



**Study on the Leather Industrial Effluents:
Chemical and Microbial Approaches to Control
Environmental Pollution**

Thesis submitted to the Institute of Environmental Science (IES)
University of Rajshahi
for the degree of
Doctor of Philosophy
in
Environmental Science

By
MANJUSHREE CHOWDHURY

**Institute of Environmental Science
University of Rajshahi
Rajshahi-6205, Bangladesh**

April, 2013



Study on the Leather Industrial Effluents: Chemical and Microbial Approaches to Control Environmental Pollution

Thesis submitted to the Institute of Environmental Science (IES)
University of Rajshahi
for the degree of
Doctor of Philosophy
in
Environmental Science

By
MANJUSHREE CHOWDHURY

Principal Supervisor

Dr. Md. Golam Mostafa
Professor
Institute of Environmental Science
University of Rajshahi
Rajshahi-6205, Bangladesh

Co-Supervisor

Dr. Ananda Kumar Saha
Professor
Department of Zoology
University of Rajshahi
Rajshahi-6205, Bangladesh

**Institute of Environmental Science
University of Rajshahi
Rajshahi-6205, Bangladesh**

April, 2013



**DEDICATED
TO
MY BELOVED PARENTS AND
FAMILY MEMBERS**

DECLARATION

I do hereby declare that the entire work as embodied in this dissertation towards the fulfillment of Ph.D. degree is the result of my own investigation except where due acknowledgement has been stated. It has not concurrently been submitted elsewhere for any degree.

M Chowdhury
22. 04. 2013
(Manjushree Chowdhury)

CERTIFICATE



This is to certify that the thesis entitled “Study on the Leather Industrial Effluents: Chemical and Microbial Approaches to Control Environmental Pollution” submitted by Manjushree Chowdhury is the outcome of her own research work carried out at the Institute of Environmental Science and Department of Zoology, University of Rajshahi, Bangladesh, under our supervision. Any part or whole of the dissertation has not been submitted elsewhere for any degree.

We are forwarding this dissertation to be examined for the degree of Doctor of Philosophy in the Institute of Environmental Science, University of Rajshahi. The data presented in this thesis are genuine and original. Mrs. Chowdhury has fulfilled all the requirements according to the rules of the University for submission of a dissertation for the Ph.D. degree and made distinct contribution to the field of Environmental science.

M. Golam Mostafa
22.4.2013

(Dr. Md. Golam Mostafa)
Professor
Institute of Environmental Science
University of Rajshahi
Rajshahi-6205, Bangladesh.

AK Saha
22.4.2013

(Dr. Ananda Kumar Saha)
Professor
Department of Zoology
University of Rajshahi
Rajshahi-6205, Bangladesh

University of Rajshahi
April, 2013

ACKNOWLEDGEMENTS

First, I would like to recollect Almighty God. All praises and thanks for the graceful creator Who has given me the strength, patience and ability to do my research work.

The research works presented in this thesis was carried out at the Univesity of Rajshahi, Rajshahi, Bangladesh during the period of July 2008 to April 2013. It is indeed great pleasure to me to express my deepest sense of gratitude to my research supervisors, Professor Dr. Md. Golam Mostofa and Professor Dr. Ananda Kumar Saha, for their constant supervision during the course of this investigation. Their proper guidance, stimulating suggestions, continuous encouragement and unbounded generosity during the research work has made this dissertation possible. I greatly acknowledge the authority of the University of Rajshahi for partial financial support.

I would like to acknowledge my gratefulness to Professor Dr. Das Basudev Kumar, Professor Dr. M. Entazul Huque, Chaiman, Department of Chemistry, University of Rajshahi, for their personal co-operation, continuous encouragement when needed, in the entire period of this work.

I am grateful and indebted to Professor Dr. Md Azizul Islam, Director and Professor Dr. Raqib Ahmed, former Director, Institute of Environmental Science, University of Rajshahi, for providing laboratory and library facilities. I am very grateful to Professor Dr. Sarwar Jahan for his generous assistance, encouragement and invaluable suggestions in carrying out the research works. My thanks are conveyed to all other honorable teachers of the Institute of Environmental Science, University of Rajshahi for their encouragement, brilliant criticisms and helpful suggestions. I also like to give thanks to the staff of the Institute of Environmental Science.

I am grateful to Dr. Biswanath Sikder, Professor, Department of Genetic Engineering for providing DNA sequencing data analysis and wise suggestion. I wish to acknowledge the Chairman, Department of Zoology, University of Rajshahi for providing the Genetic Engineering and Molecular biology laboratory facilities and Chairman, Department of Chemistry, University of Rajshahi for providing the UV spectroscopy and conductrometry measurement facilities. I also wish to acknowledge

Professor Dr. T. H. Tarafder (former Director, Central Science Laboratory, University of Rajshahi) for providing laboratory facilities during the research work.

I also wish to express thanks to all my research colleagues for the friendly environment in the laboratory, discussions, help and suggestions when needed. The authoress is truly thankful to Md. Shahnewaj Bin Mannan, Research Officer, Enteric Microbiology Laboratory, ICDDR, Bangladesh for the help of PCR Amplication, DNA Sequencing analysis. I also express thanks to Mr. Md. Akhter-E-Ekrum, Lecturer, Department of Genetic Engineering, University of Rajshahi and Mr. Amal Kanti Dev, Chairman, Department of Footware Engineering and Technology, University of Dhaka for their kind help and cooperation during the research period.

I would like to acknowledge with much appreciation the partial financial support of the NSICT (Ministry of Science, Information and Communication Technology) in the form of senior fellowship and CDMP (Comprehensive Disaster Management Programme) to contribute partial financial assistance during this research period.

Finally, I am deeply indebted particularly to my husband Mr. Tapan Kumar Biswas, who was the constant source of inspiration during my study and research work, and especially indebted to my children Dipannita and Debanjana, who continuously encouraged, helped actively and allowed me to continue this research. I greatly appreciate their patience, generosity and sacrifice for this cause. I wish to express my grateful acknowledgements to my relatives and friends for their kind cooperation and encouragement. Lastly but not least, I owe a life-long debt to my parents for their keen interest in my education, affectionate encouragement, and tremendous cooperation and for taking care of my family during this long period.

Sole responsibility due to any error inscribed in the dissertation lies on the authoress.

M. Chowdhury
22.04.2013
(Manjushree Chowdhury)

ABSTRACT

Effluents from raw hide processing tanneries, which produce wet-blue, crust leather and finished leather contain high levels of inorganic compounds including trivalent chromium (Cr) and sulphides in most cases. Organic and other ingredients are responsible for high BOD₅ (Biological Oxygen Demand in 5 days) and COD (Chemical Oxygen Demand) values and represent an immense pollution load, causing technical problems, sophisticated technologies and high costs in concern with effluent treatment. In Bangladesh, most of the leather industries discharge the effluent without any treatment into a nearby water body and cause significant pollution. The present study was conducted aiming to characterized the tannery effluents and evaluate the performances of an innovative tannery wastewater process based on a combined physico-chemical and biological degradation.

In this study, effluents were collected from different manufacturing units (MU) in the three selected leather industries and also collected composite effluents from three monitoring points around the effluents dumping area situated at Hazaribagh industrial zone in Dhaka, Bangladesh during the year 2008-2010. The effluents were stored and analyzed for various physic-chemical parameters. The study also evaluated the performance of treatment processes combining of physico-chemical and microbial approaches to reduce toxic elements from the tannery effluents. The analysis results illustrate that the collected tannery effluents at different manufacturing units (MU) in the three selected leather industries contained extremely high values of TSS, TDS, TS, BOD₅, COD, SO₄²⁻, Na, Ca, Cr, As, Cd and Pb. The results show that the highest value of SO₄²⁻ and As were found in the liming and un-hairing effluents, the highest values of TDS, Cl⁻, Cr, Cd and Pb were found in the pickling and chrome tanning effluents and the highest value of COD and BOD₅ were found in the re-tanning and dyeing effluents. These values were far above the standard permissible limits for ISW in BDS, ISI-2000 and USEPA-2000. The mean concentration of TSS, TDS, SO₄²⁻, Cl⁻, BOD₅, COD, Na, Cr, As, Cd and Pb in the collected composite tannery effluents in all monitoring points were also exceeded the standard disposal limits. The concentration of metal ions in the composite tannery effluents were observed in the order Na > Mg > Ca > Cu > Zn > Cr > Pb > Ni > Cd > Mn > As. The

pollution levels at the three monitoring points was in the order $P_1 > P_2 > P_3$. The study also reveals that the concentration of various metal ions in the sludge contained very high values, and in particular, the concentration of Cr and Na ions were about 10000 and 6000 mg/Kg, respectively. The concentration of ions in the sludge was in the order: Cr > Na > Ca > S > Mg > P > Cu > TN > Zn > Organic compound > Pb > As > Cd. The analysis results of physico-chemical parameters suggest that the untreated leather industrial effluents contained very high level of toxic pollutants and could not be discharged into surface water and land, which pose potential threats to human health, aquatic life and the entire environment.

This study was also conducted a series of treatment processes consisted of physical, chemical and biological in order to reduce the toxic pollutants level. The collected composite effluent was allowed to settle for 1 day and filtered through different filtered media namely (i) sand-stone (ii) saw-dust and (iii) sand-stone combined with saw dust. The analysis results of the filtered effluents suggest that sand-stone filtered media could be used to reduce pollution to certain levels, but the efficiencies were not enough to consider its alone use.

The study also evaluated the performance of removal efficiencies of selected coagulants namely, FeCl_3 , Fe_2O_3 and FeSO_4 . The study optimized the coagulant dose and pH for each coagulant. The coagulants FeCl_3 and FeSO_4 showed maximum removal efficiencies for majority of the parameters at an optimum dose of 150 mg/L and neutral pH. The other coagulant Fe_2O_3 showed the maximum efficiency at a dose of 100 mg/L and pH around 9. The analyses results reveal that removal efficiency of color and TSS achieved about 100% for all coagulants. The coagulant Fe_2O_3 removed Cu and Cr almost 100%. The mean removal efficiency for all parameters were 91%, 89% and 85%, for the coagulants Fe_2O_3 , FeCl_3 and FeSO_4 , respectively, indicating that Fe_2O_3 showed the best removal performance among the coagulants. It may be mentioned that Fe_2O_3 showed lower removal efficiency only for $\text{NH}_4\text{-N}$ and TN compared to the coagulant FeCl_3 . The overall removal performance of the coagulants were in the order $\text{Fe}_2\text{O}_3 > \text{FeCl}_3 > \text{FeSO}_4$. The analysis results of the chemical treated effluents illustrated that most of the physical and chemical parameters were found well below the prescribed limits.

The study also carried out microbial treatment with bacterial species for the synthetic and composite untreated tannery effluents to remove heavy metal ions. The isolated bacteria showed resourceful tolerance against some selected heavy metal ions, i.e., Cr, Cd and Pb without producing any byproduct. The isolated metal ions tolerance bacteria were applied to synthetic and composite tannery effluents. The study results revealed that Cr concentration in the treated synthetic effluent was found almost “zero” (0) mg/L and the removal efficiency was about 100% using isolated bacteria at a culture dose of 3ml (OD-1) at pH 7.2 and 37°C for an inoculation period of 10-15 days but the Cr removal efficiency showed lower, i.e., 84%, when treated the composite tannery effluent with the same bacterial isolates under the same inoculation conditions. The strain shows comparatively lower efficiencies for degrading metal ions of Cd and Pb. The bacterial isolate was identified as *Enterobacter species* by 16S rRNA sequencing and biochemical studies. The study observed that the microbial strains (*Enterobacter species*) were successfully grown and enrichment in the presence of heavy metal ions and thus could be used in degrading heavy metal ions such as Cr, Cd and Pb from the discharged tannery effluents. Finally the study concludes that a combine treatment approaches, i.e., physical, chemical and microbial processes could be applied for tannery effluents to reduce environmental pollution and save the water bodies from further deterioration.

TABLE OF CONTENTS

	Page No.
Declaration	i
Certificate	ii
Acknowledgements	iii-iv
Abstract	v-vii
Table of Contents	viii-xii
List of Tables	xiii-xiv
List of Figures	xv-vii
List of Plate	xviii
 Chapter One: General Introduction	 1-29
1. General introduction	1
1.1. Introduction	1
1.2. Leather industrial wastes and environmental hazards	6
1.2.1. Solid wastes	6
1.2.2. Gaseous pollutants	7
1.2.3. Wastewater (effluent)	7
1.2.3.1. Characteristics and environmental impact of leather industrial effluents	8
1.3. Necessity of effluent treatment	15
1.4. Present status of tannery effluents in Bangladesh	16
1.5. Physical and chemical treatment approaches	18
1.6. Microbial approach	20
1.7. Status of tannery effluents treatment in Bangladesh	26
1.8. Lack of treatment facilities, justify the work and research gap	26
1.9. Aim and objectives of the research work	28
 Chapter Two: Characterization of the Leather Industrial Effluents	 30-65
2. Characterization of the leather industrial effluents	30
2.1. Introduction	30
2.2. Materials and methods	33
2.2.1. Study area	33
2.2.2. Sample collection	33
2.2.3. Sample preparation	36

2.3. Tannery effluent characteristics and analysis methods	36
2.3.1. Color	37
2.3.2. Odor	37
2.3.3. Temperature	37
2.3.4. pH	37
2.3.5. Electrical conductivity (EC)	37
2.3.6. Total suspended Solid (TSS)	38
2.3.7. Total dissolved solid (TDS)	38
2.3.8. Total solids (TS)	38
2.3.9. DO, BOD ₅ and COD	39
2.3.10. Sulphate (SO ₄ ²⁻)	42
2.3.11. Chloride (Cl ⁻)	43
2.3.12. Determination of Ammonium-Nitrogen, Nitrite-Nitrogen, Nitrate-Nitrogen and Total Nitrogen in tannery effluents	44
2.3.13. Cationic constituents	49
2.4. Results and Discussion	51
2.4.1. Color and temperature	51
2.4.2. pH and EC	53
2.4.3. TSS, TDS and TS	55
2.4.4. BOD ₅ , COD and DO	60
2.4.5. SO ₄ ²⁻ and Cl ⁻	62
2.4.6. Ammonium, Nitrite, Nitrate and Total Nitrogen	63
2.4.7. Quantity of dissolved metal ions concentration	63
2.4.8. Characterization of sludge	64
Chapter Three: Physico-Chemical Treatment of Leather Industrial Effluents	66-117
3. Physico-chemical treatment of leather industrial effluents	66
3.1. Introduction	66
3.2. Materials and methods	70
3.2.1. Study area and sample collection	70
3.3. Different treatment processes	70
3.4. Analysis of filtered and chemically treated effluents	74
3.5. Results and Discussion	75
3.5.1. Filtered effluent analysis results	75

3.5.1.1. Color, appearance and odor	75
3.5.1.2. pH and EC	76
3.5.1.3. TSS, TDS and TS	78
3.5.1.4. DO, BOD ₅ and COD	78
3.5.1.5. NH ₄ -N, NO ₃ -N, NO ₂ -N and TN	79
3.5.1.6. SO ₄ ²⁻ and Cl ⁻	79
3.5.1.7. Metal ions concentration	79
3.5.2. Chemically treated effluent	80
3.5.2.1. FeCl ₃ treated effluent analysis results	80
3.5.2.2. Fe ₂ O ₃ treated effluent analysis results	92
3.5.2.3. FeSO ₄ treated effluent analysis results	103
3.6. Comparison with different treatment processes	114
Chapter Four: Microbial approach for removal of toxic metal ions	118-167
4. Microbial approach for removal of toxic metal ions from tannery effluents	118
4.1. Introduction	118
4.2. Materials and methods	122
4.2.1. Study area and sample collection	122
4.3. Apparatus and Chemicals	123
4.4. Culture media and Isolation of heavy metal degrading bacteria	124
4.5. Identification and characterization of the effluents bacteria	125
4.6. Microscopic Examination of Bacterial Cells	126
4.6.1. Staining technique	126
4.6.1.1. The slide preparation	126
4.6.1.2. Gram staining	126
4.6.2. Motility Test	127
4.6.2.1. Hanging drop method	127
4.6.3. Atomic force microscopic analysis	127
4.7. Determination of optimal growth conditions	127
4.8. Biochemical Studies	128
4.8.1. Carbohydrate utilization	128
4.8.2. Catalase tests	128
4.8.3. Citrate utilization tests	128
4.8.4. Methyl Red test	128
4.8.5. Oxidase test	129

4.8.5.1. MacConkey agar plate	129
4.8.5.2. Triple Sugar Iron agar slant	129
4.8.6. Indole tests	129
4.8.7. Urea hydrolysis test	129
4.8.8. Production of hydrogen	129
4.8.9. Voges-proskauer (V.P.)	130
4.9. Identification of the bacterial Isolates	130
4.9.1. Nucleotide sequencing and Phylogeny	130
4.9.2. PCR Amplification of the 16s rRNA Gene	131
4.9.3. Purification of the PCR Products	132
4.9.4. Rapid thermal ramp	133
4.9.5. Detection of the Nucleotides by ABI- PRISM 310	134
4.9.6. Sequence Analysis	135
4.10. Antibiotics Disk Sensitivity Test	135
4.11. Preservation of Bacterial Isolates	135
4.12. Extraction of Plasmid DNA	136
4.13. Determination of Minimum Inhibitory Concentration (MIC) of Gentamycin	136
4.14. Preparation of inoculums	136
4.15. Curing Experiments	137
4.16. Degradation rate of metal ions (Cr, Cd and Pb) containing effluents by bacteria	138
4.16.1. Experimental description	138
4.16.2. Preparation of standard solution	139
4.17. Results and Discussion	139
4.17.1. Isolation of bacteria	139
4.17.2. Determination of optimal growth conditions for isolated bacterial strain	143
4.17.3. Biochemical Tests	147
4.17.3.1. Utilization of sugars by isolating bacterial strain	147
4.17.3.2. Results of bio-chemical tests	152
4.17.4. Antibiotic Sensitivity	152

4.17.5. Extraction of Chromosomal DNA	155
4.17.5.1. DNA Sequencing Analysis of 16S rRNA gene	156
4.17.5.2. Analysis of sequencing results	160
4.17.6. Investigation of Plasmid DNA	160
4.17.7. Minimum Inhibitory Concentration (MIC)	160
4.17.8. Curing Experiment	162
4.17.9. Degradation rate of metal ions (Cr, Cd and Pb) by bacterial treatment	164
Conclusions	168-171
References	172-206
Appendix	207-230

LIST OF TABLES

Table No.	Title	Page No.
Table 2.1:	Physico-chemical characteristics of tannery effluents at manufacturing units of BLC Tannery	52
Table 2.2:	Physico-chemical characteristics of tannery effluents at manufacturing units at BAY Tannery	54
Table 2.3:	Physico-chemical characteristics of tannery effluents at manufacturing units in Karim Tannery	55
Table 2.4:	The physico-chemical parameters including heavy metals of the composite tannery effluents and compare with the standard limits during May – June in the year 2008-2010	57
Table 2.5:	The physico-chemical parameters including heavy metals of the composite tannery effluents and compare with the standard limits during August- September in the year 2008-2010	59
Table 2.6:	The physico-chemical parameters including heavy metals of the composite tannery effluents and compare with the standard limits during November – December in the year 2008-2010	61
Table 2.7:	Characterization of sludge collected from tannery effluents dumping area at Hazaribagh industrial zone	65
Table 3.1:	Physical characteristics (color, appearance and odor) of filtered effluents	75
Table 3.2:	The physico-chemical characteristics including metal ions of tannery effluents after filtered with different filtration media and compare with untreated effluents	77
Table 3.3:	The physical characteristics (Color, appearance and odor) of different doses of coagulants (FeCl_3 , Fe_2O_3 and FeSO_4) treated effluents in a day 1	81

