resistance to zinc than Pseudomonas and Enterococcus species for 30Um and Enterococcus strains showed resistance to 50Um than Bacillus, Pseudomonas and Enterobacter strains. The bacteria which survived in high concentrations of heavy metal are Enterococcus species.

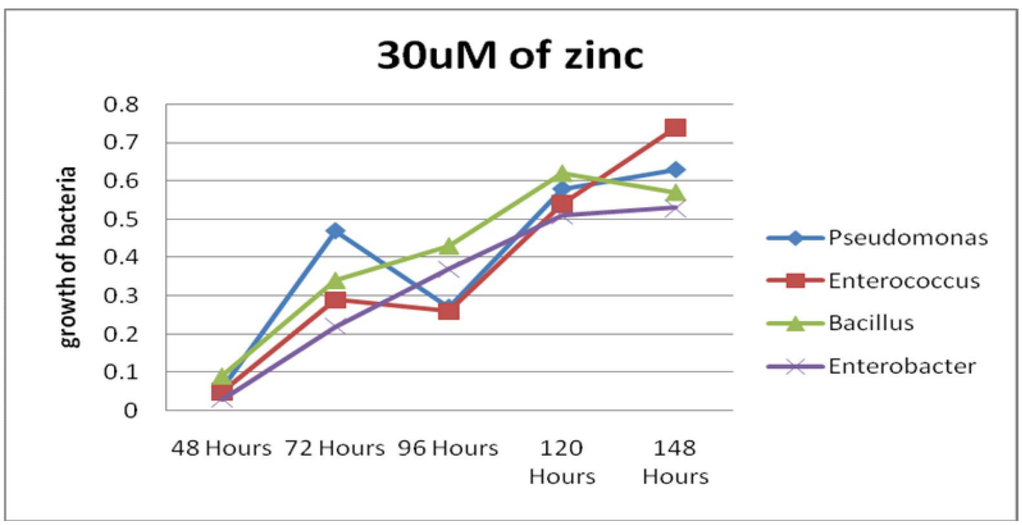
Table-1 & 2 showing 30 uM &50uM concentration of zinc resistance

