

CONTENTS

Abstract	i-vi
Declaration	vii
Certificate of the Supervisor	viii
Certificate	ix
Acknowledgement	x
Table of Contents	xi-xvi
List of Tables	xvii-xviii
List of Figures	xix-xxiv
List of Abbreviations	xxv
CHAPTER 1: Introduction	
1.1. General background	1-3
1.2. Aims and objectives	3
References	4-7
CHAPTER 2: Literature Review	
2.1. Problem and its background	1
2.2. Soil contamination	1
2.3. Sources of metals in the environment	2
2.4. Heavy metals in soil	2-3
2.5. Trends of heavy metal pollution in soils contaminated by industrial and municipal solid wastes (MSW)	4-5
2.6. Abstraction of heavy metal speciation	5-7
2.6.1. Water soluble fraction	7
2.6.2. Exchangeable fraction	7
2.6.3. Carbonate bound fraction	7
2.6.4. Fe-Mn oxide bound fraction	8
2.6.5. Organically bound fraction	8

2.6.6. Residual fraction	8
2.7. Analytical methods for determination of heavy metals in different matrices	8-9
2.7.1. Atomic absorption spectroscopy (AAS)	9
2.7.2. Inductively coupled plasma optical emission spectrometry (ICP-OES)	9-10
2.8. Indices of pollution	10
2.8.1. Mobility factor (MF)	10
2.8.2. Enrichment factor (EF)	10-11
2.8.3. Geoaccumulation index	11
2.8.4. Pollution load index	12
2.8.5. Individual and global contamination factors	12-13
2.8.6. Ecological risk assessment	13
2.9. Remediation of metal contaminated soil	14
2.10. Prospects and promises of <i>Jatropha curcas</i> L. and <i>Pongamia pinnata</i> L. Pierre for the remediation of degraded lands	14-15
References	17-25
 CHAPTER 3: Influence of paper mill wastes and municipal solid wastes on heavy metal pollution in soil	
3.1. Introduction	1
3.2. Paper mill	1-3
3.2.1. Nagaon Paper Mill: A brief description	2-3
3.3. Municipal landfill	3-4
3.3.1. Guwahati Municipal landfill: a brief description	4
3.4. Environmental impact	5-6
3.5. Materials and Methods	
3.5.1. Study area and soil sampling	6-7
3.5.2. Estimation of physical and chemical properties of soils	7-8
3.5.2.1. Soil texture (Bouyoucos Hydrometer method)	8
3.5.2.2. pH	8-9
3.5.2.3. Electrical conductivity (EC)	9
3.5.2.4. Bulk Density (BD)	9

3.5.2.5. Particle Density	13
3.5.2.6. Porosity	13
3.5.2.7. Soil organic carbon (SOC)	13-14
3.5.2.8. Available nitrogen (Av. N)	14-15
3.5.2.9. Available Phosphorus (Av. P)	15-17
3.5.2.10. Available Potassium (Av. K)	17-18
3.5.2.11. Microbial biomass carbon (MBC)	18-19
3.5.2.12. Total heavy metals in soil samples	19
3.5.2.13. Sequential partitioning of heavy metals in soil samples	20
3.6. Sample analysis and quality control	20
3.7. Statistical analysis	20-21
3.8. Results and discussion	
3.8.1. Physico-chemical properties of soil	21-32
3.8.2. Pearson correlation	32-35
3.8.3. Spatial distribution of Cd, Cu, Cr, Fe, Mn, Ni and Zn	
3.8.3.1. Paper Mill	35-40
3.8.3.2. Municipal Landfill	40-42
3.8.4. Chemical fractionation of Cd, Cr, Cu, Fe, Mn, Ni and Zn	
3.8.4.1. Paper Mill	42-47
3.8.4.2. Municipal Landfill	47-55
3.8.5. Cluster analysis	55-57
3.9. Conclusion	57-58
References	58-70
 CHAPTER 4: Heavy metal species distribution, mobility and various contamination factors in soils	
4. 1. Contamination factors	1
4.2. Background/ baseline concentrations	1
4.3. Contamination assessment	1-2
4.3.1. Pollution load index	2