Table 3.4:	Physico-chemical characteristics of FeCl ₃ treated tannery effluents	83
Table 3.5:	Physico-chemical characteristics of Fe ₂ O ₃ treated tannery effluents	93
Table 3.6:	Physico-chemical characteristics of FeSO ₄ treated tannery effluents	105
Table 3.7:	Comparison of among coagulants, FeCl ₃ , Fe ₂ O ₃ and FeSO ₄ for different parameters removal efficiencies	115
Table 3.8:	Comparison of removal percentage (present and others research findings)	116
Table 4.1:	Primer of the detection of 16s small subunit ribosomal RNA genes (Loffler <i>et al.</i> 2000)	131
Table 4.2:	The reagents used in cycle sequencing	132
Table 4.3:	Cycle Sequencing Program	133
Table 4.4:	Purification of the cycle sequence product and Detection of Nucleotides by ABI- PRISM 310	134
Table 4.5:	Morphological and physiological characteristics and Microscopic observation of the isolated Cr-tolerance bacterial strain	140
Table 4.6:	Biochemical tests for the isolated bacterial strain	148
Table 4.7:	Antibiotic sensitivity tests	153
Table 4.8:	Minimum Inhibitory Concentration of <i>Gentamycin</i>	161
Table 4.9:	The elimination of plasmid from the bacterium using various curing agents	162

LIST OF FIGURES

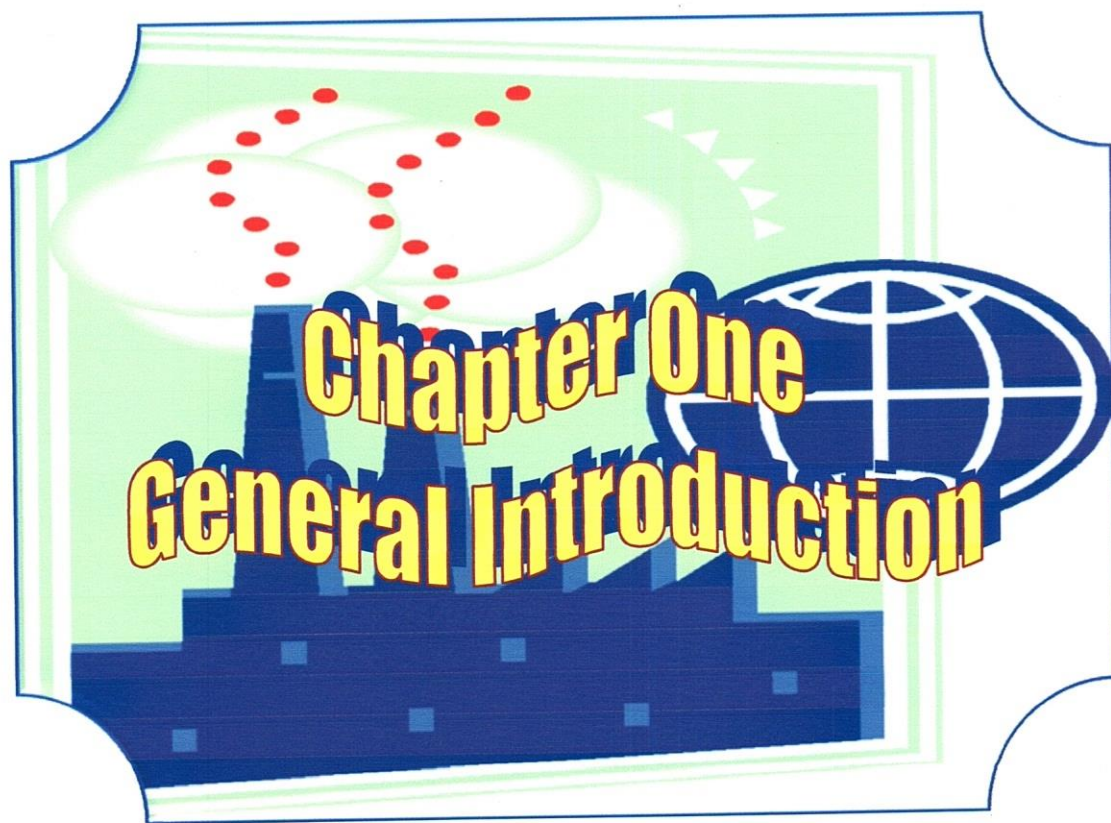
Figure No.	Title	Page No.
Figure 1.1:	Metal-microbe interactions affecting bioremediation	24
Figure 2.1:	Sampling location of leather industrial zone in Hazaribagh Thana, Dhaka, Bangladesh	35
Figure 2.2:	Schematic diagram of sample collecting points at Hazaribagh industrial zone	36
Figure 3.1:	Photograph of sand-stone filtration process (SSF)	71
Figure 3.2:	Flow chart of physico-chemical treatment process	72
Figure 3.3:	Effect of coagulant (FeCl_3) doses on pH and EC	82
Figure 3.4:	Effect of coagulant (FeCl_3) doses on TSS, TS and TDS	84
Figure 3.5:	Effect of coagulant (FeCl_3) doses on BOD_5 and COD	85
Figure 3.6:	Effect of coagulant (FeCl_3) doses on $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN	86
Figure 3.7:	Effect of coagulant (FeCl_3) doses on the removal efficiency of different parameters (BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd)	88
Figure 3.8:	Effect of pH on removal efficiency of BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd for coagulant, FeCl_3	90
Figure 3.9:	The dependency of removal efficiency of some physico-chemical parameters (TSS, COD, SO_4^{2-} , Cl^- , Cr, As and Cd) on reaction time for coagulant, FeCl_3	91
Figure 3.10:	Effect of coagulant (Fe_2O_3) doses on EC and pH	94
Figure 3.11:	Effect of coagulant (Fe_2O_3) doses on TSS, TDS and TS	95
Figure 3.12:	Effect of coagulant (Fe_2O_3) doses on BOD_5 and COD	96

Figure 3.13:	Effect of coagulant (Fe_2O_3) doses on $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN	97
Figure 3.14:	Effect of coagulant (Fe_2O_3) doses on the removal efficiency of different parameters (EC, TSS, COD, SO_4^{2-} , Cl^- Cr, As and Cd)	100
Figure 3.15:	Effect of pH on TSS, COD, SO_4^{2-} , Cl^- Cr, As and Cd for coagulant, Fe_2O_3	101
Figure 3.16:	The dependency of removal efficiency of some physico-chemical parameters (TSS, COD, SO_4^{2-} , Cl^- Cr, As, Cd and Pd) on reaction time for coagulant, Fe_2O_3	103
Figure 3.17:	Effect of coagulant (FeSO_4) doses on EC and pH	106
Figure 3.18:	Effect of coagulant (FeSO_4) doses on TDS, TS and TSS	107
Figure 3.19:	Effect of coagulant (FeSO_4) doses on BOD_5 , and COD	108
Figure 3.20:	Effect of coagulant (FeSO_4) doses on $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN	109
Figure 3.21:	Effect of coagulant (FeSO_4) doses on the removal efficiency of different parameters (BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd)	111
Figure 3.22:	Effect of pH on TSS, COD, SO_4^{2-} , Cl^- Cr, As and Cd for coagulant, FeSO_4	112
Figure 3.23:	The dependency of removal efficiency of some physico-chemical parameters (TSS, COD, SO_4^{2-} , Cl^- Cr, As and Cd) on reaction time for coagulant, FeSO_4	114
Figure 4.1:	A typical structure of plasmid pBR322 (Appendix 4.1, p 221)	
Figure 4.2:	Microscopic imaged of isolated bacteria	142
Figure 4.3:	Atomic force microscopic imaged of isolated bacteria	142
Figure 4.4:	Effect of temperature on bacterial growth (nutrient: Cr)	143

Figure 4.5:	Effect of temperature on bacterial growth (nutrient: Cd)	144
Figure 4.6:	Effect of temperature on bacterial growth (nutrient: Pb)	144
Figure 4.7:	Effect of pH on bacterial growth (nutrient: Cr)	145
Figure 4.8:	Effect of pH on bacterial growth (nutrient: Cd)	146
Figure 4.9:	Effect of pH on bacterial growth (nutrient: Pb)	146
Figure: 4.10:	Effect of inoculation age on Cr reduction.	164
Figure: 4.11:	Effect of inoculation age of Cd reduction.	165
Figure 4.12:	Effect of inoculation age of Pb reduction	165
Figure: 4.13:	Effect of inoculation age on metal ions (Cr, Cd and Pb) reduction.	166

LIST OF PLATE

Plate No.	Title	Page No.
Plate-4.1:	Isolated bacterial colonies (nutrient: Cr 20µg/mL)	141
Plate-4.2:	Isolated bacterial colonies (neutrient : Cr 50 µg/mL)	141
Plate-4.3:	Sugar Utilization (1. Glucose, 2. Galactose, 3. Maltose, 4. Sucrose, 5. Lactose, 6. Mannitol, 7. Cellulose and 8. All)	150
Plate-4.4:	Biochemical tests	151
Plate-4.5:	Biochemical tests	152
Plate-4.6:	Antibiotic disc sensitivity tests	154
Plate-4.7:	PCR Amplification of 16S rRNA gene yielded a 1500 bp PCR product, 1 kb+ DNA marker is used for size determination	155
Plate-4.8:	Agarose gel electrophoresis of the plasmid DNA isolated from bacterial sp. (lane 1, 2, 3 and 4). The DNA was resolved on 0.8 % agarose gel and stained with ethidium bromide (0.5 µg/mL)	161
Plate-4.9:	Cured strains of isolated <i>Bacterial spp.</i> (lane 3 and 4). Other lane 1 and 2 denoted that 1Kb ⁺ marker DNA, Plasmid DNA	163



1. General Introduction

1.1. Introduction

Industrial effluent treatment is concerned with environmental protection and with the reduction of production costs by reducing toxic chemicals. Industrial discharges have been growing concern as a result of untreated release of toxic chemicals through effluents, which has potential threats to the environment and life. Industrial wastewaters are generated from different processes and the huge amount of toxic wastewater released varies with its own specific industrial processes. But industrial wastewater is customarily called effluent. It is a liquid that flows out of a containing space. Wastewater is any water that has been adversely affected in quality by anthropogenic activities. Since the beginning of the industrial revolution, pollution of the biosphere with toxic metals has accelerated dramatically. Increasing industrialization and population develops the standard of living, which results in highly contaminated atmosphere due to the drainage and wastage from these industries (Tiwari *et al.*, 2008). Rapid industrialization plays an important role in polluting the environment and causes severe degradation in hydrosphere and atmosphere. The wide spread of industrialization in urban areas has drastically reduced land area for waste disposal. Disposal of untreated industrial and domestic wastewater into the environment affect both soil and ground water quality. Soil and streams have been used for multifarious purposes including waste disposal. Water used in industries creates wastes that has potential hazard for our environment because of the introduction of various contaminants such as heavy metals and different toxic chemicals into soil and water resources (Azumi and Bichi, 2010). The careless dumping of wastewater has affected the precious resources and the industrial wastewaters are discharged into the open drain without any consideration of the environmental degradation and ultimately making its way into the rivers. Thus it becomes an emerging problem not only for the environment but also the social context of the country.

Industrial estates are established to fulfill the demand for growing population in the country. The introduction of industries give useful products in one hand manufactures but at the same time generates waste products in the form of solid, liquid or gas that leads to the creation of hazards, pollution and losses of energy. Environment pollution

is becoming a global problem where a huge amount of industrial effluents is one of major sources of water pollution. The industrial effluents are being discharged into the common drainage or nearby soil or water bodies (Lokhande *et al.*, 2011) and thus ultimately pose a serious threat to human and routine functioning of ecosystem (Lakshmi *et al.*, 2010; Olajumoke *et al.*, 2010).

The most problematic industries for the water sector (surface and ground water) are textiles, leather-industries, pulp and paper mills, fertilizer, industrial chemicals production and refineries. A complex mixture of hazardous chemicals, both organic and inorganic, is discharged into the water bodies from all these industries usually without any treatment. The organic pollutants are both biodegradable and non-biodegradable in nature. The biodegradable organic components degrade water quality during decomposition by depleting dissolved oxygen. The non-biodegradable organic components persist in the water system for a long time and pass into the food chain (Ahmed and Reazuddin, 2000). Inorganic pollutants are mostly metallic salts, and basic and acidic compounds. These inorganic components undergo different chemical and biochemical interactions in the river system, and deteriorate the water quality. Leather industrial wastewaters was found to contain high salinity, high organic loading higher concentrations of total dissolved solids, chromium, chloride, ammonia, nitrate and sulphates and specific pollutants such as As, Cd and Cr and Pb etc when the samples were collected from the outlets of the lather industry (Calheiros *et al.*, 2008). Above all the chemical pollutants are received water, which may be a river, a city sewer, a lake, surface water, ground water or even the ocean, if this pollution crosses the danger level, the fish and other aquatic species cannot survive there and the receiving water becomes unfit for human and animal uses. On the other hand, the land is received this polluted water loses its fertility. In developing country, very recently it has come to the sharp focus that the tannery effluents containing pollutants have the values of standard limits of waste discharge quality of industrial units in National and International limits. In developing countries like Bangladesh, tannery industries generate huge amount environmental pollutants. So the Governments of the developing countries should come forward and take the major responsibility of tannery effluents treatment and bear its cost before discharged effluent into the open environment if the foreign exchange earnings are expected for

their nations out of leather industry. The Government of the People's Republic of Bangladesh decided to shift the tannery industries from Hazaribagh to Baliapore which is only about fifteen kilometer away from the Dhaka City (Safiullah S., 2010). The river Bongshy is flowing beside the fore coming location of tannery industrial zone at Baliapore and is also connected to the river Buriganga on its way. But the shifting is now getting delayed over the last decade due to the delay in setting up of the common effluent treatment plant (ETP) for the proposed industrial zone. Unwillingness of the owners is also a factor for the delay.

Leather industrial effluents are ranked as the highest pollutants in all environments among all the industrial wastes or effluents and it is one of the major health concerns of developing countries (Shen T.T., 1999; Soyalsan and Karaguzel., 2007; Camargo *et al.*, 2003 and Rahman MA., 1997). Leather sector is a vital component of Bangladeshi economy in terms of foreign exchange earnings since it is the third highest foreign exchange earner. This industry is generally export based. More than 95% of leather and leather products of Bangladesh are marketed abroad, mostly in the form of wet-blue, crust leather, finished leather, leather garments, and footwear. More than 300 modern tannery units are now in operation in the country. With the exception of a few tanneries located in Chittagong and other places of the country, tanneries located mostly in the Hazaribagh area of Dhaka city. The Hazaribagh is in the 25 hectors of land located on the southwestern corner of the Dhaka city, beside the River Buriganga. It is the nerve center of the entire leather industry in Bangladesh. There are more than 200 tanneries in Hazaribagh area, and off them about 95% of tanneries in Bangladesh are engaged in chrome tanning process (Azom *et al.*, 2012). Daily discharged wastes from all the tanneries in Hazaribagh industrial zone are about 22000 cubic meters of liquid wastes, 150 tones of solid wastes during pick time and 75 tones of solid wastes during off pick time (Shams *et al.*, 2009).

Bangladesh earned US \$463 million export earnings from this sector in 2007- 2008 (BBS, 2011). A recent report revealed that leather and leather products are one of the major external trade sectors which contribute up to 1.39% share on the total export earnings (EPB, 2010). However, tanning industries pose a serious environmental hazard for Dhaka city, the hugely populated capital of Bangladesh. Hazaribagh (Dhaka) and Kalurghat (Chittagong) are the two main tanning industries concentrated

zones in Bangladesh. Hazaribagh is situated on the bank of the river Buriganga and Kalurghat is on the bank of Karnaphuly river. The extremely colored tannery wastewater is acidic in nature with high chromium content including other pollution loads such as chemical oxygen demand, biochemical oxygen demand, dissolved salts etc. (Sreeram *et al.*, 2003). The discharge of the effluent prior to treatment is creating great environmental threats. Water in the river Buriganga is poisonous and black in color. The physical look and smell of the area is frightening and intolerable. People of Hazaribagh area are somehow more or less dependent on the river water for their domestic purposes especially in the dry season when there is a scarcity of water. As a result, they are always facing food poisoning too. Several studies have been carried out about the pollution status of the Hazaribagh area (Shams *et al.*, 2008). This promising sector is suffering from a host of problems including environmental pollution problems related to effluents treatment technology, management and infusion of advanced technology and innovative and integrative marketing strategy. But Without any exceptions there is no effluents treatment plant (ETP) in leather tanning industries in the country. Moreover, the owners of tannery industries are not much so concerned about human health and environmental safety. As a result, the country is facing natural resources degradation and very serious pollution threats. The environmental problems such as air pollution, water (natural water bodies and ground water) pollution, disposal of solid waste have increased manifold. Due to lack of awareness as well as the absence of strong punitive actions, the practices of circulating waste and effluent into water bodies including ponds, canals and rivers still remains widespread (Ahmed and Reazuddin, 2000).

Tanneries are typically characterized as pollutants generated industries which produce wide varieties of high strength of toxic chemicals and large quantities of water are used in tanning process of which 90% of the water is discharged as effluents (Sinha *et al.*, 2002) which are always characterized by its strong color (reddish dull brown, black and any type of dark color), high pH, high EC, and high organic and inorganic chemicals level including salinity, Ca, organic load (chemical oxygen load or demand, biological oxygen demand), inorganic matter, dissolved and suspended solids, ammonia, nitrate, nitrite and total kjeldahl nitrogen (TKN) and specific high toxic pollutants (sulphide, chromium, cadmium, lead, chloride, sodium and other salt

residues) and heavy metals (Armenta *et al.*, 2001; Cooman *et al.*, 2003; Chaudry *et al.* 1997; Tariq *et al.*, 2005; Apte *et al.*, 2005; Leghouichi *et al.* 2009; Ajmal *et al.*, 1998a). About 20-30 L effluents are discharged per kilogram of skins /hides processed, and in the case of finishing units, this quantity is about 35-40 L kg⁻¹ skins /hides (Nariagu and Pacyna, 1988; Nandy *et al.*, 2001; Colak *et al.*, 2005; Tudunwada *et al.*, 2006. According to Imamul Huq (1998), various chemicals are used during the manufacturing steps such as soaking, liming, unhairing, deliming, bating, pickling, tanning and post tanning of hides and skins. The main chemicals are used include sodium sulphite and basic chromium sulphate including non-ionic wetting agents, bactericides, soda ash, CaO, ammonium sulphide, ammonium chloride and enzymes, others sodium bisulphate, sodium chlorite, NaCl, H₂SO₄, formic acid, different type of fat, sodium bicarbonate, vegetable tannins, syntans, resins, polyurethane, dyes, fat emulsions, pigments, binders and different type of water proofing agent (Tudunwada *et al.*, 2007). Azom *et al.*, 2012 reported that about 40 heavy metals and acids are used for processing raw hides. Various types of processes and finishing solvents and auxiliaries are used, as well (Imamul Huq 1998). It has been reported that only about 20% of the large number of chemicals used in the tanning process is absorbed by leather, the rest of the chemicals is released as wastes which are polluted the environment (see the more information Appendix 1.1 UNIDO, 2005). The huge amount of unutilized process chemicals are discharged from tannery industries directly or indirectly throughout the public sewer lines and these pollutants are finally linked to the rivers. During the chrome tanning process, the pickled pelt reacts with about 40-60% of the applied chromium salt and the rest of the salts (remaining 60-40%) are usually discharged in the final effluents (Heshem *et al.*, 2010, especially spent chrome tanning solutions) causing a serious threat to the environment (Van Groenestijn *et al.* 2002; Leghouichi *et al.* 2009, Owlad *et al.*, 2008 and Fathima N.N *et al.*, 2003).