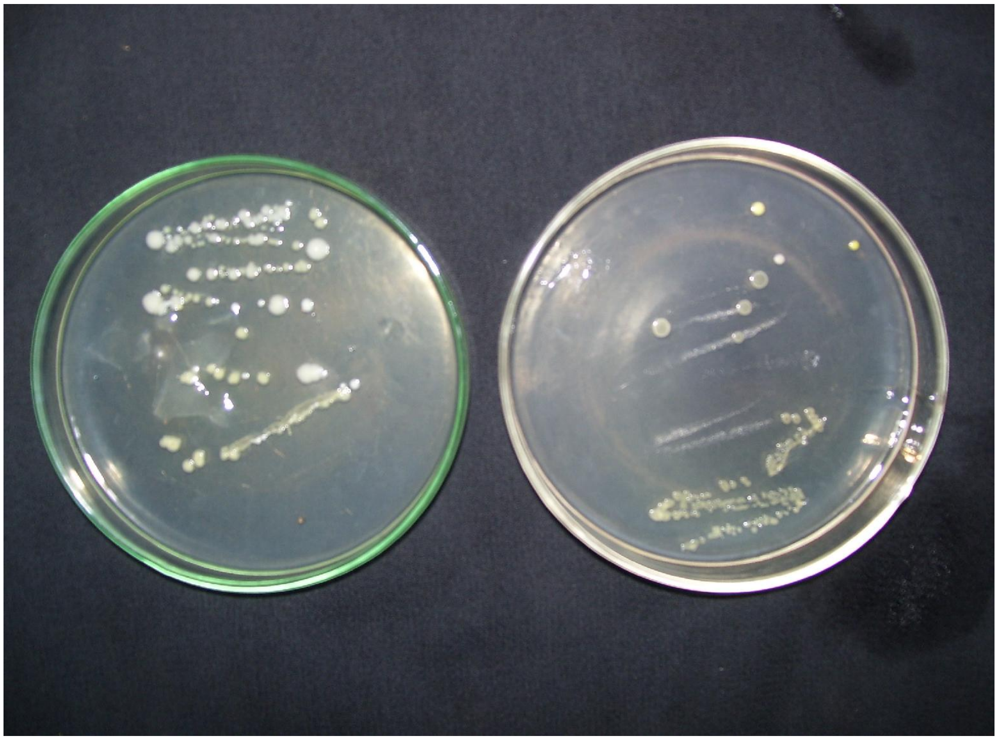
50µM of zinc concentration

Hours	Psuedomonas	Enterococcus	Bacillus	Enterobacter
48 Hours	0.05	0.4	0.09	0.11
72 Hours	0.37	0.26	0.17	0.31
96 Hours	0.28	0.24	0.44	0.39
120 Hours	0.59	0.56	0.58	0.58
148 Hours	0.75	1.01	0.92	0.61

30uM of zinc concentration

Hours	Pseudomonas	Enterococcus	Bacillus	Enterobacter
48 Hours	0.06	0.05	0.09	0.03
72 Hours	0.47	0.29	0.34	0.22
96 Hours	0.27	0.26	0.43	0.37
120 Hours	0.58	0.54	0.62	0.51
148 Hours	0.63	0.74	0.57	0.53





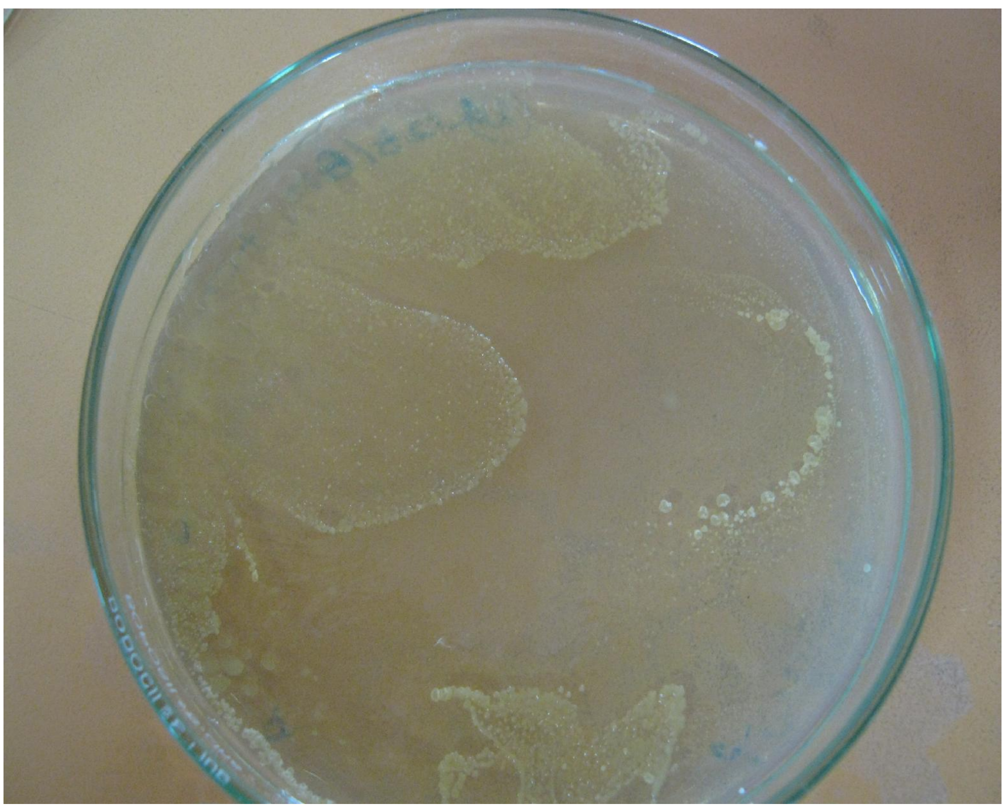
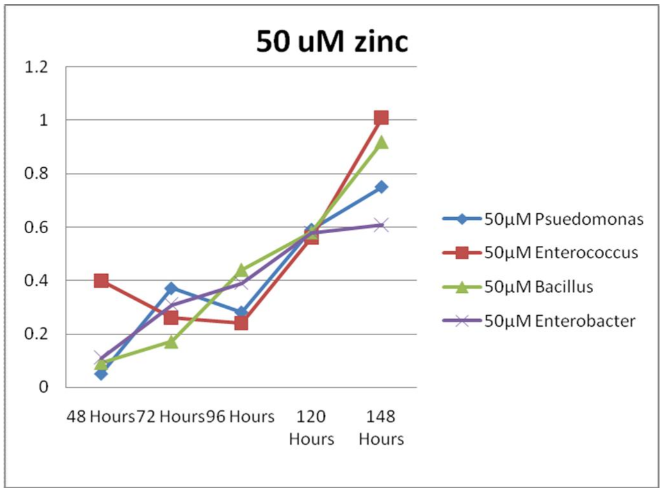