4.3.2. Individual and global contamination factors	2-3
4.3.3. Mobility factor (MF)	3
4.3.4. Enrichment factor (EF)	3-4
4.3.5. Geoaccumulation index (I_{geo})	4
4.4. Ecological risk assessment	
4.4.1. Ecological hazard index	4-5
4.5. Results and discussion	
4.5.1. Pollution load index	5-7
4.5.2. Individual and global contamination factors	7-8
4.5.3. Enrichment factors	8-11
4.5.4. Geoaccumulation index	11-12
4.5.5. Mobility factors	12-16
4.5.6. Ecological hazard index	16-18
4.6. Conclusion	22
References	23-26

**Chapter 5: Phyto-remediation potential of different plant species
in heavy metal contaminated soil**

5.1. Introduction	1
5.1.1. Effects of abiotic stress factors on plants	1
5.1.2. Effect of metals on plants	1-3
5.1.3. Toxicity of heavy metals	3-4
5.1.3.1. Cadmium (Cd)	4
5.1.3.2. Chromium (Cr)	4-5
5.1.3.3. Copper (Cu)	5-6
5.1.3.4. Iron (Fe)	6-7
5.1.3.5. Manganese (Mn)	7
5.1.3.6. Nickel (Ni)	7-8
5.1.3.7. Zinc (Zn)	8-9
5.1.4. Remediation techniques used for metal contaminated soil	9-10
5.1.5. Biofuel plants for phytoremediation	10-13
5.1.5.1. <i>Jatropha curcas</i> L.	13
5.1.5.2. <i>Pongamia pinnata</i> L.	14-15
5.2. Materials or methods	

5.2.1. Selection of the experimental site and field experiment	15-17
5.2.2. Physico-chemical analysis of soil samples	17-22
5.2.3. Growth performance	22
5.2.4. Fresh/Dry weight (Biomass content)	22
5.2.5. Bioassay for Chlorophyll and Carotenoids	22-23
5.2.6. Proline	23
5.2.7. Nitrate reductase (NR) activity	24
5.2.8. Heavy metals in plant sample	24-25
5.3. Statistical analysis	25
5.4. Indicators of phyto-tolerance	25-26
5.5. Results and Discussions	26
5.5.1. Physico-chemical properties of Paper mill and MSW contaminated soils	26
5.5.2. Phytoremediation potential of the plants species in pot experiment	
5.5.2.1. Impact of various combination of polluted soil on the physico-chemical properties of soil	27-28
5.5.2.2. Plant morphology and growth	28-32
5.5.2.3. Biochemical parameters	32-46
5.5.2.4. Phytoextraction efficiency of the studied plants	46-58
5.5.2.5. Survival rate and phyto-tolerance of the studied plants	59
5.5.2.6. Metal removal (%)	59-63
5.5.3. Field experiment	
5.5.3.1. Physico-chemical properties of soil in the field condition	63-65
5.5.3.2. Biochemical parameters	65-69
5.5.3.3. Accumulation of heavy metals in <i>Pongamia pinnata</i>	69-73
5.5.3.4. Metal removal (%)	75
5.6. Conclusion	83
References	84-92
Chapter 6	
6.1. Summary and conclusion	1-4

6.2. Future Scope	4
List of Publications	1

LIST OF TABLES

Table no. no	Table captions	Page no.
2.1	An indicative list of plants used for Phytoremediation	16
3.1	Locations of the sampling sites (Nagaon Paper Mill and Guwahati Municipal Landfill)	12
3.2	Selected physico-chemical properties of soil in paper mill site (values are in mean of three replications with standard deviation)	29-30
3.3	Selected physico-chemical properties of soil in municipal landfill site (values are in mean of three replications with standard deviation)	30-31
3.4	Pearson's correlation matrix of the physico-chemical parameters of the soils from the paper mill sites	36
3.5	Pearson's correlation matrix of the physico-chemical parameters of the soils from the municipal landfill sites	38
4.1	Individual contamination factors (ICF) and Global contamination factors (GCF) of the studied metals of the soil samples	10
4.2	Mean Igeo and its classes of the studied metals for all sampling sites of paper mill	13
4.3	Mean Igeo and its classes of the studied metals for all sampling sites of municipal landfill	14
4.4	Ecological risk factor (E^i_r) and risk index (RI) associated with Cd, Cr, Cu, Mn, Ni and Zn of the study area (Paper mill)	19-20
4.5	Ecological risk factor (E^i_r) and risk index (RI) associated with Cd, Cr, Cu, Mn, Ni and Zn of the study area (Municipal landfill)	20-21
5.1	Various phyto-remediation process	11
5.2	Physico-chemical properties of soils around paper mill and municipal landfill sites (before initiation of pot experiment)	29