The exposure to chromium, pentachlorophenol and other toxic pollutants increases the risk of dermatitis, ulcer nasal septum perforation and lung cancer (Carlos *et al.*, 2002, Barnhart *et al.*, 1997; Barceloux *et al.*, 1999, Bajza and Vrcek 2001; Kornhauser *et al.*, 2002 and Stoyanva *et al.*, 2004). The tannery effluents is being also contaminated with high levels of metals such as Cu, Zn, Mn, Ni and As which consumed as a result

causes serious health hazards (Salt *et al.*, 1995; Lakshmi *et al.*, 2010). The major chemical constituents of the wastes from the post tanning and finishing industry are different type of syntan fat liquor, different type of dyes, pigments, cationic fat, oils and binders etc. These chemicals mixed with water are discharged from the tanneries and polluted the ground water permanently and making it unfit for drinking, irrigation and general consumption. Above the discussion, pollution is defined in various ways. It is considered as the release of unwanted substances to the environment by man in quantities that damage either the health or the resource itself (Tripathi *et al.*, 2007). Environmental pollution caused by heavy metals is increasing along with the increase in the usage of chemicals in industry and agriculture. Such pollution is apparent in streams and lakes and in ground water which is replenished directly from surface water (Harriman *et al.*, 2001).

1.2. Leather industrial wastes and environmental hazards

Tannery Industries generate all categories of wastes including solid, liquids and gaseous, these are described below:

1.2.1. Solid wastes

The major solid wastes of tannery industries consist of dusted curing salt, wet trimmings, dry trimmings, wet shaving dusts, buffing dusts, etc. These wastes are generally separated at the source. During handling of raw skins, adhered dusted salt, which is contaminated with blood, hair, dirt and certain type of bacteria is removed and dumped. Trimmings are cuttings of edges of raw skins. Fleshing is the flesh material of the limed skins generated during fleshing operation. Chrome wet shaving dust is produced when skins are shaved for proper thickness (for buyer requirements) after chrome tanning. Except most dusted salt of the solid wastes, remaining chrome shaving dusts are sold in the local market as poultry feed manufacturers due to the protein content of the solid wastes in chrome shaving dust. The main problem with these wastes is their high Chromium content. The Chrome tanned waste contains Chromium in trivalent form but it was found that when the solid wastes are used in making poultry feed the chromium is converted to hexavalent form due to high temperature and pressure which is the most toxic and carcinogenic. The mixing of this

metal in poultry feed could produce serious human health problems. It is estimated that for a tannery producing on an average 10,000 kilograms of skins per day, a total of about 5,500 kg of solid waste would be produced per day (see more information Appendix 1.2).

1.2.2. Gaseous pollutants

Out of the three wastes (Solid, Gas, and Liquid), gaseous pollutants, i.e., air emissions are very low as compared to the standards mentioned in National Environmental Quality Standards (NEQS) (ECR, 1997) in Bangladesh. In tanneries, gaseous pollutants, i.e., air emissions are produced from the stacks of boilers and generators and during the processing of leather. Emissions from the stacks are well within the limits and pose no serious environmental impact. But hydrogen sulphide, sulfur-dioxide and ammonia gases which are emitted during different processes are a health hazard for the workers. Hydrogen sulphide gas is highly toxic and its smell like, rotten egg which causes headache, skin disease, nose irritation, respiration difficulties etc. Sulphur dioxide gas causes nausea, diarrhea, ear, nose and throat irritation etc. Air pollutant like sulphur dioxide “acid gases” may have adverse effects on human health, ecosystems such as forests and surface water.

1.2.3. Wastewater (effluent)

Water is used as the carrier for mentioned chemicals to render the cleaning of raw hides and skins. The water after completion of the process is drained out in the same quantity as it was used in the process. Normally water consumption of 50 liters /kg is recommended for tanneries but it is found that tanneries generally consume more water and in some cases it is found to be as high as 150 liter/kg. Samples of water were taken from different processes of leather manufacturing and were analyzed. The leather industrial wastewater or effluents come from leather industry which is a serious threat to the environment. Tanneries are disposing of their wastewater into drains, which finally find its way into natural water bodies. Therefore major environmental problems are linked with the wastewater of tanneries. Tannery wastewater is highly polluted in terms of Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Total suspended solids, Total dissolved solid, Total Nitrogen, Electrical conductivity, Sulphate, Chloride, and metal ions. The quantity of

these pollutants in the water is compared to the quantities mentioned in the NEQS (National Environmental Quality Standards) set by the government of Bangladesh. Considerable quantity of sludge is also found in the waste water. Wastewater of each tannery process consists of pollution of varying pH values. The process water, after completion of the process, is drained out as wastewater in the same quantity as it is added in the processes. The discharged wastewater containing unused process chemicals are disposed off without any treatment into the local environment. Tannery's wastewater is highly contaminated and the contamination observed is many times beyond the limits set by Environmental Quality Standards for all wastewater parameters and impact on environment are discussed mention the below. A comparison of average quantities of Pollutants (liquid) from Tanneries with NEQS in Bangladesh is shown in Appendix 1.3 (see more information). If the quantity of raw sheep & goat skin-finished materials is 12000 kg/day then volume of wastewater is 600 m³/day. Another think, the quantity of raw cow hides - finished materials is 55000 kg/day then volume of wastewater is 814m³/day and wet-blue sheep and goatskin-finished materials quantity would be 10000 kg/day, then wastewater volume is to be 110 m³/day.

1.2.3.1. Characteristics and environmental impact of leather industrial effluents

The common characteristics of wastewater/effluents which are important in assessing the potential for water pollution shows that it is high pH, high EC, highly polluted with Biochemical Oxygen Demand (BOD₅), Chemical Oxygen Demand (COD), suspended solids, settle-able solids, total Nitrogen, Sulphate and Chromium, Chloride, Lead etc. which values were given appendix 1.3. A considerable quantity of sludge is also present in the wastewater. Values of these parameters vary from tannery to tannery due to different processes and raw material utilization. Due to the high pollution level in wastewater; very severe environmental impacts are associated with its discharge into the environment without applying any measures. Untreated tannery effluent in surface water can bring about a rapid deterioration of their physical, chemical and biological qualities.

The effluent released from tannery industry was brown in color and had an offensive odor. The color of the effluent might be due to the presence of biodegradable and non

biodegradable high molecular weight organic compounds and high amount of chemicals used during the processing and the odors may be due to the processing of skins and hides by soaking and liming.

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 2005). The turbidity of the effluent might be due to the discharge of high concentrations of carbonate, bicarbonate, chloride, calcium, magnesium and sodium used in tanning industry (Chakrapani, 2005). The higher electrical conductivity value of the effluent indicates that the discharge of chemicals as cations and anions were higher in the waste water. The higher conductivity alters the chelating properties of water bodies and creates an imbalance of free metal availability for flora and fauna (Akan *et al.*, 2007). Venkatesh *et al.* (2008) recorded that the electrical conductivity in tannery effluent were much higher than the tolerance limits for industrial effluent discharged into land surface.

The pH of directly discharged tannery effluents is varied 2.6 to 13.5 (UNIDO-2000). Water with a low pH is corrosive to water-carrying systems and in unfavorable circumstances, can lead to the dissolution of heavy metals in the wastewater. The high pH in tannery wastewater is produced by lime because it is used in excess quantities and this causes scaling in sewers. On the other hand, the low pH of wastewater is caused by use of acids in different tannery processes. A large fluctuation in pH exerts stress on aquatic environment which may kill some sensitive species of plants and animals living there. The NEQS recommends a value of pH in the range of 6 to 9.

Total suspended solids, apart from being societal nuisance, have their main effect when they settle. The layer so formed on the bottom of the watercourse, covers the natural fauna on which aquatic life depends. This can lead to a localized depletion of oxygen supplies in the bottom waters. A further secondary effect is the reduced light penetration and consequent reduction in photosynthesis due to the increased turbidity of water. Tanneries discharge wastewater containing 820 –7000 mg/L of suspended solids. The Bangladesh sewage contains 500-900 mg/L suspended solids, whereas the NEQS-2000 recommends a value of 500 mg/L. The effluent showed a higher level of total dissolved solids (TDS) (550-156000 mg/L). The value was much greater than the

tolerance limits (2100 mg/L) prescribed by Inland Surface Water in Bangladesh Standards (ISW-BDS) and Indian Standard Institute (ISI- 2000). Total dissolved solids are mainly due to carbonates, bicarbonates, chlorides, sulphates, phosphates, nitrates, nitrogen, calcium, sodium, potassium and iron (Kannan *et al.*, 2005). In the liming section of tanning process, protein, hair, skin and emulsified fats are removed from the hides, which are released in the effluent and therefore increase the total dissolved solids (Bhalli and Khan *et al.*, 2006). Large quantities of proteins and their degraded products form the largest single constituent group in the effluent. They effect the environment which can be expressed by two composite parameters; Biochemical Oxygen Demand (BOD₅) and Chemical Oxygen demand (COD). BOD₅ is a measure of the oxygen consuming capacity of water containing organic matter. Organic matter by itself does not cause direct harm to aquatic environment, but it exerts an indirect effect there by depressing the dissolved oxygen content of the water. The oxygen content is an essential water quality parameter and its reduction causes stress on the ecosystem. As an extreme example, a total lack of dissolved oxygen as a result of high BOD₅ can kill all natural life in an effected area. Tanneries discharged wastewater containing the value of BOD₅ in the range of 714-14827 mg/L. whereas the NEQS recommends the value of BOD₅ is 250 mg/L. Therefore, tannery wastewater is carrying about 3 - 60 times higher than the recommended NEQS limit. The Chemical Oxygen Demand (COD) is a measure of oxygen equivalent to that portion of the organic matter in a sample which is susceptible to oxidation by a strong chemical oxidant. It is an important, rapidly measured parameter for stream and industrial waste studies and for control of waste water treatment plants. Along with the organic compounds immediately available to the stream organism, it also determines biological compounds that are not a part of immediate biochemical load on the oxygen assets of the receiving water. With certain wastes containing toxic substances, this test or total organic carbon (TOC) determination may be the only method for determining the organic load. Where wastes contain only readily available organic bacterial food and no toxic matter, the results can be used to approximate the ultimate carbonaceous BOD₅ values. The major pollutants of the post-tanning are Chrome, salts, dyestuff residues, fat liquoring agents, syntans and other organic matter, typically measured by COD (Bajza and Vrcek, 2001; UNIDO, 2000). It is the amount of oxygen required for the oxidation of inorganic matter using a strong

chemical oxidant. COD is tested to determine the degree of pollution in the effluent samples (Bhalli and Khan, *et al.*, 2006; Awan A. M. 2004). Some researchers also reported that COD values in the effluent were found to be 12 times higher than that of the tolerance limits. Some reports show that composite wastewater of tanneries carries a COD value in a range of 2000-43000 mg/L. whereas according to the NEQS in BDS, a value of 450 mg/l has been recommended for COD. So the COD value of untreated composite tannery wastewater is carrying about 5 to 95 times higher than the standards prescribed limits (UNEP, 1999; ETPI, 1998; FAO, 1996). Cooman *et al.*, 2002)

Tanneries generate sulfate-rich wastewaters due to its adding the excess sulfuric acid in pickling and sulfide in the unhairing process, which is oxidized to sulfate before its discharge into sewer. Due to sulphide discharged from the unhairing process, hydrogen sulphide is released at a pH value lower than 8.5. This gas has an unpleasant smell even in trace quantities and is highly toxic to many forms of life. In higher concentrations, fish mortality may occur at a sulphide concentration of 10 mg/L. Sewage contains sulphide in the range of 15-20 mg/L and composite tannery effluents contain 290 mg/L, whereas, the NEQS recommends a value of 1.0 mg/L for sulphide discharged. Sodium chloride used in the tannery industries has no effect if the effluents discharged into estuaries or the sea, but can certainly harm aquatic life in fresh water bodies when its (salts) concentration in a stream or lake becomes very high. There is no economically viable way of removing salt from the effluent. One the other dissolved anion, sulphate is one of the major occurring in natural water. Sulphate being a stable, highly oxidized, soluble form of sulphur, is generally present in natural surface and ground waters. The normal levels of sulphate are more than adequate to meet plants need. When water is over loaded with organic waste to point that oxygen is removed then sulphate as an electron acceptor is often used for breakdown of organic matter to produce H_2S and produce rotten egg smell (Welch, 1980). On the other hand the high concentrations of chloride in water damages plants. And pesticides kill fish and have human health effects if found for example in drinking water. The Chloride content of tanneries' composite wastewater ranges from 1260 to 6000 mg/L and the sulphate content varies from 800 to 6480 mg/L, whereas the NEQS recommends value of 600 and 1000 mg/L, for Cl^- and SO_4^{2-} respectively.

Nitrogen is contained in several different components of tannery effluents as a part of their chemical structure. The most common chemicals are ammonia (from deliming materials) and the protein-aqueous materials (from liming/ unhairing operation). These sources of nitrogen pose two direct problems. Firstly, the plants require nitrogen in order to grow but the high levels released by substances containing nitrogen over stimulate the growth. Water –based plants and algae grow too rapidly, whereupon water ways become clogged and flows are impaired. As the plants die, a disproportionately high amount of organic matter has to be broken-down. If the load outstrips the natural supply of oxygen from the river, plants, fish and aerobic bacteria die and ultimately anaerobic conditions develop; and secondly, the nitrogen released through protein break-down and de-liming process is in the form of ammonia. Bacteria can convert the latter over several stages into water and nitrogen gas which is ultimately released into the atmosphere. Both of these break-down products are non-toxic, yet large volumes of oxygen are needed in the process. If oxygen demand is greater than the level supplied naturally by the watercourse, toxic and anaerobic conditions may rapidly develop.

Two types of tanning systems are mainly used in leather industry. One, vegetable tanning which is not chromium based and chrome tanning, the most common type of tanning processes where large amount of 33% basic chrome powder and liquor are used (Sreeram *et al.*, 2003; Islam *et al.*, 2011). In the second method, the collagen parts of hides form complexes with chromium and changes to leather. Chromium salts (particularly chromium sulphate) are the most widely used tanning substances and hides tanned with chromium salts exhibit a good mechanical resistance.

Trivalent chromium is released during the chrome tanning process. It is much less toxic than hexavalent chromium. Chromium (Cr) is an essential trace element for all living organisms. It is necessary for fat and glucose metabolism and proper functioning of insulin (Thacker *et al.*, 2007). Hexavalent chromium is easily soluble and 100-fold more toxic than trivalent one. Hexavalent chromium it has been recognized as one of the most dangerous environmental pollutants due to its ability to cause mutations, irritation, corrosion of the skin and respiratory tract to most microorganisms; it also causes lung carcinoma in humans (Liu *et al.*, 2005; Bhinde *et al.*, 1996). At present, tanneries are discharging chromium (133 mg/L) in composite

wastewater and in sludge (3 to 17.5 gm/kg). Chrome tanning process shares, about 2 % of the total wastewater, which contains 1865- 7000 mg/L of chromium (Cr) whereas the National Environmental Quality Standard (NEQS) in BDS recommends only 2.0 mg/L. In humans, exposure to hexavalent chromium salts for periods of 2 to 26 years has been implicated as a cause of cancer of the digestive tract. High levels of chromium in soil have been correlated with regional incidences of stomach cancer. Based on exposure to chromium via inhalation, the International Agency for Research on Cancer has categorized "chromium and certain chromium compounds" in Group 1: sufficient evidence for carcinogenicity in humans and animals. These metals reduce the growth of plants, decreased net photosynthesis and the biomass of plants (Tripathi, 1996; Chain *et al.*, 1987 and Chadderton, 2003). The main release to waste water originates from wet processing in the beam house, the tanyard, and the post-tanning operations. The main release to air is due to the dry-finishing processes, although gaseous emissions may also arise in all other parts of the tannery. The main sources of solid wastes originate from fleshing, splitting, shaving and trimming (Buljan *et al.*, 1994). Poultry feed manufacturers use materials of tanneries as it contains and use it due to the high protein content of the solid wastes e.g. fleshing, raw trimming, chrome shaving, buffing dusts etc. The wastes contain Cr in the range of 14-26 gm/kg. Chromium in the waste is in trivalent form which is less toxic than hexavalent form. Rafiquallah *et al.* (2008) showed that poultry feed made using solid wastes (chrome shaving dust) which contain hexvalent form of chromium also in our country. It seems that during the feed preparation, transition of trivalent chromium to hexvalent chromium takes place, which poses a serious threat to human health (Javed M., 2005).

Other pollutants concerned with tannery industries waste include Azodyes, Cadmium compounds, Cobalt, Copper, Antimony, Barium, Lead, Selenium, Mercury, Zinc, Arsenic, Polychlorinated Biphenyl's (PCB), Nickel, formaldehyde resins and Pesticides residues. Because tannery wastewater contains a mixture of pollutants including different heavy metals and chlorinated phenols as indicated earlier, it is vital to dissect the toxic nature of such wastewater both to understand its environmental impacts and identify potential remediation strategies. Furthermore, there are strict regulations imposed on the environmental control of pollutants such as heavy metals which might be discharged from tanneries and whose discharge may be subjected to

statutory limits include aluminum, cadmium, arsenic and lead. Depending on the chemical species, these metals have different toxicities that are also affected by the presence of inorganic and organic matter, complex agents and pH of the water. Aluminum, in particular, is known to inhibit the growth of green of algae; crustaceans are sensitive to low concentration. The other chemicals found in tannery effluents are discussed below:

Cadmium is a non-essential trace element that enters the environment via anthropogenic activities such as industrial effluent, sewage-sludge, fertilizers and pesticides (DWAF, 1996). Cadmium adsorbs strongly to sediments and organic matter (Sanders *et al.*, 1999). Cadmium has a range of negative physiological effects on organism, such as decreased growth rates and negative effects on embryonic development (Newman and McIntosh, 1991). Cadmium, sometimes used in yellow pigments is considered to be highly toxic. It is accumulative and has a chronic effect on a wide range of organisms. If present in drinking water, it can induce brittleness in bones (Brierley *et al.*, 1986 and 1993).

Lead is a non-essential trace element. The toxicity of lead is dependent on the life stage of the organism and the presence of organic material and decreases in water pH can increase the bio-availability of lead in the system (Hellowell, 1986). Lead in aquatic environment is risky to life since aquatic organisms used as food are particularly very sensitive to lead and often retain about a percent of ingested lead which could be taken up by man through the food chain (Ewers and Schlipkoter, 1991). Lead can cause damage to the nervous system and the kidneys and it is suspected to be carcinogenic (Radojevic and Bashkin, 1999). Children exposed to high lead levels are particularly at risk. Akhan *et al.* (2009) reported that the levels of lead in the analyzed waste water and sediment samples showed that the limiting values by (United Standard Environmental Protection Agency) USEPA of 0.01 mg/L and 40 µg/g respectively were exceeded, indicating contamination of River water and may pose a hazard to the aquatic biota.