FIGURE 1 & 2. Bacillus & Pseudomonas showing 30 and 50 **uM** of zinc.



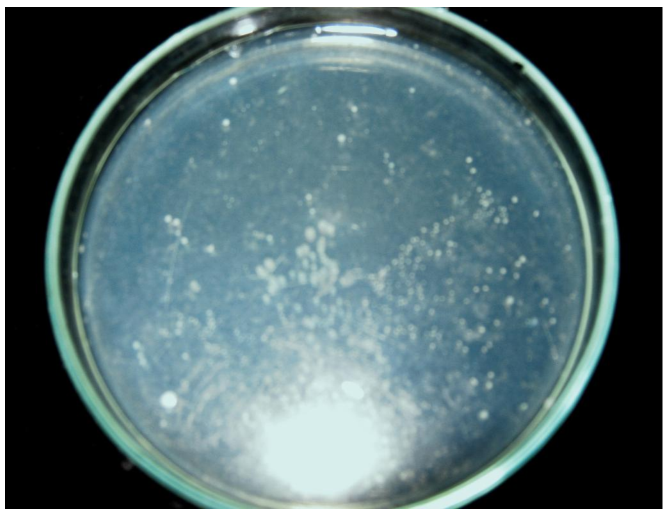


FIGURE 3. ENTEROBACTER showing resistant to zinc metal.

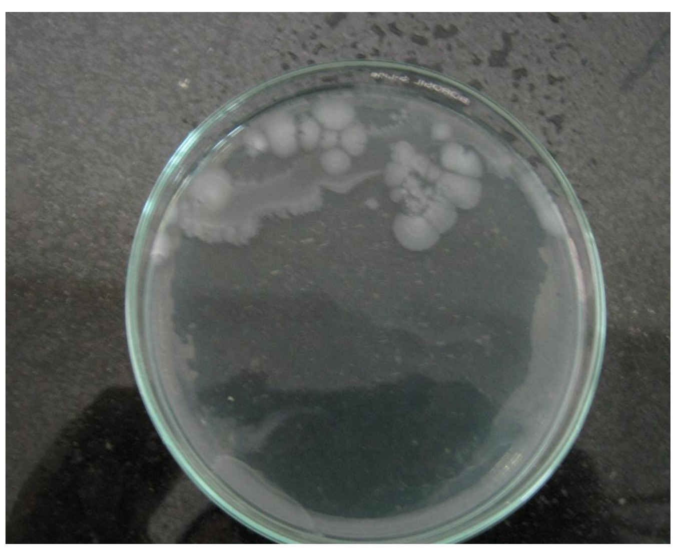


FIGURE 4 ENTEROCOCCUS showing resistance to zinc metals.

Many investigations were carried out regarding accumulation of heavy metals from effluents.Wood *et al*., (1985) isolated *Pesudomonas putida* strains from copper factory in Canada that could accumulate 6.5% of Cu in dry biomass. Mclean *et al.,* (2001) isolated a *pseudomonas* strain that reduce chromate (VI) to chromites (III). This study emphasizes the understanding of zinc resistance in the bacteria. Furthermore, *Enterococcus* could be a good candidate for biotechnological applications, such as a heavy metal biosensor.*Bacillus* which showed resistance to 30 uM of zinc whereas Enterococcus showed resistance to 50 uM of zinc. Bacillus and Enterococcus are prospective organisms for soil bioremediation of zinc-contaminated environments.

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