5.3	Various physico-chemical characteristics of the control soil and various combinations of soils (n=3, Mean \pm SD) (before plantation)	35
5.4	Heavy metal concentration (mg kg^{-1}) in the control and various combinations of soils (n=3, Mean \pm SD) (before pot experiment)	35
5.5	Correlation between chlorophyll, carotenoid (Carot), proline and nitrate reductase activity (NRA) and conc. of various metals in the leaves of <i>Jatropha</i> after 4 months of observation	47
5.6	Correlation between chlorophyll, carotenoid (Carot), proline and nitrate reductase activity (NRA) and conc. of various metals in the leaves of <i>Pongamia</i> after 4 months of observation	48
5.7	Heavy metal concentration (mg kg^{-1}) in the control and various combinations of soils (n=3, Mean \pm SD) (post harvest)	49
5.8	Depth-wise periodic physico-chemical characteristics in the soils collected near <i>Pongamia</i> plantation from both the sites-Paper mill and Municipal landfill site (n=3, Mean \pm SD)	66
5.9	Depth-wise periodic physico-chemical characteristics in the soils collected near <i>Pongamia</i> plantation from control site (n=3, Mean \pm SD)	67
5.10	Depth-wise periodic metal concentration (mg kg^{-1}) in the soils collected near <i>Pongamia</i> plantation from control site (n=3, Mean \pm SD)	68
5.11	Depth-wise periodic metal concentration (mg kg^{-1}) in the soils collected near <i>Pongamia</i> plantation from both the	72

LIST OF FIGURES

Figure no.	Figure captions	Page no.
2.1	Hypothetical Dose-Response Curves depicting the effect of heavy metal in plants	3
2.2	Heavy metal toxicity in plants	5
3.1	Map showing the sampling sites around the paper mill, Jagiroad, Assam, India	10
3.2	Map showing the sampling sites around the municipal landfill, Guwahati, Assam, India	11
3.3	Spatial distribution maps for (a) cadmium (Cd), (b) chromium (Cr), (c) Copper (Cu), (d) iron (Fe), (e) manganese (Mn), (f) nickel (Ni) and (g) zinc (Zn) in the sampling area in and around the paper mill	22-24
3.4	Spatial distribution maps for (a) cadmium (Cd), (b) chromium (Cr), (c) Copper (Cu), (d) iron (Fe), (e) manganese (Mn), (f) nickel (Ni) and (g) zinc (Zn) in the sampling area in and around municipal landfill	25-26
3.5	Total concentration of the studied metals (a) Cd, Cr, Mn and Zn) and (b) Cu, Fe and Ni in paper mill site (results presented here are the mean of three replicates and error bars indicate standard error of mean)	39
3.6	Total concentration of the studied metals (a) Cd, Cr, Cu and Zn) and (b) Fe, Mn and Ni in municipal landfill site (results presented here are the mean of three replicates and error bars indicate standard error of mean)	41
3.7	Percentage contribution of studied metals (a) Cd, (b) Cr, (c) Cu and (d) Fe in six different fractions of soils (F1), (F2), (F3), (F4), (F5) and (F6) at paper mill sites (results presented here are the mean of three replicates and error bars indicate standard error of mean)	49
3.8	Percentage contribution of studied metals (a) Mn, (b) Ni and (c) Zn in six different fractions of soils (F1), (F2),	50