Arsenic is a highly toxic metalloid element (Rodrigues *et al.*, 2003; Pizzaro *et al.*, 2003). It is widely distributed as a trace element in rocks and soils and is mainly mobilised by microbial activities (Garcia-Sanchez and Alvarez-Ayuso, 2003). The

high concentration of nickel of untreated leather industrial effluents were exceeded than standard limits in ISW-BDS (Appendix 1.2), then that industrial zone will be extremely polluted. However, nickel limiting levels were exceeded and River water could be said to be contaminated by nickel. Long term exposure can cause decreased body weight, heart and liver damage and skin irritation.

Copper is a common environmental metal and is essential in cellular metabolism but at high concentrations it can be highly toxic to fish (Grosell *et al.*, 1997). Copper is an essential substance to human life, however, in high concentrations, it can cause anemia, liver and kidney damage, stomach and intestinal irritation (Turnland, 1988). Copper is generally re-mobilized with acid-base ion exchange or oxidation mechanism (Gomez *et al.*, 2000). Long term exposure of copper may lead to liver and kidney damage (EPA, 1999).

Zinc is equally an essential element in the human diet. Zn deficiency in the diet may be more detrimental to human health than too much of it in the diet (ATSDR, 1994). But in aquatic ecosystem, Zn is highly toxic to some aquatic organisms. Although above mentioned Zn is not a human carcinogenic, ingestion of large doses can cause death (ATSDR, 1994). Zinc is also an essential micronutrient for all organisms and forms the active site for various metallo-enzymes (DWAF, 1996). Excessive intake of Zn may lead to vomiting, dehydration, abdominal pain, nausea, lethargy and dizziness (ATSDR, 1994).

1.3. Necessity of effluent treatment

To have long and better life style and meet the demand of increasing population, urbanization, industrialization, traffic and construction activities are increasing very rapidly day by day. Industrialization in Bangladesh is going on without any proper planning and management. As a result, the country is facing severe problems in natural resources management and pollution control. The Hazaribagh area of Dhaka city is one of the worst affected areas of Bangladesh. Leather tanning industry is notorious for its heavy pollution through effluents containing heavy metals, various acids, salts, trace gases which have effects on: a. Human health b. Surface water c. Ground water d. Land e. the atmospheric condition. So, it is imperative to have a

proper planning and management to reduce tannery pollutants of the Hazaribagh leather industrial zone. Azom *et al.* (2012) reported that the European Commission is considering about banning the leather imported from Hazaribagh, Bangladesh as the tanneries discharge extremely hazardous chemicals into the open environment. So, it is very important to know the pollution level for the protection of environment and natural resources and to develop sustainable treatment processes.

1.4. Present status of tannery effluents in Bangladesh

Due to high correlation between chrome tanning and its environmental impact, leather industrial effluents are urgently needed for treatment. Unfortunately, the effluents of all leather industries in Hazaribagh leather industrial zone are discharged without any treatment into open drains and surroundings lands and ultimately making its way into the Buriganga River. The River Buriganga, which runs part of Dhaka City, is at present one of the most polluted rivers in Bangladesh. The river is seriously polluted by discharge of leather industrial effluents into river water, indiscriminate throwing of household, clinical, pathological and commercial wastes, and discharge of fuel and human excreta. In fact, the river has become a dumping ground of all kinds of solid, liquid and chemical waste of bank-side population. These activities on the River Buriganga have caused narrowing of the river and disruption of its normal flow of water. The water of the river has become so polluted that its aquatic life has almost been extinguished (DHV, 1998). A survey in 1999 revealed that the water of Buriganga had been completely polluted. The report concluded that the water of these rivers posed a serious threat to public life and was unfit for human use, which was stated in the Appendix 1.4. People, living near the rivers, use the water because they are unaware of the health risks and also having no other alternative. This causes incidents of water borne and skins diseases (DPCD, 2003). Leather industrial effluents and solid waste generated at different steps of leather processing trekking through the low-lying area of Hazaribagh contaminated by chromium, the old wounds take a longer time to heal. Long term chromium contamination may causes cancer. Laboratory tests carried out by Department of Environment (DoE, 2001) show that chromium is a carcinogenic agent, has seeped into the aquifer at some place of Hazaribagh flow into the Buriganga River. Liquid waste is contaminating the waste of the Buriganga River on the surface as well as the ground water resource base. During

the lean season, the Buriganga River turns deadly for fish and other aquatic organisms. When solid waste and effluents run into the river, the Biological Oxygen Demand (BOD) in the water rises, creating oxygen is calamitous for the sub aqueous life. Among others, effluents of tannery factories lower the dissolved oxygen (DO) content of the river Buriganga water below the critical level of 4 ppm. The concerns over water quality relate not just to water itself, but also to danger of diffusion of toxic substances into other ecosystems. The aquatic environment for living organisms can be affected and bioaccumulation of harmful substances in the water dependent food chain can occur (DoE, 1997). The quality of surface water is seriously deteriorated in the dry season because of the toxic chemicals intrusions in this region and pollution problems in industrial areas are significant. In particular, quality scenario along the *surrounding rivers* of Dhaka City can no longer be considered as a source of water supply for human consumption (DoE, 2001). Industrial effluents contain chromium, cadmium, lead, sulphides, ammonia, chlorides and other salts, in addition to a large quantity of organic compounds and these toxic compounds discharge into air, water and soil get in to food chain from the environment. When toxic substances accumulate in the environment and in food chains, they can profoundly disrupt biological processes (Gupta, 1998). Effluents pollute the natural eco-system of river, which actually act as a sink. They thereby create serious environmental hazards, endanger human health and cause problems to aquatic lives (Ahmed and Reazuddin, 2000).

The effects of water pollution are not only devastating to people but also to animals, birds and fish. Furthermore, contamination of soil and ground water may be caused through accidental releases, spillages and leakages of certain agents as well as by the treatment of effluents and wastes. A further aspect to be aware of in all tanneries is the potential hazard to human health and the environment from handling, storing, transporting and packing of chemicals. Most of the wastes and effluents are subjected to natural decomposition in the environment, causing serious pollution problems affecting soil, water, air and human life. According to Imamul Huq (1998), about 90% of the finished trimming wastes are used by local shoemakers. The government of Bangladesh made an attempt to relocate the tanneries from the present area to a proposed site outside of Dhaka (Saver, Baliapore) and offered a compensation of US

\$1.4 million for this purpose. An Asian Development Bank study stated that US \$18.4 million would be needed to develop an area with waste treatment facilities; but due to lack of funding, the relocation scheme has been abandoned. Instead of, the Government has decided to establish a common effluent treatment plant with the assistance of the United Nations Industrial Development Organization (UNIDO) for the 200 tanneries in Hazaribagh. But such a huge treatment unit would create several problems and not be a realistic approach for tannery effluents management. Hence, an individual treatment plant for each and every tannery industry would have to be installed to save from further environmental degradation of the area.

1.5. Physical and chemical treatment approaches

A number of research work carried out around the world regarding the treatment of tannery effluents using different technology. For example, Dhungana and Yadav (2009), worked on adsorption of hexavalent chromium from tannery effluents using formaldehyde treated sawdust and charcoal of sugarcane bagasses. Again some of the authors, studied on the treatment tannery waste water using filtration, ultra-filtration and reverse osmosis process with a view to reuse it again (Krishnamoorthi *et al.*, 2009). Several studies had been carried out for the treatment of industrial effluents through coagulation and flocculation processes (Shouli *et al.*, 1992; Stephenson and Duff, 1996). Wastewater treatment methods are broadly classified into physical, chemical and biological processes (Metcalf and Eddy, 1979; Matsumoto *et al.*, 2003).

Various treatment for tannery wastewater like physico-chemical methods such as sedimentation (Song *et al.*, 2000), Electro floatation (Murugananthan *et al.*, 2004), Filtration (Cassano *et al.*, 1999; Tiglyene *et al.*, 2008) and Membrane filtration (Justina *et al.*, 2009), Precipitation (Kabdasli *et al.*, 2003; Esmaeili *et al.*, 2005) and coagulation (Jing-Wei *et al.*, 2007; Haydar *et al.*, 2009; Zhi *et al.*, 2009; Espinoza-Quinones *et al.*, 2009; Sengil *et al.*, 2009), Adsorption (Santosa *et al.*, 2008; Covarrubias *et al.*, 2008), Ion exchange (Tiravanti *et al.*, 1997; Kabir and Ogbeide, 2008), Sequencing batch reactor treatment (Andualem Mekonnen Hiruy., 2000) and Biological methods such as (Martinez *et al.*, 2003; Farabegoli *et al.*, 2004; Murat *et al.*, 2006; Lefebvre *et al.*, 2006; Banu and Kaliappan, 2007; Munz *et al.*, 2008; Zupancic and Jemec, 2010). Schrank *et al.*, (2004) has suggested that many organic

compounds contained in wastewater are resistant to conventional chemical and/or biological treatment. The degradation of inhibitor substance such as tannin during the anaerobic digestion was investigated by Banu and Kaliappan (2007). The objective here is the removal of settle able organic and inorganic solids by sedimentation, and the removal of materials that will float (scum) by skimming. Approximately 25-50% of the incoming biochemical oxygen demand (BOD_5), 50-70% of total suspended solids (TSS), and 65% of the oil and grease are removed during primary treatment. The effluent and sludge from primary sedimentation are referred to as primary effluent and sludge. This treatment system comprises of mechanical screening, pH equalization (collection of effluent of different processes in a common tank), coagulation and flocculation and sedimentation.

Coagulation or chemical precipitation has been known for treatment of wastewater, since the previous century in England, where lime was used as coagulant alone or in combination with calcium chloride or magnesium (Genovese and Gonzale, 1998). On the other hand, a few studies focused on bio-remediation of tannery effluents (Haydar *et al.*, 2007; Nourisepehr *et al.*, 2005). Sumathi and Umagowrie, (2012) studied on the treatment of leather industrial effluents using combined technology, Shukla *et al.* (2011) performed the treatment of tannery effluents using only chemical coagulants that produce sludge where disposal becomes difficult and contaminates the dumpsite. Polyelectrolyte precipitation was not yield satisfactory results for the treatment of leather tanning industrial wastewaters (Kabdasli *et al.*, 2003). Generally, tannery effluents contain mostly sodium sulphide and (or) sodium hydrosulphide (EPA, 1990; Valeika *et al.*, 2006) which has significantly contributing for environmental pollution. The presence of sulphide in wastewaters may dramatically interfere with microbial activities and consequently disturb the function of the system (Mesdaghinia *et al.*, 1991). Biological process can be carried out only when concentration of sulphides is not exceeding 50 mg/L. Aerobic treatment was not effective method for treatment of tannery effluent (Sekaran *et al.*, 1996; Ganesh and Ramanujam, 2009). The researchers have reported that anaerobic treatment of tannery wastewater in high rate close type reactors leaves sulfides in the range 31-795 mg/L, COD 395-1886 mg/L, BOD 65-450 mg/L and total organic carbon (TOC) 65-605 mg/L. So the high value of toxic chemicals present in the treated wastewater may not be suitable for aerobic

biological treatment. Shanker *et al.* (2005) and Yoochatchaval *et al.* (2008) has reported that a cost effective method was needed to remove the residual organics in biological treated wastewater as it received public attention from the neighborhood of discharge.

Removal of course, particulates, flesh and hair is achieved by means of perforated screens. The contribution of screening in reducing the BOD load is high, but the primary purpose is to prevent the blockage of pumps and sewers required for further treatment of the tannery effluent. The wide fluctuation in the effluent's pH value as a result of different requirements of the tannery processes can be reduced by means of an equalization tank. Coagulation and flocculation processes are applied to the tannery effluents in order to remove the suspended solid of the tannery effluents. This is carried out by the addition of coagulating and flocculating to the wastewater.

1.6. Microbial approach

Microbial approach is the most effective management tool to manage the polluted environment and recover contaminated environment (Ahemad *et al.*, 2011). In the other words, microbial approach is an alternative way that offers the possibility to destroy or render harmless various contaminants including heavy metal ions using natural biological activity. It was used relatively low-cost techniques which generally, have a high public acceptance and can often be carried out on site (Vidali *et al.*, 2001). Compared to other methods (physical and chemical), microbial approach is a more promising and less expensive, high efficiency and minimum sludge produce way for cleaning up the contaminated environment (Ahemad *et al.*, 2011; Kamaludeen *et al.*, 2003). Microbial approach is used to biological agents, mainly microorganisms, e.g. yeast, fungi or bacteria to clean up the contaminated environment (Strong *et al.*, 2008; Kumar *et al.*, 2011). In secondary treatment, biological processes are used to remove most of the organic matter from the wastewater. This is achieved by using micro-organisms (bacteria) to convert the organic matter into different gases and into cell tissues. Cell tissues have a specific gravity slightly greater than that of the water, the resulting tissue is removed from the treated liquid by gravity settling. For secondary or biological treatment of tannery effluent, the most widely used processes are aerobic. However, anaerobic process is

also used for this purpose. The most essential parameters required for microbial approaches are the nature of pollutants, sludge structure, temperature, pH, moisture content, hydrogeology, the nutritional state, redox-potential, and microbial diversity of the site (Dua *et al.*, 2002; Zaidi *et al.*, 2009). Aksu *et al.* (2005) also reported that temperature does not enough influence the microbial processes in the range of 20-35°C. However, pH seems to be the most important influencing factors in the microbial processes. It affects the solution chemistry of the heavy metal ions, the activity of the functional groups in the biomass and the competition of the metallic ions. In microbial processes, microorganisms are used the contaminants as nutrient or energy sources (Tang *et al.*, 2006; Ahmed *et al.*, 2010). Microbial activity through microbe is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities (Ma *et al.*, 2007; Baldwin *et al.*, 2008). Many researchers reported that some metal ions such as, Zn, Cu, Ni and Cr are essential or beneficial micronutrients for microorganisms while others (e.g., Cd, Hg and Pb) have no known biological and/or physiological functions (Olson *et al.*, 2001; Gadd GM., 1992 and Giller *et al.*, 1998 Gray *et al.*, 2005). They also stated that the higher concentration of these metal ions have great effects on the microbial communities in soils in several ways- (i) it may lead to a reduction of total microbial biomass (ii) it decreases numbers of specific populations or (iii) it may change microbial community structure. Generally, the higher concentration of the metal ions (Cd, Hg and Pb) above standard limit has deleterious impact on the functional activities of microbial communities in soils or sludge. Some literature reports showed that microorganisms exposed to the higher concentrations of toxic metal ions may develop resistance against the elevated levels of these metals (Habi *et al.*, 2009). Microorganisms are also inhabiting in metal ions polluted soils have evolved various strategies to resist themselves against metal stress (Baquero *et al.*, 1998). Such metal ions resistant microorganisms can be used as successful bioremediation agents (Khan *et al.*, 2009). Munees, (2012) also studied that at high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activates or organisms can develop resistance to the elevated levels of metals. Generally tolerance may be defined as the ability to cope with pollutant – toxicity by means of intrinsic properties of the microorganism.

In contrast, resistance is the ability of microbes to survive in higher concentrations of toxic substances by detoxification mechanisms, activated in direct response to the presence of the same pollutant (Ahmed *et al.*, 2009). Toxic heavy metals therefore, need to be either completely removed from the contaminated environment, transformed or to be immobilized, producing much less or non-toxic species. However, in order to survive and proliferate in metal contaminated environment, tolerance has to be present both in microbes and their associative hosts. For survival under metal-stressed environment, bacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions (Nies *et al.*, 1999). These mechanisms include (1) exclusion-the metal ions are kept away from the target sites (2) extrusion-the metals are pushed out of the cell through chromosomal/plasmid mediated events (3) accommodation-metals form complex with the metal binding proteins (e.g. metallothienins, a low molecular weight proteins) (Kao *et al.*, 2006; Umrانيا *et al.*, 2006) or other cell components (4) bio-transformation-toxic metal is reduced to less toxic forms and (5) methylation and demethylation. One or more of these defense mechanisms allows these microorganisms to function metabolically in environment polluted by metals. These mechanisms could be constitutive or inducible. The bacterial resistance mechanisms are encoded generally on plasmids and transposons, and it is probably by gene transfer or spontaneous mutation that bacteria acquire their resistance to heavy metals. For example, in Gram-negative bacteria (e.g. *Ralstonia eutropha*), the *czc* system is responsible for the resistance to cadmium, zinc and cobalt.

Kumar *et al.* (2011) stated that heavy metal ions are difficult to be removed from the contaminated environment. These metals cannot be degraded biologically and are the ultimately indestructible, though the speciation and bioavailability of metals may change with variation in the environmental factors (Rani *et al.*, 2009). Many researchers have already identified a number of potential bacterial species have capability accumulating of heavy metal ions from aqueous environment. Brierley *et al.* (1986) reported that *Bacillus* species have a high potential of metal sequestration which are used in commercial bio-sorbent preparation. Seki *et al.* (2005) also reported that bacterial species could be recovered heavy metal ions from the aqueous

environment. The bio-sorption characteristics of Cd and Pb were determined with purple non-sulphur bacteria, *Rhodobacter sphaeroides*, and hydrogen bacteria, *Alcaligenes eutrophus* H16. Ilhan *et al.* (2004) stated that *Staphylococcus saprophyticus* could remove Cr, Pb and Cu ions from the industrial wastewater at an optimum pH values for Cr, Pb and Cu biosorption were found to be 2, 4.5 and 3.5, respectively. Ozdemir *et al.* (2004) also stated that Gram negative bacterium species, *Pantoea TEM 18* which was isolated from wastewater of a petrochemical industry exhibited the greatest Cu tolerance. Gram negative bacterial cell walls are thinner than the Gram positive ones and are not also heavily cross-linked. They have an outer layer of lipo-polysaccharide (LPC), phospholipids and proteins. Gourdon *et al.* (1990) studied compared the Cd ion biosorption capacities of Gram positive and Gram negative bacteria. Glycoproteins present on the outer side of Gram positive bacterial cell walls were suggested to have more potential binding sites for Cd ions than the phospholipids and LPS and hence are responsible for the observed difference in capacity. Rabbani *et al.* (2005) stated that 17 isolated bacterial strains from Ramser warm spring, Iran could be biosorbed Cr (III). A new strain of gram positive *coccobacilli* bacteria (*NRC-BT-2*) was found to be highly capable for the biosorption of Cr (III). In the batch experiments with various initial concentrations of Cr ions to obtain the sorption capacity and isotherms, the biosorption of Cr (III) and Cr (VI) onto cell surface of *Pseudomonas aeruginosa* was investigated. Fundamentally, biosorption of metal ions by bacterial cells is based on non-enzymatic processes such as, adsorption. Adsorption is characterized by the non-specific binding of metal ions to extracellular/ cell surface associated polysaccharides and proteins (Rani *et al.*, 2009; Mullan *et al.*, 1989). In a nut shell, biosorption is defined as an attribute of the inactive or dead microbial biomass to bind the concentrate metal ions even from highly dilute solutions (Vasudevan *et al.*, 2011). The metal uptake by the microbial biosorbent may be an active or passive process or exhibit both active and passive processes depending upon the microbial species. Moreover, passive uptake is a rapid and reversible process and is independent of cellular metabolisms, physical conditions such as pH and ionic strength. However, the passive process is relatively nonspecific with respect to the metal species. Conversely, the active process is comparatively slow method and depends on the cellular metabolism. In this process, metal ions form complexes with specific proteins like metallo-thionins. However, it is affected by

metabolic inhibitors, uncouplers and temperature. Microorganisms can interact with metals via many mechanisms [Fig. 1.1], some of which may be used as the basis of potential bioremediation strategies.

