



BIOREMEDIATION OF HEAVY METAL CONTAMINATED FISH AQUACULTURE USING BACTERIA

Thesis

*Submitted for the degree of philosophy of
science*

Botany (Microbiology)

BY

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Abstract

The main objective of this study is the isolation and identification of some heavy metal resistance bacteria from fish farms water sources contaminated with heavy metals, and studies the effects of the bacterial isolates under different culture conditions as temperature, pH and incubation period on removal of heavy metals as Lead, Cadmium, and Copper. Heavy metals pollution with Pb^{+2} , Cd^{+2} and Cu^{+2} ions were determined in industrial and sewage water while, agricultural and fresh water did not showed any pollution with these heavy metals. Two bacterial isolates were identified morphologically and physiologically and confirmed genetically using 16 sRNA techniques as *Bacillus Subtilis* N₁ and *Pseudomonas fluorescens* N₂. The minimum inhibitory concentrations of the two bacterial isolates were; 80, 6.0 and 20.0 mg/l for Pb^{+2} , Cd^{+2} and Cu^{+2} respectively, with using *B. subtilis* N₁ and (350, 6.5 and 40 mg/l) with using *Ps. fluorescens* N₂ for Pb^{+2} , Cd^{+2} and Cu^{+2} respectively. The environmental conditions as incubation period, incubation temperature and pH values were affected the growth rate and heavy metals uptake of the two bacterial isolates. The maximum uptake of Pb^{+2} (70 mg/l) by *B. subtilis* N₁ was achieved after one day of incubation at 30°C and pH7, however, the highest growth rate and uptake of Pb^{+2} (300 mg/l) by *Ps. fluorescens* N₂ was achieved after one day of incubation at 30°C and pH6. The maximum growth of *B. subtilis* N1 and *Ps. fluorescens* N₂ in the presence of 5 mg/l Cd^{+2} was achieved when incubated for four days at 25-30°C in medium with pH (7 and 8) respectively. At 10 mg/l Cu^{+2} contraction, the *B. subtilis* N₁ became more efficient for bioremediation after 3 days of incubation, at 25°C and pH 8. But, in case of *Ps. fluorescens* N₂ the maximum uptake of 30 mg/l of Cu^{+2} was achieved after 4 days of incubation at 30°C and pH8. Not only living cells, but also, dead cells of the two isolates had the ability to reduce heavy metal ions after only few mints. TEM examination showed that mechanism of Pb^{+2} ions uptake by *B. subtilis* N1 were dependent intra-cellular and accumulated outside the cells. It could be concluded that both living and nonliving strains of *B. subtilis* N1, and *Ps. fluorescens* N2 had been used in removing toxic metal ions, Pb^{+2} , Cd^{+2} and Cu^{+2} at high concentration. Also, the biosorptive capacity of metal is greatly affected by pH and temperature and incubation period.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
APHA	American Public Health Association
Cd	Cadmium
CFU	Colony Forming Units
Cu	Copper
EPS	Extracellular Polymeric Substances
FAO	Food and Agriculture Organization
MDA	Malon di aldehyde
MIC	Minimum Inhibitory Concentration
NO	Number
NO₂	Nitrite
NO₃	Nitrate
O.D	Optical Density
Pb	Lead
PCR	Polymerase chain reaction
pH	Concentration Of Hydrogen Ions
ROS	Reactive Oxygen Species
TBE	Tris Borate EDETA
TEM	Transmission Electron Microscope

TSA	Tryptic Soya Agar Medium
WHO	World Health Organization
Zn	Zinc

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