	(F3), (F4), (F5) and (F6) at paper mill sites (results presented here are the mean of three replicates and error bars indicate standard error of mean)	
3.9	Percentage contribution of studied metals (a) Cd, (b) Cr, (c) Cu and (d) Fe in six different fractions of soils (F1), (F2), (F3), (F4), (F5) and (F6) at municipal landfill sites (results presented here are the mean of three replicates and error bars indicate standard error of mean)	53
3.10	Percentage contribution of studied metals (a) Mn, (b) Ni and (c) Zn in six different fractions of soils (F1), (F2), (F3), (F4), (F5) and (F6) at municipal landfill sites (results presented here are the mean of three replicates and error bars indicate standard error of mean)	54
3.11	Dendrogram obtained by the clustering of paper mill	56
3.12	Dendrogram obtained by the clustering of municipal landfill	56
4.1	(a): Box-plot model of Pollution load index (P_i) and integrated pollution index (IP_i) of the studied soil samples (1 st quartile represents 25 percentile and the 3 rd quartile represents 75 percentile)	6
	(b): Box-plot model of Pollution load index (P_i) and integrated pollution index (IP_i) of the studied soil samples (1 st quartile represents 25 percentile and the 3 rd quartile represents 75 percentile)	7
4.2	The maximum, minimum and average enrichment factor % (EF) for soil samples of (a) paper mill and (b) municipal landfill	9
4.3	The mobility factor (MF %) for the studied metals in the soil samples of (a) Cd, Cr, Mn, Zn and (b) Cu, Fe and Ni in paper mill site	15
4.4	The mobility factor (MF %) for the studied metals in the soil samples of (a) Cd, Cr, Cu, Zn and (b) Fe, Mn, Ni in	17

	municipal landfill site	
5.1	The relationship between environmental stress and either plant survival or death	2
5.2	Reactive oxygen species generation by heavy metals	3
5.3	Schematic representation of phyto-remediation processes	10
5.4	<i>Jatropha curcas</i> in different habitat	13
5.5	<i>Pongamia pinnata</i> in different habitat	14
5.6	Flow diagram of field experiment	15
5.7	Flow diagram showing the experimental setup of pot experiment	19
5.8	(a) Different growth stages of <i>Jatropha curcas</i> in pot Experiment from germination stage to 120 DAS (days after sowing) in control + paper mill contaminated soil	18
	(b) Different growth stages <i>Jatropha curcas</i> in pot experiment from germination stage to 120 DAS (days after sowing) in control + municipal landfill soil	20
5.9	(a) Different growth stages of <i>Pongamia pinnata</i> in pot Experiment from germination stage to 120 DAS (days after sowing) in control + paper mill contaminated soil	21
	(b) Different growth stages <i>Pongamia pinnata</i> in pot experiment from germination stage to 120 DAS (days after sowing) in control + municipal landfill soil	27
5.10	Sampling sites in and around Guwahati municipal landfill	
5.11	Sampling sites in and around Nagaon paper mill	28
5.12	Morphological aspect of <i>Jatropha curcas</i> subjected to different combinations of forest soil and polluted soil during 4 months	30
5.13	Morphological aspect of <i>Pongamia pinnata</i> subjected to different combinations of forest soil and polluted soil during 4 months	31
5.14	Pictures showing the visible symptoms of phytotoxicity in <i>Jatropha curcas</i> in metal contaminated soil in the pot experiment	33