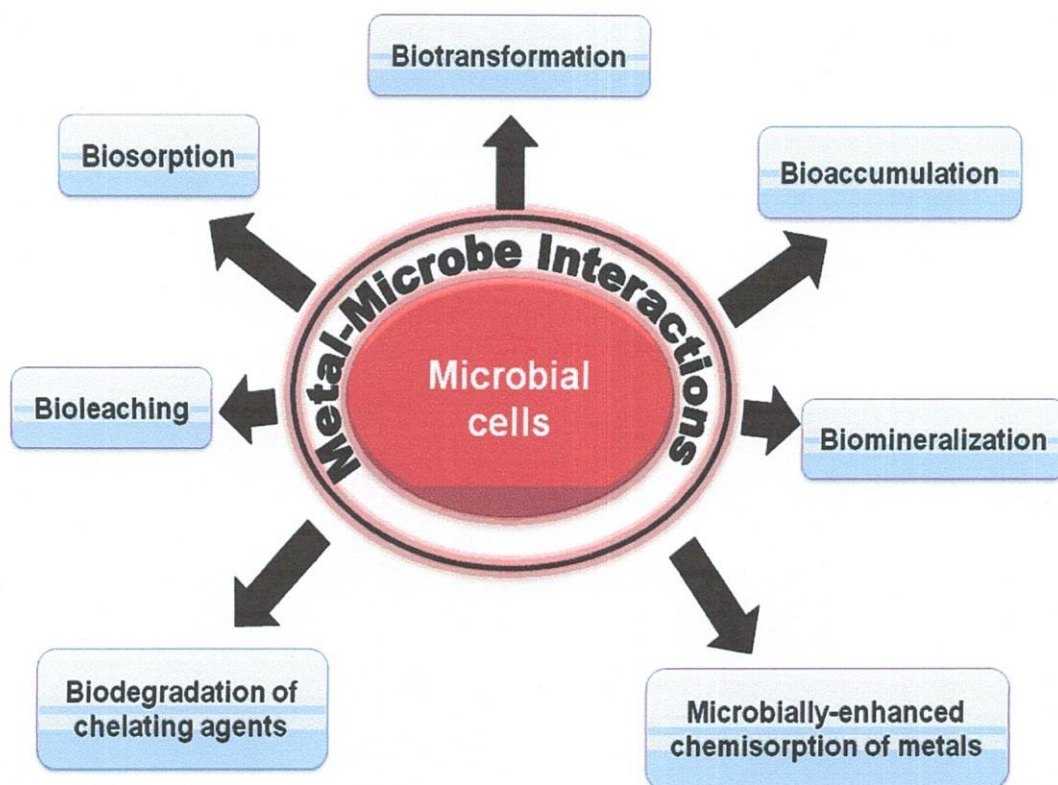


Figure 1.1: Metal-microbe interactions affecting bioremediation (source Munees, 2012)

The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to innocuous products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

Microorganisms play an important role to degrade the toxic pollutants. The vast majority of natural, synthetic inorganic and organic compounds are metabolized by microorganisms (Gibson, 1984). It is imperative that mechanisms exist by which the elements trapped in organic materials, either naturally occurring or man-made, are returned to the global elemental cycle. The role of microorganisms in this process is paramount and when one considers the large number of chemicals, which must

potentially mineralized, it is not surprising that microorganisms are among the most metabolically diverse group on earth.

One reason that such metabolic potency has arisen is the plasmid-borne nature of many degradative genes, which allows the evolution of novel catabolic enzymes. The genes that specify the enzymes responsible for degradation have frequently been shown to be borne by plasmids (often termed as catabolic or degradative plasmids). Interest has developed in recent years the ability of microorganisms to degrade and detoxify the large amount of toxic pollutants, which are introduced in to the environment through industrial activities of man. One of the most important aspects concerning the environmental impact of xenobiotics is biological toxicity, which can be established by following the fate of the particular xenobiotics. Before studying the pathway(s), which the microorganisms used to degrade toxic pollutants, it is necessary to understand how these organisms have developed this ability. It is possible that during the evolutionary process microorganisms have been exposed to a wide variety of synthetic organic and inorganic compounds, which have chemical structures analogous to naturally occurring compounds. Thus, the microorganisms have genetically modified, or acquired *de novo* from other microorganisms, genes coding for enzyme systems capable of degrading them (Carniglia, 1981).

Plasmids seem to play a major role in the adaptation of bacteria to xenobiotic and in the acquisition of new genetic traits due to pollution. A particularly important aspect is the occurrence of some broad host range plasmids specialized in the degradation of synthetic chemicals. Modern ecological biotechnology attempts to solve the problems of pollution by screening for and molecularly breeding microbial strains that are capable of degrading recalcitrant. Research in this direction is in good shape. This investigation aimed to apply microbial genetic technique to induce recombinants from bacteria to be testing for maximal accumulation of heavy metals from tannery effluents to improve the quality of irrigated water in the industrial regions. This enhancement of bio-sorption which would be resulting in a decrease of environmental loading pollutants such as contamination of groundwater and also receiving surface waters would be less threatened.

1.7. Status of tannery effluent treatment in Bangladesh

In developing countries like Bangladesh, many industrial units are operating in a small and medium scale. The leather industrial units can generate a considerable pollution load by discharging untreated effluents directly into the environment due to the poor enforcement of law. According to a recent estimate, about 60,000 tons of raw hides and skins are processed in these tanneries every year, which release nearly 95,000 L of untreated effluents into the open environment daily (Rusal *et al.*, 2006). There is an increasing society concern with regard to the importance of the preservation of our environment and the corresponding legislation which regulates effluents discharges. So any contaminating substance which, though having been processed, is not of free disposition, represents a “mortgage” for the industry. There are considerable dissimilarities in the concentration range of pollutants in tannery wastewaters given by different authors (Ates *et al.*, 1997; Balasubramian *et al.*, 1999 and 2000; Bartkiewicz, 2000; Dhungana and Yadav, 2009; Krishnamoorthi *et al.*, 2009; Nourisepehr *et al.*, 2005), but all of the authors observed that the tannery industry causes horrendous environmental pollution and high environmental impact of tannery effluents makes its treatment an essential fact, mainly due to its volume, nature and concentration of pollutants such as tanning agents (chromium and tannin), color, organic matter and others (Mohanta *et al.*, 2010). So leather industrial effluents contained pollutants remediation is of great need, since they damage the normal functioning of the ecosystem in environment. To reduce the toxicity of soil, water, air and the entire atmosphere as a whole caused due to the discharge of inorganic, organic and heavy metal ions and contaminants from leather industries, various treatment procedures are widely used in developed country. In Bangladesh, most of the work regarding tannery effluents is related to their characterization and impact assessment (Das *et al.*, 2006; Vasudevan *et al.*, 2011; Mohanta *et al.*, 2010; SEHD, 2002 and Azom *et al.*, 2012).

1.8. Lack of treatment facilities, justify the work and research gap

In Bangladesh, most of the works on tannery effluents have done to characterization and impact assessment (Das *et al.*, 2006; Mohanta *et al.*, 2010; SEHD, 2002; Azom *et al.*, 2012). No work has so far been reported on tannery effluents treatment of Hazaribagh tanneries in Bangladesh. Although several studies have been conducted, in the developed countries, into the treatment of water and wastewater through

granular activated carbons (Bansoda *et al.*, 2004; Ahmedna *et al.*, 2000), adsorbents (Pala *et al.*, 2000; Gouhua *et al.*, 2004), Fenton's reagent (Martinez *et al.*, 2003), wet oxidation (Hung *et al.*, 2003), coagulation- electro-oxidation (Xiong *et al.*, 2000), and advanced oxidation with biological oxidation (Gogate *et al.*, 2004). On the other hand, a few studies focused on bio-remediation of tannery effluents (Haydar *et al.*, 2007; Nourisepehr *et al.*, 2005; Mythili *et al.*, 2011). Sumathi and Umagowrie, 2012 studied on the treatment of leather industrial effluents using combined technology, Shukla *et al.*, 2011 performed the treatment of tannery effluents using only chemical coagulants that produce sludge where disposal becomes difficult and contaminates the dumpsite. Polyelectrolyte precipitation was not yield satisfactory results for the treatment of leather tanning industrial wastewaters (Kabdasli *et al.*, 1999). Generally, tannery effluents contain mostly sodium sulphide and (or) sodium hydrosulphide (EPA, 1990; Valeika *et al.*, 2006) which has significantly contributing for environmental pollution. The presence of sulphide in wastewaters may dramatically interfere with microbial activities and consequently disturb the function of the system (Mesdaghinia *et al.*, 1991). Biological process can be carried out only when concentration of sulphides is not exceeding 50 mg/l (Valeika *et al.*, 2006). Aerobic treatment was not effective method for treatment of tannery effluent. Some reporters have reported that anaerobic treatment of tannery wastewater in high rate close type reactors leaves sulfides in the range 31-795 mg/l, COD 395-1886 mg/l, BOD 65-450 mg/l and total organic carbon (TOC) 65-605 mg/l. So the high value of toxic chemicals present in the treated wastewater may not be suitable for aerobic biological treatment.

The choice of wastewater treatment process depends on several factors like maximum removal efficiency of pollutants, cost effective, easy to operate, energy saving treatment and Environmental capability (Costa and Olivi, 2009). Moreover, the wastewater characteristics should also be considered when choosing the best process (Costa and Olivi, 2006). Few authors have previously reviewed the various techniques for treatment of tannery wastewater (Cassano *et al.*, 2001; Aravindhan *et al.*, 2004; Sharma *et al.*, 2007). Mostly, the sulphides are removed by using H_2O_2 and Electro oxidation process (Valeika *et al.*, 2006; Anglada *et al.*, 2009). Oxidation of sulfide by air using activated carbon as catalyst gained importance for its removal of COD, BOD₅ and TOC in addition to elimination of sulfide in wastewater (Sekaran *et al.*, 1996). But all of these leather industries in developing country, like Bangladesh still

lack of basic technology of wastewater treatment and have no adequate, sustainable and suitable treatment plant without any proper planning of environmental pollution. So, for the first time in this study, an attempt has been taken to analyze the efficiency of different type of filtrate materials and different type of coagulants to treat the tannery waste water of Bangladesh. There are also other advanced treatment processes available around the world, yet in this study low cost coagulant has been chosen for the treatment of tannery effluents considering the socio-economic aspect of Bangladesh. Of these alternatives, sand-stone and salt of Iron (FeCl_3 , FeSO_4 and Fe_2O_3) seem to be more economically attractive because it is abundant. The ability of sand-stone to refine TSS, TDS, Cl and As etc has been well known. Recently, sand-stone has been accepted as one of the appropriate low-cost adsorbents for the removal of considerable parameters from wastewater. Sand-stone is the main components of the mineral fraction of soils. They are effective natural adsorbents due to their small particle sizes, lamellar structures, and negatively charged surfaces, which make them good cation adsorbents with large reactive surface areas for ion exchange or electrostatic attraction. Adsorption using sand-stone as an adsorbent is an inexpensive methods since some sand derivatives can be easily prepared and regenerated. Sand-stone derivatives may be promising adsorbents for environmental purification purposes (Gürses *et al.*, 2004). So far, there have been no reports of a combined tannery effluent treatment processes attempted to reduce toxicity from tannery effluents in the country. Thus the study, an attempt has been taken to analyze the efficiency of different physical adsorbents (sand-stone, saw-dust and combined) different type of coagulants (salt of iron) to treat the tannery waste water of Bangladesh and finally the study was also conducted a microbial approach for removal of toxic metal ions from the tannery effluents. Combined physical, chemical and microbial processes hold also great promise to provide alternative for better treatment. To protect our environment is using these methods (Physical, Chemical and Microbial treatment) aim at reducing the toxicity of the toxic inorganic and organic compounds and metal ions containing effluents before discharge into environment. The main objectives of the study was to reduce the literature gaps, characterization of leather industrial effluents (manufacturing units wise and composite) and evaluate the treatment performance (physical, chemical and microbial).

1.9. Aim and objectives of the research work

The tanneries of Hazaribagh discharge toxic chemicals causing severe ecological imbalance. There is pressing need to remediate the contaminated environment with eco-friendly techniques.

The main aim of this study was to reduce toxicity due to discharge of untreated tannery effluents into the environment. Therefore, the main objective of the present study is to consider sustainable effluents treatment processes using indigenous and low cost materials for tannery industries in Bangladesh. The present study considered a detail characterization of tannery industrial effluents and evaluated the pollution status for discharging untreated effluents from leather tanning industries situated at Hazaribagh of Dhaka City. The study also considered a combination of physical, chemical and biological treatment processes and evaluated the effectiveness of such an integrated-process for treating tannery wastewater to realize the aim and objectives.

To achieve the main objective of the present research was undertaken to satisfy and clean up the environment and other objectives also be realized, and these are as follows:

1. Biodiversity would be less threatened.
2. Polluted cultivated land would regain its fertility.
3. Freshwater organisms would be getting benefited.
4. Aquatic life would be healthier than previous period.
5. Human health consequences would be reduced.

So the objectives of the present investigation were characterized of untreated effluents then treated with different adsorbents and coagulants. And finally to screen the chromium, cadmium and lead resistant bacteria for their ability to reduce heavy metals biologically, for environment cleanup and bioremediation of metallic ions from contaminated tannery effluents. The selection of the technology depends on many factors like capital cost, availability of land, operation and maintenance cost, efficiency of the process etc. In the following sections, technical viability as per the available tannery data, along with a brief process description of these technologies has been discussed.



2. Characterization of the leather industrial effluents

2. 1. Introduction

The tanning industry belongs to one of the most polluting industrial sectors. Leather tanning has been ranked as one of the most polluting industries compared to other manufacturing sector. Tannery wastewater owes its pollution load to a massive presence of chloride, ammonia, sulphate and various organic, inorganic substances and different tanning materials. The effluents discharged from tanneries are voluminous, highly colored, contained toxic metallic compounds, chemicals, biologically oxidizable materials and large quantities of putrefying suspended matter. The discharged tannery effluents, damages the normal life of the receiving water bodies and land surface (ETPI, 1998; GATT, 1994; Khan *et al.*, 1999). In Bangladesh, about 90% of tannery industry has been used in chrome tanning process for the excellent properties that it renders to the leather along with simplicity of operation. The characteristics of tannery wastewater vary widely depending on the nature of the adopted tanning process, and the amount of water used at different tanning stages and tanning processing capacity. The tanning process is almost wholly a wet process that consumes high amounts of water and also generates about 90% of the water as effluent (World Bank, 1998; IFC, 2007). Process water consumption, and consequently wastewater effluent discharges, varies greatly between tanneries, based on the processes involved, raw materials, and products (Wiegant *et al.*, 1999; UNIDO, 2000). Generally, water consumption is greatest in the pre-tanning areas, but significant amounts of water are also consumed in the post-tanning processes (ETPI, 1998). The amount of wastewater generated in each process is shown in Appendix 2.1.

Conventional methods of pre-tanning, tanning and post-tanning discharged effluents (many unused process chemicals such as Cr, sodium chloride dye-stuffs, different type of re-tanning materials, acids and different type of fats etc. containing effluents) are contained enormous amounts of pollutants together with effluents (ETPI, 1998; UNEP, 1991). Tannery effluents from the beam house processed and associated rinsing process may contain hide substance, dirt, blood, or drug and therefore have significant loads of organic matter and total suspended solids. This stage is the most

polluting section of tanning process, contributing around 50-55% of the total pollution load of the tanning industry. In the liming section of tanning process, protein, hair, skin and emulsified fats are removed from the hides, which are released in the effluent and therefore increase the total solids. Tannery effluents from tan- yard processes, deliming and bating contain sulfides, ammonium salts, and calcium salts and the effluent is weakly alkaline. The pickling and chrome tanning effluents contained chrome, chlorides, sulfuric acid, sodium bicarbonate and sulphates (UNIDO, 2000). Several reports show that beamhouse processed effluents contained high concentration of chloride, total solids, sulfide, sulphate and chromium (Cooman *et al.*, 2002; Gupta, 2003; Junior *et al.*, 2006).

The major pollutants of the post-tanning are chrome salts, dyestuff residues, fat liquoring agents, syntans and other organic matter, typically measured by COD (Bajza and Vrcek, 2001; UNIDO, 2000). Therefore, finally the composite tannery effluents (wastewater) is characterized by high load of organic matter originating from the hides as well as from the added chemical, strong color, turbid, foul smelling, high pH, dissolved and suspended solids, organic nitrogen and ammonia (Cooman *et al.*, 2003; World Bank, 1998; Boshoff *et al.*, 2004; UNIDO, 2000). The characteristics of the different streams of wastewater generated during the tanning process and the leather tanning industry generates about 50 to 60 m³ wastewater per ton of raw wet salted animal hides, usually processed batch wise.

Many researchers showed that pH mainly depends on chemicals used at different stages. Electrical conductivity of tannery effluents contains very high values that indicate the presence of cations and anions in the effluents. The higher conductivity alters the chelating properties of water bodies and creates an imbalance of free metal availability for flora and fauna. Some reporters stated that the electrical conductivity, pH, chloride, sulfides, biological oxygen demand and chemical oxygen demand in tannery effluent were much higher than the tolerance limits for industrial effluent discharged into land surface (Vajpayee *et al.*, 1999; Tunay *et al.*, 1994 and Tripathi *et al.*, 1996). Kannan *et al.* (2008) showed that tannery effluents contained a higher level of total dissolved solids (TDS) which are mainly due to carbonates, bicarbonates,

chlorides, sulphates, phosphates, nitrates, nitrogen, calcium, sodium, potassium and iron. Akan *et al.* (2007) also reported that higher levels of BOD, COD and TSS in tannery effluent. Industrial effluents carry organic and inorganic substances which utilize dissolved oxygen resulting in oxygen depletion. Nearly 70% of the emission loads of biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total dissolved solids (TDS) emanate from the pre-tanning operations. Reports show that COD values in tannery effluents were found higher than the tolerance limits for inland surface water (Akan *et al.*, 2008). Tannery wastewater release high amount of organic matter to the environments. Organic compounds which are present in these wastes are; polyphenolic compounds, acrylic acids condensates, aliphatic ethoxylates, fatty acids, dyes, proteins, soluble carbohydrates (Szpryokowicz *et al.*, 1995; Naumczyk *et al.*, 1996). Organic matter in water bodies can be oxidized by oxygen. Therefore, when released into a water body, organic matters consume oxygen and leave the system in an oxygen-deprived state. This anoxic conditions lead to low electric potential environment and can change the chemistry of the entire system. The loss of oxygen also creates stress on many aquatic organisms including fish (UNEP, 1999; Nicholas, 1996; Pittier and Chudoba, 1990; Nicholson, 1998).

