

CONTENTS

	Page No.
Title page.....	i
Certificate.....	ii
Dedication.....	iii
Abstract.....	iv-v
Declaration.....	vi
Acknowledgements.....	vii-viii
Contents.....	ix-xiv
List of Tables.....	xv-xvi
List of Figures.....	xvii-xix
List of symbols and abbreviations.....	xx-xxi

	Page No.
Chapter 1: Introduction.....	1-18
Chapter 2: Review of literature.....	19-42
2.1. Physicochemical parameters of river water.....	19
2.2. Geomorphology of Subarnarekha river.....	20
2.3. Biodiversity of fungi.....	20
2.4. Ecological role of fungus.....	21
2.5. River pollution.....	22
2.5.1. Heavy metals	
2.6. Fungi versus heavy metal.....	23
2.6.1. Metal toxicity of fungi	
2.6.2. Resistance and tolerance	
2.6.3. Environmental effect on heavy metal toxicity to fungi	
2.6.4. Fungi in polluted habitats	

2.6.5. Interactions between toxic metals and fungi	
2.7. Extracellular precipitation and complexation.....	28
2.7.1. Metal binding to cell walls	
2.7.2. Transport of toxic metal cation	
2.8. Metal transformations.....	29
2.9. Evolution of bioremediation.....	30
2.10. Bioremediation.....	31
2.10.1 Introducing microbes based clean up system	
2.10.2. Fungal based bioremediation	
2.10.3 Mechanisms of bioremediation	
2.10.4 Bioremediation and biochemical entities of fungal EPS	
2.11. Microbial exopolysaccharide: variety and potential applications.....	36
2.11.1. Anti-microbial activities of fungus	
2.12. Eco-management and Eco-monitoring	37
2.13. Metal uptake by living cells	40
Chapter 3: Objectives	43
Chapter 4: Materials and Methods	44-70
4.1. Selection of study sites	44
4.2. Collection of water samples	45
4.3. Estimation and the recording of water quality parameter	
4.3.1. Physicochemical analysis of river water	
4.3.2 Biological parameters of water	
4.4 Collection of soil sample.....	51
4.5. Analysis of physico-chemical parameters of soil	

4.5.1. Analysis of soil texture	
4.5.2. Analysis of soil P _H	
4.5.3. Determination of soil moisture	
4.5.4. Estimation of soil organic carbon by dry combustion method	
4.6. Heavy Metals analysis of water and soil	52
4.7. Analysis of bacterial load of water	53
4.7.1. Standard plate count (SPC) method	
4.7.2. Membrane filter technique	
4.7.3. Most probable number method	
4.8. Preparation of microbial culture medium for bacteria and fungi	56
4.9. Methods of isolation of fungi	
4.10. Development of pure culture of fungi	
4.11. Pure Culture maintenance	
4.12.. Selection of fungal isolates to be used in the experimental set up for studying tolerance to heavy metals against Pb, Cd and Hg	58
4.13. Microscopic identification of fungi	57
4.14. Determination of heavy metal resistant fungal diversity in different study sites	57
4.15. Selection of metal tolerance fungi according to their maximum tolerance ability	58
4.16. Selection of efficient strain for heavy metal tolerance	58
4.17. Identification of fungi by genome sequencing	58
4.18. Study of growth rate of <i>Aspergillus penicillioides</i> (F12) against two heavy metals	60
4.19. Heavy metal tolerance activity of F-12 fungus in liquid culture	60
4.20. Determination of MICs (Minimum Inhibitory Concentrations)	61
4.21. Uptake of heavy metals (Cd II, Pb II and Hg II) by fungal isolates from liquid media	62
4.22. Production and separation of extracellular polymeric substances (EPS) from <i>Aspergillus penicillioides</i> (F12)	62
4.23. Optimization of parameters for metal removal study	63

4.23.1. Fungal Growth optimization with heavy metals	
4.23.2. Parameters optimization (for simultaneously heavy metal Pb II and Cd II removal by dry biomass and EPS)	
4.23.3. Optimization of heavy metal Pb (II) absorption by EPS using box–behnken design	
4.24. Heavy metal absorption by biomass and EPS determined by SEM and EDEX	66
4.25. Determination of heavy metal binding region of fungi by Fourier transforms infrared spectroscopy (FTIR) method.....	66
4.26. Fungal metabolites extraction	67
4.27. Estimation of emulsifying activity of EPS	67
4.28. Estimation of flocculating activity of EPS	68
4.29. Determination of antibacterial activity of the fungal extract (F12)	68
4.30. Statistical Analysis	69
Chapter 5: Results and Discussion	71-127
5.1. Physiography of Subarnarekha rive	71
5.2. Water and Soil quality parameters: Seasonal study on selected study sites	72
5.2.1. Seasonal fluctuation of water quality parameters in Subarnarekha river	
5.2.1.1 pH	
5.2.1.2. Temperature (°C)	
5.2.1.3. Alkalinity (mg/l as CaCO ₃)	
5.2.1.4. Ca-Hardness (mg/l as CaCO ₃)	
5.2.1.5. Mg- Hardness (mg/l as CaCO ₃)	
5.2.1.6. Total Hardness (mg/l as CaCO ₃)	
5.2.1.7. Total dissolved solid (mg/l)	
5.2.1.8. Total suspended solid (mg/l)	
5.2.1.9. Dissolved oxygen	

5.2.1.10. Chemical oxygen demand	
5.2.1.11. Biological oxygen demand	
5.2.1.12. Total kjeldahl nitrogen	
5.2.1.13. Chloride content	
5.2.1.14. Total phosphate phosphorous	
5.2.1.15. Total coliform and faecal coliform	
5.3. Composition of soil sediment	84
5.4. Identification of both point and nonpoint sources of pollution	84
5.4.1. Non- point source	
5.4.2. Point source	
5.5. Heavy metal analysis and their seasonal concentration at study site	87
5.5.1. Heavy metal analysis of water	
5.5.2. Heavy metal analysis of soil	
5.6. Fungal diversity of Subarnarekha river, India	93
5.7. Diversity of metal tolerance fungi in different study sites of Subarnarekha river	95
5.8. Selection of metal tolerant fungus <i>Aspergillus penicillioides</i> for detailed study	97
5.9. Tolerance index of <i>Aspergillus penicillioides</i>	99
5.10. Fungus with potential heavy metal removing activity	100
5.11. Minimum inhibitory concentration (MIC) assay of heavy metals	102
5.12. Optimized process parameters for Cd (II) and Pb (II) absorption by fungal biomass and exo polysaccharide (EPS)	102
5.13. Optimization of Pb (II) absorption percentage of EPS	104
5.13.1 Generation of response curves and regression modelling	
5.13.2. Optimal processing conditions	
5.14. Light microscope and phase contrast microscopic view of <i>Aspergillus penicillioides</i>	108

5.15. Scanning electron microscopic (SEM) view and energy-dispersive X-ray (EDEX) analysis.....	108
5.16. Fourier transform infrared spectroscopy (FTIR) study	114
5.17. Emulsifying activity of fungal EPS	116
5.18. Flocculation activity of fungal EPS	117
5.19. Antibacterial activity of fungal extract	119
5.20. Statistical analysis	120
5.20.1. Correlation between physicochemical parameters with total fungal count	
5.20.2. Cluster analysis (CA)	
Conclusions	128-131
Summary	132-143
References	144-173
List of publications from the present study.....	174