5.15	Pictures showing the visible symptoms of phytotoxicity in <i>Pongamia pinnata</i> in metal contaminated soil in the pot experiment	33
5.16	Showing leaves, shoot, root dry weight (g) and biomass content (g) of (a) <i>Jatropha</i> and (b) <i>Pongamia</i>	36
5.17	Collar diameter (cm), height (cm), leaf number and leaf area (cm ²) of <i>Jatropha</i> growing in control + paper mill contaminated soil	37
5.18	Collar diameter (cm), height (cm), leaf number and leaf area (cm ²) of <i>Jatropha</i> growing in control + municipal landfill soil	38
5.19	Collar diameter (cm), height (cm), leaf number and leaf area (cm ²) of <i>Pongamia</i> growing in control + paper mill contaminated soil	39
5.20	Collar diameter (cm), height (cm), leaf number and leaf area (cm ²) of <i>Pongamia</i> growing in control + municipal landfill	40
5.21	Chl a, Chl b, carotenoid and Total Chl content of <i>Jatropha</i> for 2 nd and 4 th months	42
5.22	Chl a, Chl b, carotenoid and Total Chl content of <i>Pongamia</i> for 2 nd and 4 th months	43
5.23	Nitrate reductase (NR) activity and proline content of <i>Jatropha</i> and for 2 nd and 4 th months	44
5.24	Nitrate reductase (NR) activity and proline content of <i>Pongamia</i> for 2 nd and 4 th months	45
5.25	<i>Jatropha</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	51
5.26	Post harvest Cu and Fe content in different parts of <i>Jatropha</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	52
5.27	Post harvest Mn and Ni content in different parts of <i>Jatropha</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	53
5.28	Post harvest Zn content in different parts of <i>Jatropha</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	54
5.29	Post harvest Cd and Cr content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	55

5.30	Post harvest Cu and Fe content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	56
5.31	Post harvest Mn and Ni content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	57
5.32	Post harvest Zn content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in control and different combinations of control and polluted soils	58
5.33	Bioaccumulation factors for different soil combinations in <i>Jatropha</i> and <i>Pongamia</i> grown on different degrees of multi-metal contaminated soils. Values are mean ± SE (n = 3)	60
5.34	Translocation factor (TF) of the heavy metals in <i>Jatropha</i> and <i>Pongamia</i> in control and different combinations of control and contaminated soils	61
5.35	Fresh weight tolerance index (FWTI), root length tolerance index (RLTI) and tolerance index of <i>Jatropha</i> and <i>Pongamia</i> in different soil combinations (control + paper mill contaminated soil) and (control + municipal landfill soil)	63
5.36	Metal removal (%) of <i>Jatropha</i> and <i>Pongamia</i> in control and combination of soils (control+ paper mill contaminated soil)	64
5.37	Comparative chlorophyll a, b, total and carotenoid content in leaves of <i>Pongamia</i> grown in the field sites- paper mill, municipal landfill and control sites	70
5.38	Proline (µmol g ⁻¹) and nitrate reductase activity (NRA) (NO ₂ g ⁻¹ fw hr ⁻¹ in the leaves of <i>Pongamia</i> growing in the field sites- paper mill, municipal landfill and control	71
5.39	Heavy metal content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in paper mill sites	74-75
5.40	Heavy metal content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in municipal landfill sites	76-77
5.41	Heavy metal content in different parts of <i>Pongamia</i> (mg kg ⁻¹) in control	77-78
5.42	Bioaccumulation factor (BAF) of the selected HMs in <i>Pongamia</i> in the field sites- paper mill, municipal landfill and control	79

5.43	Translocation factor (TF) of the selected HMs in <i>Pongamia</i> in the field sites- paper mill, municipal	80
5.44	Depth-wise metal removal (%) of different metals by <i>Pongamia</i> in paper mill site	81
5.45	Depth-wise metal removal (%) of different metals by <i>Pongamia</i> in municipal landfill and control sites	82

LIST OF ABBREVIATIONS

HM	Heavy metal
PM	Paper mill
MSW	Municipal solid waste
AAS	Atomic absorption spectroscopy
ICP-OES	Plasma optical emission spectrometry
GPS	Global positioning system
MF	Mobility factor
EF	Enrichment factor
I_{geo}	Geoaccumulation index
P_i	Pollution load index
IP_i	Integrated pollution load index
ICF	Individual contamination factor
GCF	Global contamination factor
PERI or E_{ir}	Potential ecological risk factor
RI	Ecological risk index
BDL	Below detectable limit
HCA	Hierarchical cluster analysis
CRD	Completely randomized design
BAF	Bioaccumulation factor
TF	Translocation factor
TI	Tolerance index
RLTI	Root length tolerance index
FWTI	Fresh weight tolerance index
CCME	Canadian Council of Ministers of the Environment
WHO	World Health Organization
US EPA	United States Environmental Protection Agency
PCBA	Pollution Control Board Assam