Balusubramanian *et al.* (2000) and Dorman *et al.* (2000) observed that biological decomposition of organic matter from leather industrial effluents, as well as sulphide emissions (also due to bacterial reduction of sulphate), were responsible for the objectionable odors in tannery areas in the form of H_2S . Hydrogen sulfide formed due to the presence of sulfide in the effluent and chromium is highly toxic to many forms of life (Cooman *et al.*, 2003; ETPI, 1998). Potential sources of odour include sulphide emissions from dehairing and liming; ammonia emissions from de-hairing and de-liming liquors and fleshings. The effluents discharged from tanneries are voluminous chloride, sodium, calcium highly oils and grease, color compounds contain a heavy sediment load including toxic metallic compounds, chemicals, biologically oxidizable materials and large quantities of putrefying suspended matter and when discharged tannery effluents, damages the normal life of the receiving water bodies and land surface. The lack of effective implementation of legislative control, poor processing practices and use of unrefined conventional leather processing methods have further aggravated the pollution problem caused by the tanning industry. In addition it shows

high concentration of toxic chemicals including heavy metals are frequently toxic and persistent, and affect both human health and the environment. Essentially now it is recognized that direct contact with leather industrial chemicals can potentially cause disability, illness (toxigenic/carcinogenic) and death in humans. Solvents from degreasing and finishing are a source of exposure through vapors. Human health can also be affected by toxic hazards through the unskilled and unprotected handling of pesticides, tanning chemicals and treated hides and skins. Also visual impacts, excessive noise and air emissions are known to be associated with the tanning industry. It is unfortunate that there are no reliable estimates of the quantity and types of hazardous waste generated in the most developing countries. In Bangladesh, few reports have been found on composite and manufacturing unit-wise tannery effluents in particular. Now a day, there is a growing environmental pressure against the leather processing activity because of rising salinity and different toxic chemicals including heavy metals in the tannery effluents (Saravanabhavan *et al.*, 2005; Bernel *et al.*, 2006).

The main objective of this part of the study was to reduce the literature gaps and evaluate the status of pollution due to discharged tannery effluent. The other objectives were:

- ❖ To investigate the pollution level due to chromium and other heavy metals in effluent discharged
- ❖ To evaluate the effects of anions due to effluent discharged
- ❖ To determine the effect of the untreated tannery effluents on sediment quality
- ❖ To indentify the sources pollutants and their effects on environment

Therefore, to reduce the toxicity and improve human health quality and other environmental conditions, a detail characterization of tannery effluents of each individual process streams (unit-wise) and composite effluents should be treated and made to reveal the present pollution status and to step forward towards sustainability of the environment.

2.2. Materials and methods

2.2.1. Study area

The study area is located in the tannery Industrial zone in Hazaribagh Thana situated on the Southwestern part of Dhaka city (**Fig. 2.1**). It lies between 23°45' to 23°44' north latitudes and 90°21.85" to 90°22.15" east longitudes.

2.2.2. Sample collection

The study was conducted during the year 2008 to 2010 and the tannery effluents were collected three times in a year from different points in Hagaribagh industrial zone in Dhaka, Bangladesh. Mainly two types of tannery effluents were collected, i.e., samples from different Manufacturing Units indicated MU of a tannery industry (inside an industry) and a composite mixture of tannery effluent (out let of a tannery industry). On the other hand, two types of sludge soil were collected from Hazaribagh adjacent leather industrial zone, i.e., sluice gate area, and 100 meters away from the sluice gate area. Three industries namely BLC Ltd, BAY Tannery Ltd and Karim tannery Ltd were selected for collecting samples from seven different units inside of each industry, i.e., I) soaking; II) Liming- un-hairing ; III) De-liming and bating ; IV) Pickling and chrome-tanning ; V) Re-tanning and dyeing ; VI) Fat-liquoring VII) Post-tanning and finishing (manufacturing units wise discharged effluents). The composite effluent mixtures also were collected from three different points of Hazaribagh leather industrial zone. Firstly, the samples from the final discharge point, where all stages of leather processing effluents were mixed, named as monitoring point indicated **P₁**. Secondly the samples from the main drain of tannery industries named as monitoring point indicated **P₂** and finally from the Rayerbazer sluice gate area, named as monitoring point indicated **P₃**. The map of Hazaribagh Thana is shown in **Fig. 2.1**.

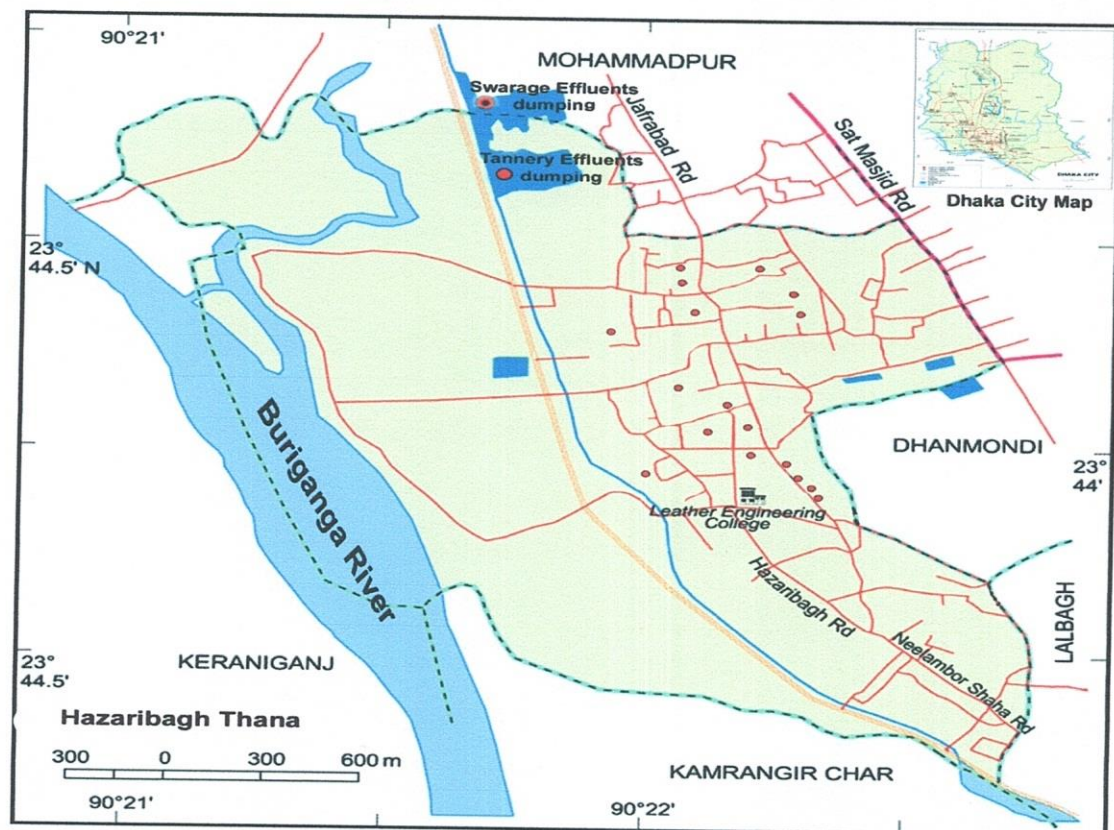


Figure 2.1: Sampling location of leather industrial zone in Hazaribagh Thana, Dhaka, Bangladesh

Each time, 20 liters of effluent samples were collected in several screw capped sterilized polyethylene containers previously cleaned by washing in non-ionic detergent, rinsed with tap water and later soaked in 10% HNO_3 for 24 hours. Finally the container rinsed with de-ionized water and duly sun dried with turning their upside down prior to usage. During sampling, sample containers were rinsed with sampled water three times and then filled to the brim at the depth of one meter below the wastewater from each of all the designated sampling points. The samples were labeled and transported to the laboratory, stored in the refrigerator at about $4 \pm 1^\circ\text{C}$ prior to analysis. The sample collection processes were followed the Pearson method ((Pearson *et al.*, 1987). Sludge samples were also collected from two different locations, i.e., a) from Rayer bazar sluice gate and b) 100 meters from the sluice gate once in a year. The sludge samples were collected in a dried polythene bag and analyses were done at Soil Research Institute, Rajshahi. All the samples are collecting from the mentioning points P_1 , P_2 and P_3 which are stated in Fig. 2.2.

A schematic diagram of sampling points is shown in Fig. 2.2.

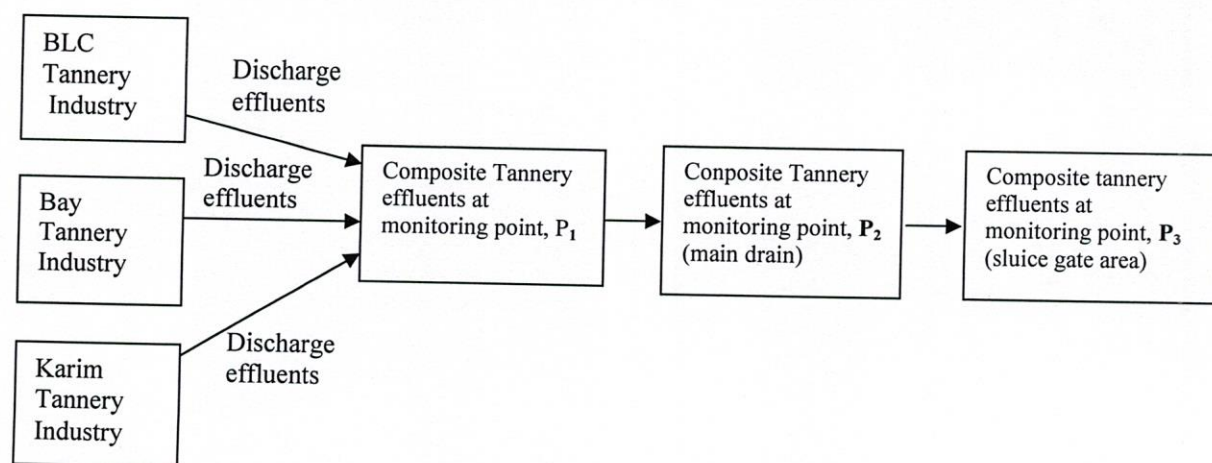


Figure 2.2: Schematic diagram of sample collecting points at Hazaribagh Leather Industrial zone

2.2.3. Sample preparation

In laboratory the collected samples were filtered using filter paper (Whatman 41, Diameter 125 mm) to remove large materials present in the effluents. The filtrate was used to analyze different physico-chemical parameters.

2.3. Tannery effluent characteristics and analysis methods

The physical parameters including color, appearance, odor, temperature ($^{\circ}\text{C}$), pH, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), biological oxygen demand (BOD_5) in 5 days at 20°C , chemical oxygen demand (COD) of all collected samples (manufacturing units and composite) were measured. The samples collected at different stages in each industry were analyzed for chloride (Cl^-), sulphate (SO_4^{2-}), chromium (Cr), cadmium (Cd), Lead (Pb) and arsenic (As). But, chemical parameters including dissolve oxygen (DO), sulphate (SO_4^{2-}), chloride (Cl^-), ammonium nitrogen ($\text{NH}_4\text{-N}$), nitrate nitrogen ($\text{NO}_3\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$), total nitrogen (TN), and sodium (Na), calcium (Ca) and heavy metals such as chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), manganese (Mn), copper (Cu), zinc (Zn) and arsenic (As) of composite effluents were analyzed. All these parameters were analyzed using different standard methods of analyses. The methods used in this study are described briefly as follows.

2.3.1. Color

Color of the all collected samples (Manufacturing Unit Wise Discharged, i.e., MU and composites effluents) were measured by color measurement scale in Pt-Co.

2.3.2. Odor

Odor was detected through sniffing. It was sensed that all of the collected tannery effluents were highly unpleasant and offensive. In fact the whole leather industrial zone was strongly pervaded by odious odors.

A 75 ml of collected effluent was taken in a 100 ml beaker and checked for odor by smelling. In this way, the odor of the entire collected samples was measured.

2.3.3. Temperature

Temperature was measured using a sensitive mercury thermometer in degree Celsius (Gallenkamp Model N/F BS 1704, Made in England) by dipping the thermometer bulb into the sample.

2.3.4. pH

The pH values of all collected samples were determined using a portable digital pH meter (Model KRK, KP-5z, Japan). The portable digital pH meter was switched on for about 6 seconds and the temperature was adjusted. The instrument was calibrated with an electrode in 3M KCl + AgCl₂ electrolyte. The electrode was calibrated using buffers of pH 4.0, 7.0 and 10.0. The electrode of pH meter was immersed in the samples (MU and composite) and waited for a stable reading. The electrode was thoroughly rinsed with de-ionized water after each measurement.

2.3.5. Electrical Conductivity (EC)

EC values of collected effluents (MU and composite) were measured by a digital multi range conductivity meter (Model Hanna HI 9033, Singapore) at 25°C. EC was directly analyzed by dipping the conductance cell in the samples (MU and composite). The power key of the Electrical conductivity meter was switched on. Temperature was adjusted and the instrument was calibrated with 0.001M KCl to give a value of 14.7 μ S/m at 25°C. The electrode of EC meter was dipped into the collected effluents (MU and composite) and allowed for getting a stable reading. The electrode was thoroughly rinsed with de-ionized water after each measurement.

2.3.6. Total suspended Solid (TSS)

TSS values of all tannery effluents (MU and composite effluents) were measured as follows:

- a. **Apparatus** Filter papers, filtration apparatus, funnel, platinum-weighing dishes, drying oven, analytical digital balance.
- b. **Procedure** The funnel was washed with de-ionized water and dried in an oven. The filter paper was also dried in an oven at 103-105°C for 1 hour. Then it was cooled and weighed. Now 50 ml of collected tannery effluents was taken for filtration process through the filter paper in a funnel. After filtration, the filter paper with residue was removed from filtration apparatus and transferred to a previously weighed porcelain crucible. The crucible was dried in an oven at 103-105°C for 1 hour. Then it was cooled and weighted. The cycle of drying, cooling and weighing processes were repeated until a constant weight was obtained.

- c. **Calculation**

$$\text{TSS} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{(B - A) \times 1000}{\text{taken sample (ml)}}$$

where, A = Weight of filter paper (mg)

B = Weight of filter paper with dried residue, (mg)

2.3.7. Total dissolved solid (TDS)

Total dissolved solids are defined as the amount of organic and inorganic substances present in the effluents in dissolved state. TDS values of the collected samples were measured using a digital multi range TDS meter (Model Hanna HI 9033, Singapore). TDS meter was calibrated using potassium chloride solution provided by the manufacturer (HACH-1997). The electrode of TDS meter was immersed below the surface of the collected (MU and composite) effluents. Time was allowed for the reading to be stabilized and reading was recorded. The electrode was thoroughly rinsed with distilled water after each measurement.

2.3.8. Total solids (TS)

Total solids are considered to be the sum of total dissolved solids (TDS) and total suspended solids (TSS). In effluents, the dissolved solids which usually predominate

consist mainly of inorganic salts, small amount of organic matter and dissolved gases. The suspended solids contain much of the organic matter; any increase thereof tends to increase the degree of pollution in water.

2.3.9. DO, BOD₅ and COD

The measurement of DO, BOD₅ and COD of the all collected tannery effluents (MU and composite) were done using the methods, described below:

Determination of dissolved oxygen (DO)

Dissolved oxygen (DO)

DO of collected tannery (composite effluents collected from the monitoring point P₁, P₂ and P₃) effluents was determined by a DO meter (Model KRK, DO-5Z, Japan). Dissolved oxygen (DO) meter was calibrated prior to measurement with the appropriate traceable calibration solution (5% HCl) in accordance with the manufacturer's instruction. The electrode of DO meter was immersed below the surface of the collected effluents. Time was allowed for the reading to be stabilized and reading was recorded. The electrode was thoroughly rinsed with de-ionized water after each measurement.

Determination of Biological Oxygen Demand (BOD₅)

Water pollution is measured by biochemical oxygen demand (BOD₅), which is a standardized measurement of amount of molecular oxygen required by microorganisms to cause the breakdown or decomposition of organic matter in effluents. This method is applicable to determine biochemical oxygen demand (BOD₅) of all collected tannery effluents samples. BOD₅ values of collected samples (MU and composite effluents) were determined using Oxitop instruction manual.

Apparatus and Equipments

- i. Oxitop measuring system
- ii. Inductive stirring system
- iii. Incubator thermostatic box (temperature 20°C ± 1)
- iv. Sample bottles brown (nominal volume 500ml)

- v. Stirring rods and stirring rod remover
- vi. Suitable overflow measuring beakers.
- vii. Rubber quivers

Reagent: Sodium-hydroxide

Environmental conditions: Temperature at $20 \pm 2^\circ\text{C}$ and relative Humidity 45-70%.

Preparation of Sample

43.5 ml of collected samples was taken in a previously washed 500 ml BOD₅ bottles and adjusted pH 6.5-7.5 using 0.1N sulphuric acid and 0.1 N NaOH and kept at 4°C for 48 hours.

Procedure

BOD bottles with collected samples were incubated at $20^\circ\text{C} \pm 1$ for five (5) days. All the bottles were equipped with a magnetic stirrer and a rubber quiver. Then 2 pellets of sodium hydroxide (NaOH) were put into the rubber quiver with a tweezers. Finally the sample bottles were tightly closed with screw of the Oxitop machine.

Start Measurement

Two display buttons S and M (S shows days and M shows BOD₅ value) were pressed simultaneously until the display showed 00. The sample was continuously stirred for 5 days and measure BOD₅ values for all the sample bottles after 5 days have passed.

Calculation

$$\text{BOD}_5 = M \times F \text{ (mg/L)}$$

where M = measured value

F= factor (F= 50 for the sample volume 43.5 ml)

Determination of Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is the amount of oxygen required for the oxidation of organic matter using a strong chemical oxidant. COD indicates the degree of pollution in effluent samples. COD was determined using Closed Reflux-Titrimetric method (Chen *et al.*, 2001) as stated below.

Reagents preparation

Sulfuric acid, H_2SO_4 : Concentrated H_2SO_4 (Reagent grade; contained 1084 g/l) was directly used for COD measurement.

0.25N standard potassium dichromate solution

12.259 g of $\text{K}_2\text{Cr}_2\text{O}_7$ (AR grade, previously dried at 103°C for 2 hours) was taken in a 1-liter beaker and gradually added in de-ionized water and stirred thoroughly. Finally the solution was made up to the mark with de-ionized water.

Ferroin indicator solution

1.485g of 1,10-phenanthroline monohydrate and 0.695 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were taken in a 100 ml volumetric flask and gradually added de-ionized water with occasional shaking and finally made the volume up to the mark.

Apparatus

- (a) Reflux unit consisting of 250ml Erlenmeyer flask with ground glass B-24 neck and 30cm Liebig condenser.
- (b) Standard volumetric glassware
- (c) Personal protective equipments (PPE such as gloves, face shield, shoes and helmet etc) are required.

Procedure

Pipette out 2 ml of sample was taken into a 250 ml Erlenmeyer flask then de-ionized water was added to make a volume about 20 ml. 1 g of mercuric sulphate (powder) was added to it. A pinch of silver sulphate (powder) was also added gradually with shaking well and exactly 10 ml $\text{K}_2\text{Cr}_2\text{O}_7$ solution was added. Then 30 ml of sulphuric acid were added to each flask carefully. Boiling chips were added to it, connected the Liebig condenser to the open end of the flask and allowed the flow of water through the outlet jacket of the Liebig condenser. The reflux was started and continued for 2.5 hours. The flux was cooled and thoroughly washed the condenser with about 50 ml of de-ionized water. The washings were collected in the flask. The collected content was titrated with the ferrous ammonium sulphate (FAS) (0.25N) solution using 3-4 drops of ferroin as an indicator. The color changes were observed from blue green to reddish brown at the end point.

b. A blank sample was prepared with de-ionized water in the similar manner excluding the sample.

Calculation

Determination of the COD of the collected sample (mg/L)

Normality of Ferrous Ammonium Sulphate (FAS) solution:

$$\text{Normality of FAS solution (N)} = \frac{0.25N \times 10}{A}$$

where

$$\text{Normality of } K_2Cr_2O_7 = 0.25N$$

$$\text{Volume of } K_2Cr_2O_7 = 10\text{ml}$$

Volume of ferrous ammonium sulfate solution required for blank titration = A ml

$$\text{To calculate the COD} \left(\text{as mg } \frac{O_2}{L} \right) = \frac{(A - B) N \times 8 \times 100}{V}$$

where, A = Volume of the FAS solution required for blank

B = Volume of the FAS solution required for sample

N = Normality the FAS solution used

V = Volume of sample taken for analysis

Equiavalent weight of oxygen = 8

2.3.10. Sulphate (SO_4^{2-})

Sulphate ion concentrations of all collected samples (MU and composite) were determined using turbidity metric method as stated below.

Apparatus

Magnetic stirrer, Ultra-violet visible Spectrophotometer (Model No.1650 PC, Shimadzu, Japan) for use at 420 nm wave lengths and the quantity of sulphate was calculated from the calibration curve.

Reagents**Buffer solution**

30 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g of sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$), 1 g of KNO_3 , 0.111 g of sodium sulphate (NaSO_4) and 20 ml of acetic acid were taken in a 1-liter beaker and gradually added 500 ml of de-ionized water and mixed thoroughly using a magnetic stirrer. Finally the solution was made up to the mark with de-ionized water.

Barium chloride

BaCl_2 crystal 20 to 50 mesh sizes used to make uniform turbidity.

Standard sulphate solution

0.1479 g of anhydrous Na_2SO_4 was taken in a 1-liter volumetric flask and dissolved in double-distilled water and diluted up to 1000 ml, where 1 ml contained 100 μg sulphate. 4, 8, 12, 16 and 20 ml solution were taken in separate 100 ml flasks to make 4, 8, 12, 16 and 20 mg/L of sulphate solⁿ. The calibration curve is shown in the Appendix 2.2.

Procedure**Barium sulphate suspension**

100 ml sample was taken in a 250 ml Erlenmeyer flask. 20 ml buffer solution was added and mixed with stirring apparatus. At the time of stirring, a pinch of BaCl_2 crystal was added and solution was stirred for 1 min. After stirring, the solution was poured into an absorption cell (cubet) of spectrophotometer and absorbance was measured within 5 minutes using 420 nm wave-lengths.

Calculation

$$\text{Sulphate}(\text{SO}_4^{2-}) \text{ mg/L} = \frac{\text{Sulphate(mg)} (\text{from calibration curve})}{\text{taken sample(L)}}$$

2.3.11. Chloride (Cl)**Determination of chloride****Preparation of ferric ammonium sulfate (0.25M)**

30.125 g of hydrated ammonium iron (iii) sulphate [$\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$] was taken in a 250 ml volumetric flask and dissolved in 100 ml 9M HNO_3 and mixed thoroughly using a magnetic stirrer. Finally the solution was made up to the mark with de-ionized water.

Saturated mercury (II) thio-cyanate solution

1 g of $\text{Hg}(\text{SCN})_2$ was taken in a 1-liter volumetric flask and dissolved in ethanol and finally added ethanol up to the mark. The solution was filtered after 24 hours. The solution was stored in a glass bottle at room temperature.

Procedure

10 ml of collected sample was taken into a 25 ml volumetric flask and then 2 ml of 0.25 M ferric ammonium sulphate solution was added followed by 2 ml of a saturate solution of mercury(ii) thio-cyanate and made up to the mark with de-ionized water. The red color of ferric thio-cyanate complex was formed and absorbance was measured at 460 nm against a reagent blank and referred it to the calibration curve.

The calibration curve was constructed by using standard sodium chloride solutions containing 1, 2, 3, 4, 5, 6 and 7 mg/L of chloride ions. The calibration curve is shown in the Appendix 2.3.

Calculation

$$\text{Chloride content (mg/L)} = \frac{\text{Cl}^- \text{ (mg) (from calibration curve)}}{\text{taken sample (L)}}$$

Calibration of the AAS

The Atomic Absorption Spectrophotometer (AAS) (Model No.1650 PC, Shimadzu, Japan) for anions (Cl^- and SO_4^{2-}) determination were checked for malfunctioning by passing standard solutions of the anions measured. Blank samples (de-ionized water) were passed between every three measurements of effluents samples to check for any eventual contamination or abnormal response of equipment.

2.3.12. Determination of Ammonium-Nitrogen, Nitrite-Nitrogen, Nitrate-Nitrogen and Total Nitrogen in tannery effluents**Ammonium-Nitrogen**

Apparatus: Ultra violet visible Spectrophotometer for use at 640 nm with a light path of 1 cm.

Reagents

Phenol solution: 11.1 ml liquefied phenol about 95% was mixed with 95% v/v ethyl alcohol to a final volume of 100ml.

Sodium Nitroprusside (0.05% w/v)

0.05g of sodium nitro-prusside was taken in a 100ml volumetric flask and gradually added de-ionized water to it and thoroughly mixed using a magnetic stirrer. Finally the solution was made up with de-ionized water up to the mark. It was stored in amber bottle and used within 1 month.

Alkaline citrate

200g of tri-sodium citrate and 10 gm of sodium hydroxide were taken in a 1 liter beaker and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up to the mark with de-ionized water.

Sodium hypochlorite: 5% commercial solution was used.

Oxidizing solution: 100ml alkaline citrate solution was mixed with 25 ml sodium hypochlorite. It was freshly prepared daily.

Stock ammonium solution: 3.819 g of anhydrous (dried at 100°C) NH_4Cl was taken in a 1-liter beaker and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up with de-ionized water up to the mark;

1 ml = 0.1 mg N = 1.214mg NH_3 .

Standard ammonium solution

The calibration curve was made in the usual manner using six standards ammonium chloride solutions containing 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 mg/L of ammonia nitrogen. The calibration curve is shown in the appendix 2.4.

Procedure

25 ml sample was taken in a 50 ml Erlenmeyer flask. 1ml phenol solution, 1 ml sodium nitro-prusside solution and 2.5 ml oxidizing solution were added. The solution was mixed thoroughly after each addition. Samples were covered with a paraffin wrapper film. It was allowed to develop color formation at room temperature in subdued light for at least 1 hour. Color was stable for 24 hours and the absorbance was measured at 640 nm. A blank and other standards solution was prepared by diluting stock ammonia solution within the sample concentration range. The standards were treated the same as samples.

Calculation: Sample concentration was calculated by comparing sample absorbance with the standard curve.

$$\text{Ammonia-nitrogen (mg/L)} = \frac{\text{mg in NH}_3 - \text{N (from standard curve)}}{\text{taken sample (L)}}$$

$$\text{Ammonium-Nitrogen (mg/L)} = \text{Ammonia-Nitrogen (mg/L)} \times 1.214$$

Nitrate –Nitrogen

Apparatus: Spectrophotometer using at 220nm and 275 nm with matched silica cells of 1 cm was used.

Reagents

Stock nitrate solution

Potassium nitrate (KNO_3) was dried in an oven at 105°C for 24 hours. 0.7218 g of KNO_3 was taken in a 1-liter beaker and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up to the mark with de-ionized water. $1\text{ ml} = 100\text{ }\mu\text{g NO}_3\text{-N}$ (0.1 mg/L). 2 ml CHCl_3 was used per liter of solution to preserve for at least 6 months.

Intermediate nitrate solution

Hydrochloric acid solution (1 N HCl)

8.0765 ml of concentrated HCl was taken in a 100ml Erlenmeyer flask and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up to the mark with de-ionized water.

Procedure

Treatment of sample

1 ml HCl (1N) solution was mixed thoroughly in 50 ml collected sample.

Preparation of standard curve

The calibration curve was previously constructed by using 0.4, 0.8, 1.2, 1.6 and 2 mg/L of potassium nitrate (KNO_3). The calibration curve is stated in the appendix 2.5.

Spectrometric measurement

The absorbance was read against de-ionized water (blank) set at zero absorbance. A wavelength of 220nm was used to obtain NO_3^- -N reading and a wavelength of 275 nm was used to determine interference due to dissolved organic matter.

Calculation

For samples and standards, always the absorbance readings at 275 nm were subtracted from the reading at 220nm to get absorbance of NO_3^- -N. A standard curve was prepared and the sample concentrations were obtained directly from the standard curve.

$$\text{Nitrate} - \text{nitrogen} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\mu\text{g in NO}_3 - \text{N (from standard curve)}}{\text{taken sample (ml)}}$$

$$\text{Nitrate} \left(\frac{\text{mg}}{\text{L}} \right) = \text{NO}_3 \left(\frac{\text{mg}}{\text{L}} \right) \times 4.429$$

Nitrite-Nitrogen

Apparatus: Spectrophotometer, for use at 543nm, was providing a light path of 1 cm.

Color reagent

85% phosphoric acid of 100 ml and 10 gm of sulfanilamide was added in 800ml water. After completely dissolving sulfanilamide, 1 g N-(1-naphthyl)-ethylene-diamine dihydro-chloride was added. It was dissolved and diluted to 1000 ml. It was stored in refrigerator with dark bottle for about a month.

Sodium oxalate, 0.025M (0.05N)

3.35 g of $\text{Na}_2\text{C}_2\text{O}_4$ (primary standard grade) was taken in a 1 liter beaker and gradually added in de-ionized water and mixed thoroughly with magnetic stirrer. Finally the solution was made up to the mark with de-ionized water.

Ferrous ammonium sulphate, (FAS) 0.05M (0.05N)

19.607 g of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 20 ml of conc. H_2SO_4 was taken in a 1 liter beaker and gradually added de-ionized water and mixed well. Finally the solution was made up to the mark with de-ionized water.

Stock nitrite solution

1.232 g of NaNO_2 (generally commercial grade NaNO_2 contains 99%) was taken in a 1 liter beaker and gradually added de-ionized water and stirred thoroughly, finally the solution was made up to the mark. $1 \text{ ml} = 250 \mu\text{g N}$.

1 ml CHCl_3 was added for preservation.

Preparation of standard solution

For the construction of calibration curve, five standard sodium nitrite solutions containing 1, 2, 3, 4 and 5 mg/L of nitrite nitrogen were used. The calibration curve is accumulated in the appendix 2.6.

Procedure**Color development**

50 ml sample was taken in a 100ml Erlenmeyer flask and 2 ml color reagent was added and mixed thoroughly.

Photometric measurement

The color was measured between 10 min to 2 hours after adding color reagent at absorbance at 543 nm wavelengths.

Calculation

$$\text{Nitrite - nitrogen} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{\mu\text{g NO}_2 - \text{N (from standard curve)}}{\text{taken sample (ml)}}$$

$$\text{Nitrite (NO}_2\text{)} \frac{\text{mg}}{\text{L}} = \text{NO}_2 - \text{N} \left(\frac{\text{mg}}{\text{L}} \right) \times 3.286$$

Total Nitrogen

The estimation of total nitrogen as nitrite, nitrate and ammonia are of great importance in effluent and polluted water as all of these forms cause pollution in effluents.

Define total nitrogen

The National Pollutant Inventory (NPI) and National Environment Protection Measure (NEPM) stated that total nitrogen refers only to those amounts of nitrogen that give rise to nitrate and nitrite ions. Since this may be possible for a range of nitrogen compounds in a range of environmental conditions, the following pragmatic definition can be applied: Total Nitrogen is the sum of nitrate (NO_3), nitrite (NO_2) and ammonia (all expressed as N).

2.3.13. Cationic constituents

Determination of calcium (Ca), sodium (Na) and heavy metals such as arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), manganese (Mn), cobalt (Co), zinc (Zn) and copper (Cu) in collected samples were analyzed by Atomic Absorption Spectrometric method. The method is described briefly as follows:

Sample preparation**Acid digestion of effluent samples**

100 ml sample was transferred into a 200 ml volumetric flask and added 5ml concentrated HNO_3 into it. The flask with the content was placed on a hot plate and heated boiling at $103\text{-}105^\circ\text{C}$ for 6 hours and evaporated to reduce its volume to about 15-20 ml. The flask was cool and added 3 ml concentrated HClO_4 . The flask was covered with a watch glass and returned to the hot plate. The heating was continued, and then small portion (5/6 drops) of HNO_3 was added until the solution appeared light colored and checked pH 2-3. Outside- wall of the flask and watch glass were washed with de- ionized water and the sample was filtered with $0.45\ \mu\text{m}$ membrane filter paper to remove any insoluble materials that could clog the atomizer. The volume was adjusted to 100ml with de-ionized water (Ademoroti, 1996). For Ca^{2+} ion determination, 1 drop of 0.2% lanthanum solution was added to each of 100ml samples and standard solutions. The concentration of samples was measured using Atomic Absorption Spectrophotometer (AAS).

Standardization

Every standard metal solution was taken and selected to bracket the expected metal concentration of a sample. In this way at least four to five concentrations of each standard metal ion solution were taken. The instrument was aspirated blank and set to

zero. Then each standard was aspirated in turn into flame and absorbance was recorded. In the present work, calibration curve method was adopted for the analysis of water and environmental samples. From 1000 mg/L standard metal stock solutions (supplied with the Shimadzu AA-6650 AAS from Japan), 4 to 5 different (for example, in case of As 5, 10, 20, 40 and 100 ppb) standard solutions were prepared using $V_1S_1=V_2S_2$ formula, where, V and S denote their usual significance. These standard metal solutions were aspirated directly into the AAS. A standard calibration curve was constructed with the absorbance as ordinate and concentration as abscissa. For 11 metals solution, 11 calibration curves were constructed Figures which is incorporated appendix No. 2.7 to 2.17 as an example for standard calibration curve for an especial metals.

Calibration of the AAS

Calibration of the AAS for cationic measurement was followed the same as was done in anionic calibration.

Calculation

The prepared sample solution was aspirated directly (as with standard solution) into the flame of AAS and absorbance was measured. The concentration of cations in the sample was thus obtained from the respective standard calibration curves. The concentrations of metal ions in the samples were obtained using the following relation:

$$\text{Metal ions} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{d \times f}{v}$$

where,

d = concentration of measured cations (mg/L);

f = dilution factor;

v = volume of the taken sample (L).

All parameters were analyzed in accordance with standard methods of APHA (1998 and 2005). All the instruments were calibrated with standard solution before use; all the chemicals were used in analytical grade (Sigma Aldrich Company Ltd., Germany,

and BDH Ltd., England) and dissolved in de-ionized water. All the experiments were performed at least three times to minimize analytical error. Each time, three readings were taken, and their mean value and standard error of the mean were calculated. The mean value of all physico-chemical parameters of the SWD and composite effluents were tabulated. The analysis results were compared with the values of National and International Environmental Quality Standards (NEQS -2000; ISI-2000), United States–Environmental Protection Agency (US-EPA) and Federal Environmental Protection Agency (FEPA) and Inland surface water for Bangladesh standards (ISW in BDS) for wastewater to evaluate the efficacy of this work.

Data analysis

Computer programs were used to analyze the data obtained from Laboratory test. Microsoft Excel 2007 and SPSS were used for statistical analysis. Finally the effluents quality data of different sources were compared with the standard permissible limits, to identify the most suitable and sustainable option for treating tannery effluents and thus helping clean up the environment.

2.4. Results and Discussion

2.4.1. Color and temperature

The analyses results of seven different manufacturing units (samples are denoted by numbers between 1 to 7) of each of the three industries (namely BLC, BAY and Karim Tannery Ltd) are shown in Tables 2.1, 2.2 and 2.3. Differences in color were observed in manufacturing unit wise discharged (MUWD) effluents. The dark – brownish color of soaking effluents was due to contain blood, dirt, vessels and excessive sodium chloride, white color of liming and un-hairing effluents was due to excessive lime water, creamy white color was due to its excessive bleaching agent and bat powder, dark blue color of chrome-tanning effluents was due to excessive basicity of chromium salt, black color of re-tanning dyeing effluents was due to unfix dyeing materials and different type of vegetable materials, light black color of fat-liquoring effluents was due to unfix dye, black color of post-tanning effluents was due to pigment color. The color appearance of the composite tannery effluents at monitoring points P₂ and P₃ were observed the same results, i.e., light brown color. The average color range of the composite tannery effluent at monitoring point P₁ was

found 1760 Pt-Co units and yellowish-brown in color, which was 117 times higher than the all the recommended values of standard limits (15 Pt-Co units). The analysis results indicate that the composite effluents contained highly colored compound which have the potential to cause significant harm to the environment. Verma, *et al.* (2008) reported that the yellowish brown color might be hindering the penetration of sunlight causing depletion in the rate of oxidation process. Chhatwal, *et al.* (1989) also reported that the yellowish brown color contained α -mesosaprobic type water which indicates the presence of large amounts of algae and large numbers of bacteria. The results obtained in this study are also similar in Maiti (2002) and Dhungana and Yadav (2009).

Table 2.1: Physico-chemical characteristics of tannery effluents at manufacturing units of BLC Tannery

Parameters	Sample No.1	Sample No.2	Sample No.3	Sample No.4	Sample no.5	Sample No.6	Sample No.7
Color	Dark brownish	white	Creamy white	Dark blue	Black	Light black	Black
Temperature (°C)	27	25	35	25	40	50	30
pH	8.18	12.5	6.5	3.8	4.02	4.01	4.2-4.0
EC mS/cm)	19	13	12	285	142	94	8.2
TSS(mg/L)	6080	10800	10800	1000	850	820	1240
TDS(mg/L)	14500	12500	12000	156200	50950	4100	8100
TS(mg/L)	20580	23300	22800	157200	51800	4920	9340
COD(mg/L)	10560	4800	4200	49000	71040	17000	7200
BOD ₅ (mg/L)	1200	700	700	2000	2300	1900	1000
Sulphate(mg/L)	-	26200	1200	2100	1000	1200	-
Chloride(mg/L)	45800	1500	1000	58700	500	510	300
Cr(mg/L)	-	-	-	1975	30	20	35
Cd(mg/L)	0.5	0.6	0.8	3.5	2.1	0.9	0.5
Pb(mg/L)	0.4	0.6	0.8	4.12	1.5	0.15	0.2
As(mg/L)	0.3	2.36	1.02	1.6	0.4	0.12	0.15

Sample no. 1: after soaking , 2: liming and un-hairing, 3: de-liming and bating, 4: pickling and chrome-tanning, 5: re-tanning and dyeing, 6: fat-liquoring and 7: post-tanning and finishing operational effluents respectively. All analyzed tabulated

parameters unit in mg/L except in Color (pt-co), Temperature °C, pH and EC (at 25°C). The average value denoted the table.

The temperature of effluents at different leather manufacturing units ranged from 25-50°C which is shown in Tables 2.1, 2.2 and 2.3. The highest temperature (50°C) was found in fat-liquoring effluents while the lowest one (25°C) was found in pickling and chrome-tanning effluents. The temperature of composite effluents at monitoring point 2 and 3 were the same (25°C). In this study, the average temperature of composite effluent at monitoring point P₁ was found to be 34°C which were well below within the permissible limits of National Environmental Quality Standard (NEQS- 2000,), United State of Environmental Protection Agency (USEPA), Indian Standard Institute (ISI-2000), Inland Surface Water in Bangladesh (ISW-BDS-2000). Temperature is an important indicator of water quality with regards to survival of aquatic organisms. Research reports showed that the effluents temperature depends on the production process in the industry and it is usually with a range of 32 to 40°C (Khan and Noor. 2002; Tariq *et al.*, 2005; Rován *et al.*, 1998).

2.4.2. pH and EC

The study results reveal that the pH values of the effluents at various leather manufacturing units ranged from 3.8-13, which are given in Tables 2.1, 2.2 and 2.3. The chrome-tanning effluents had the lowest pH of 3.8, which was well below the prescribed standard limits but may adversely affect the aquatic life due to high acidic level. The low pH of the chrome-tanning effluents may be due to add sulphuric acid during pickling step for preparing the hides. On the other hand, the highest pH (13) was found in liming effluents may be due to the excessive use of lime and sodium sulphide in the production of lime pelt, which was strong basic level and far above the standard limits. A large fluctuation in pH value exerts stress on aquatic environment, which may kill some sensitive species of plants life and animals living there. The mean pH values of composite samples at monitoring points, P₁, P₂ and P₃ were found around 7 (neutral) which were well below the standard limits.

Table 2.2: Physico-chemical characteristics of tannery effluents at manufacturing units at BAY Tannery

Parameters	Sample No.1	Sample No.2	Sample No.3	Sample No.4	Sample no.5	Sample No.6	Sample No.7
Color	Dark brownish	white	Creamy white	Dark blue	Black	Light black	black
Temperature(°C)	25	25-30	28-35	25-27	40-42	48-50	30-35
pH	7.8-8	11.5-13	6-6.5	3.6-3.8	4.02-4.2	3.8-4.01	4.0-4.2
EC(mS/cm)	22	14	12	295	145	95	8.2
TSS(mg/L)	6800	11000	10800	1200	850	860	1240
TDS(mg/L)	15000	13000	12500	156700	59050	4100	8300
TS(mg/L)	21800	24000	23300	157900	59900	4960	9540
COD(mg/L)	10600	5000	4300	49500	71540	16180	7300
BOD5(mg/L)	1200	800	750	2050	2400	1600	1000
Sulphate(mg/L)	-	27200	1200	2200	1000	1200	-
Chloride(mg/L)	46000	1600	1050	60000	500	510	300
Cr(mg/L)	-	-	-	2001	30	20	35
Cd(mg/L)	0.56	0.67	0.85	3.45	2.51	1.09	1.5
Pb(mg/L)	0.45	0.65	0.86	4.12	1.5	0.15	0.2
As(mg/L)	0.35	2.46	1.36	0.86	0.46	0.12	0.15

Sample no. 1: after soaking , 2: liming and un-hairing, 3: de-liming and bating, 4: pickling and chrome-tanning, 5: re-tanning and dyeing, 6: fat-liquoring and 7: post-tanning and finishing operational effluents respectively. All analyzed tabulated parameters unit in mg/L except in Color (pt-co), Temperature °C, pH and EC (mS/cm at 25°C).

The average EC values of the effluents at different leather manufacturing units ranged from 8.2-295 mS/cm, which are shown in Tables 2.1, 2.2 and 2.3. The highest mean value of EC was found to be 295mS/cm in pickling and chrome tanning effluents at BAY tannery (Table 2.2). It is mainly due to add huge amount of sodium salts during pickling process to avoid acidic hide swelling. So the higher electrical conductivity values of the effluents indicate the high level of salts in the effluents. On the other hand, the EC of the post-tanning and finishing effluents contained value 8.2mS/cm which is higher than the prescribed standard limits. The study indicates that the EC values of composite effluents at monitoring points P₁, P₂ and P₃ were ranged from 8 - 20.65 mS/cm which are shown in Tables 2.4, 2.5 and 2.6. The present study illustrated that the EC of composite tannery effluents (P₁) were higher values (18.65, 20.65 and

18.65 mS/cm) than the others monitoring points (P_2 and P_3). The value of P_1 is high because of its salinity or salt content is very high in the effluents and directly come from the tannery industry; on the other hand decreases toward points P_2 and P_3 due to sedimentation and connect to sewage system. The higher conductivity depends on the chelating properties of water bodies and creates an imbalance of free metal availability for flora and fauna (Akan *et al.*, 2008; Verma T. *et al.*, 2008). The EC of all collected samples in tannery effluents were much higher than the tolerance limits for industrial effluent discharged to surface water.

Table 2.3: Physico-chemical characteristics of tannery effluents at manufacturing units in Karim Tannery

Parameters	Sample No.1	Sample No.2	Sample No.3	Sample No.4	Sample no.5	Sample No.6	Sample No.7
Color	Dark brownish	white	Creamy white	Dark blue	Black	Light black	Black
Temperature(°C)	27	25	35	25	40	50	30
pH	7.5	12	6.5-8.5	3.8-3.6	4.02	3.8- 4.01	4.2
EC (mS/cm)	19	13	12	275	135	85	8.2
TSS(mg/L)	6080	10800	10000	1000	850	820	1240
TDS(mg/L)	14500	12500	12000	156000	50950	4100	8100
TS	20580	23300	23800	157000	51800	4920	9340
COD(mg/L)	10160	5800	4400	49200	71040	17040	7200
BOD ₅ (mg/L)	1200	900	800	2000	2300	1900	1000
Sulphate(mg/L)	-	26200	1200	2100	1000	1200	-
Chloride(mg/L)	45800	1500	1000	58000	500	510	300
Cr (mg/L)	-	-	-	1875	30	20	35
Cd (mg/L)	0.65	0.6	0.88	3.05	2.01	0.9	0.5
Pb (mg/L)	0.45	0.6	0.85	4.12	1.5	0.15	0.2
As (mg/L)	0.36	2.36	1.2	1.6	0.4	0.12	0.15

Sample no. 1: after soaking , 2: liming and un-hairing, 3: de-liming and bating, 4: pickling and chrome-tanning, 5: re-tanning and dyeing, 6: fat-liquoring and 7: post-tanning and finishing operational effluents respectively. All analyzed tabulated parameters unit in mg/L except in Color (pt-co), Temperature °C, pH and EC (at 25°C).

2.4.3. TSS, TDS and TS

The study results illustrate that the total suspended solid (TSS) of different leather manufacturing units (MU) effluents in three different industries contained in the range from 820-11000 mg/L which are shown in Tables 2.1, 2.2 and 2.3. It is evident from the study results that the effluents of all leather manufacturing unit contained high TSS values which were above all the permissible standards limits. The study observes that the average TSS values of composite tannery effluents at monitoring point P₁, P₂ and P₃ are ranged from 980-6800 mg/L which are tabulated in Tables 2.4, 2.5 and 2.6. The study reveals that the TSS of P₁ were higher values (about 6800, mg/L) than the others monitoring points P₂ and P₃.

The TSS values were exceeds the all standards limits for effluents discharge. Moreover presence of total suspended solids lead to turbidity resulting in poor penetration of light in aquatic system, thereby curtailing the light for photosynthesis activity. Further the settling of suspended particles on soil, soil fauna might lead to various damages like change in soil porosity, soil texture and water holding capacity (Narasimha *et al.*, 1999; Jayasingh *et al.*, 2005). The study suggests that tannery effluents could be classified as strong wastewater according to Sachchida Nand Singh *et al.*, (2012) and could not be discharged to surface water and environment.

The present study reveals that the average TDS concentrations of discharged effluents at different manufacturing units (MU) in three selected industries are ranged from 8100-156700 mg/L which are shown in Tables 2.1, 2.2 and 2.3. The study results show that the pickling and chrome tanning effluents contained very high TDS around 156000 mg/L in three industries on the other hand post-tanning and finishing effluents contained minimum TDS value around 8000 mg/L in three industries. The study result indicated that the mean TDS value of composite tannery effluents at monitoring point P₁, P₂ and P₃ are ranged from 7050-14080mg/L which are shown in Tables 4, 5 and 6. The study illustrates that the TDS value of different composite tannery effluents at various monitoring points are in the following order: P₁ > P₂ > P₃.

Table 2.4: The physico-chemical parameters including heavy metals of the composite tannery effluents and compare with the standard limits during May – June in the year 2008-2010

Parameters	CompositeTannery Effluents P₁	CompositeTannery Effluents P₂ (Main drain of leather industrial zone)	CompositeTannery Effluents P₃ (Sluice gate area)	Standard Permissible Limits		
				ISI - 2000	NEQS – 2000 (USEPA)	ISW in BDS
Color (Pt- Co)	Yellowish Brown, 1760± 5.5	Light brownish	Light brownish	15	15	15
Odor (TON)	Bad smell	Bad smell	Bad smell	1	1	1
Temperature(°C)	34 ±0.5	25±0.5	25±0.5	40	40	40
Turbidity (FTU)	780 ±1.25	-	-		20	-
pH	7.5 ±0.11	7.04±0.1	7.05±0.05	6- 9	6-9	6- 9
EC(mS/cm)	20.65± 0.69	10.2±0.5	10±0.05	0.85	0.288	-
TSS (mg/L)	6880±30.5	1300±5	1260±5.5	100	150	500
TDS (mg/L)	14080±50.5	7820±20.5	7780±15.5	2100	3500	-
TS (mg/L)	20880±55	9120±20.12	9040±10.5	2200	3650	-
Alkalinity(mg/L)	1900±5.5	-	-	500		-
DO ((mg/L)	0.0±0.05	0.0±0.05	0.8±0.2	4.0-6.0	4- 6	-
BOD ₅ (mg/L)	920±15.81	910±10.55	905±10.5	30	80	250
COD (mg/L)	3980±29.66	3280±15.5	3200±11	250	200	400
Sulphate mg/L)	4000±14.32	2060±10.54	2001±9.53	1000		-
Chloride(mg/L)	5005±13.05	2405±5.56	2280±6.54	600		-
NH ₄ -N (mg/L)	621.36± 2.5	-	-	50		-
NO ₃ -N (mg/L)	77.97±5.5	-	-	10	50	-
NO ₂ -N (mg/L)	111.25±2.5	-	-			-
TN (mg/L)	810.58±0.52	-	-	100		-
Ca (mg/L)	785±4.12	-	-		200	-
Mg (mg/L)	981±3.2	-	-	200	50	-
Na (mg/L)	3840±37.42	1920±2.5	1890±2.5			-
Cr (mg/L)	9.81±0.63	8.5±0.55	8.4±0.5	0.1	0.05	2
Pb (mg/L)	5.35±0.19	3.51±0.15	2.8±0.15	0.2	0.01	-
Cd (mg/L)	3.45±0.45	1.8±0.5	1.6±0.05	0.1	0.05	-
Mn (mg/L)	3.15±0.63	-	-	0.2	0.05	-
Ni (mg/L)	4.21±0.56	-	-	3.0	1	-
Cu (mg/L)	46.88±5.25	6.5±0.51	6.05±.52	2	1.5	-
Zn (mg/L)	17.78±1.23	-	-	5	5	-
As (mg/L)	2.15±0.15	1.80±.51	1.75±.52	0.1	0.05	

ISI-2000 = Indian Standard Institute-2000, NEQS- 2000 = Nature / National of Environmental quality Standards-2000 (USEPA; United State Environmental Protection Agency), ISW-BDS = Inland Surface Water in Bangladesh (GOB, 1997; ECR, 1997).

The TDS values obtained in all samples were higher than the prescribed limits of 2100 mg/L for discharge of wastewater to surface water. High level of TDS in the effluent renders it unsuitable for irrigation.

The present study reveals that the average TS value of the effluents at different manufacturing units (MU) in three different industries are ranged from 9340-157900 mg/L, while the highest TS concentration are observed in pickling and chrome-tanning operation (sample no.4) and low concentrations of TS among the parameters observed in post-tanning and finishing operation (sample no.7) which are shown in Tables 2.1, 2.2 and 2.3. The mean value of TS in composite tannery effluents at monitoring points (P_1 , P_2 and P_3) were ranged from 8030- 20880 mg/L which are shown in Tables 2.4, 2.5 and 2.6. The results of the study show that TS of the composite tannery effluents at monitoring point (P_1) was higher than the other monitoring points (P_2 and P_3). TS level in the entire sampling point was higher than all of the standard limits for discharges of wastewater to surface water bodies and the adjacent area. The analysis results that the alkalinity of the composite tannery effluents at monitoring points (P_1) were in the range 1850-1900 mg/L (Tables 2.4, 2.5 and 2.6).

The total alkalinity of the collected sample depends on its high carbonate and bicarbonate value. The high values of alkalinity in P_1 may be due to add the exceed sodium bicarbonate salt during the process of after pickling to produce tanning condition for pickled pelt. The levels of alkalinity in the entire sampling points were higher than the all standards permissible limits for the discharged of wastewater into river and the adjacent area.

Table 2.5: The physico-chemical parameters including heavy metals of the composite tannery effluents and compare with the standard limits during August- September in the year 2008-2010

Parameters	CompositeTannery Effluents P₁	CompositeTannery Effluents P₂ (Main drain of leather industrial zone)	Composite Tannery Effluents P₃ (Sluice gate area)	Standard Permissible Limits		
				ISI -2000	NEQS – 2000 (USEPA)	ISW in BDS
Color (Pt- Co)	Yellowish Brown, 1760 ±5.5	Light brownish	Light brownish	15	15	15
Odor (TON)	Bad smell	Bad smell	Bad smell	1	1	1
Temperature(° C)	34±0.5	25±0.5	25±0.5	40	40	40
Turbidity (FTU)	750±1.25	-	-		20	-
pH	7.5±0.11	8.5±0.25	8.4±0.24	6- 9	6-9	6- 9
EC(mS/cm)	18.65±0.65	8±0.21	8±0.23	0.85	0.288	-
TSS (mg/L)	6800±35..35	1000±5.5	980±5.5	100	150	500
TDS (mg/L)	14000±1.5	7060±.65	7050±1	2100	3500	-
TS (mg/L)	20800±55.01	8060±35	8030±25.5	2200	3650	-
Alkalinity(mg/L)	1850±5.5	-	-	500		-
DO ((mg/L)	0.8±0.05	2.7±0.5	2.6±0.55	4.0-6.0	4- 6	-
BOD ₅ (mg/L)	920±15.8	840±10.5	820±10.54	30	80	250
COD (mg/L)	3980±29.66	2740±15.5	2700±15.45	250	200	400
Sulphate (mg/L)	4000±14.32	1190±5.5	1100±5.24	1000		-
Chloride(mg/L)	5005±13.05	1820±2.5	1810±2.4	600		-
NH ₄ -N (mg/L)	621.36±2.5	-	-	50		-
NO ₃ -N (mg/L)	77.97±5.5	-	-	10	50	-
NO ₂ -N (mg/L)	111.25±2.5	-	-			-
TN (mg/L)	810.58±0.52	-	-	100		-
Ca (mg/L)	785±4.12	-	-		200	-
Mg (mg/L)	981±8.55	-	-	200	50	-
Na (mg/L)	3840±37.42	1230±5.63	1200±5.54	-	-	-
Cr (mg/L)	9.81±0.63	8.5±1.22	6.4±1.25	0.1	0.05	2
Pb (mg/L)	5.35±0.20	1.6±0.25	1.5±0.24	0.2	0.01	-
Cd (mg/L)	3.45±0.05	1±0.05	0.96±0.05	0.1	0.05	-
Mn (mg/L)	3.15±1.2	-	-	0.2	0.05	-
Ni (mg/L)	4.21±0.56	-	-	3.0	1	-
Cu (mg/L)	46.88±5.25	6.6±0.55	6.5±0.56	2	1.5	-
Zn (mg/L)	17.78±1.5	-	-	5	5	-
As (mg/L)	1.65±0.5	0.70±0.05	0.67±0.05	0.1	0.05	

ISI-2000 = Indian Standard Institute-2000, NEQS- 2000 = Nature / National of Environmental quality Standards-2000 (USEPA; United State Environmental Protection Agency), ISW-BDS = Inland Surface Water in Bangladesh (GOB, 1997; ECR, 1997).

2.4.4. BOD₅, COD and DO

The mean BOD₅ and COD of the effluents at different manufacturing unit (MU) in three industries were in the range 700-2400 mg/L and 7200- 71540 mg/L, respectively which are tabulated in Tables 2.1, 2.2 and 2.3. The results of the study indicate that re-tanning and dyeing (sample no. 5) effluents contained highest BOD₅ and COD values (about 2400 mg/L and 70000 mg/L) due to add many organic vegetable tannin materials and inorganic chemicals (such as mimosa, myrab chobraso and different type of bleaching agent) and de-liming and bating (sample no. 3) effluents contained lowest BOD₅ and COD values (700 mg/L and 7000 mg/L) among the parameters in three industries. These observations were supported by Bajza and Vrcek, (2001), where the results of COD and BOD₅ showed higher values (2000 mg/L and 70000 mg/L) in an Indian tannery industry. The mean value of BOD₅ and COD in composite tannery effluents at monitoring points, P₁, P₂ and P₃ were in the range 820 -920 mg/L and 2700-3980 mg/L, respectively (Tables 2.4, 2.5 and 2.6). The concentrations of BOD₅ and COD in all samples were much higher than the standard limits. Reports showed that BOD₅ and COD values in the tannery effluent were found higher than the tolerance limits for inland surface water. The results of the study illustrate that the high BOD₅ values indicated the high organic load in the effluents. Akan *et al.* (2007) also reported that higher levels of BOD₅ and COD in tannery effluents contained organic and inorganic substances which utilize dissolved oxygen resulting in oxygen depletion, may cause hypoxic conditions with consequent adverse effects on aquatic biota. Under such a condition no aquatic life can survive, except anaerobic micro-organisms.

Further the presence of organic matter could promote anaerobic action leading to the accumulation of toxic compounds in the water bodies (Trivedy and Goel, 1986) and the presence of high amount of inorganic compound which are not affected by the bacterial decomposition.

Table 2.6: The physico-chemical parameters including heavy metals of the composite tannery effluents and compare with the standard limits during November – December in the year 2008-2010

Parameters	Composite Tannery Effluents P₁	Composite Tannery Effluents P₂ (Main drain of leather industrial zone)	Composite Tannery Effluents P₃ (Sluice gate area)	Standard Permissible Limits		
				ISI - 2000	NEQS – 2000 (USEPA)	ISW in BDS
Color (Pt- Co)	Yellowish Brown, 1760±5.5	Light brownish	Light brownish	15	15	15
Odor (TON)	Bad smell	Bad smell	Bad smell	1	1	1
Temperature(° C)	34±0.5	25±0.45	25±0.45	40	40	40
Turbidity (FTU)	750±1.25	-	-		20	-
pH	7.50±0.11	7.1±0.1	7.05±.11	6- 9	6-9	6- 9
EC (mS/cm)	18.65±0.65	8.2±0.54	8±0.54	0.85	0.288	-
TSS (mg/L)	6800±35.35	1200±5.25	1000±5.25	100	150	500
TDS (mg/L)	14000±1.25	7520±1.02	7480±1.01	2100	3500	-
TS (mg/L)	20800±55.01	8720±45.05	8480±40.05	2200	3650	-
Alkalinity(mg/L)	1856±5.52	-	-	500		-
DO ((mg/L)	0.8±0.03	2.1±0.05	2.2±0.06	4.0-6.0	4- 6	-
BOD ₅ (mg/L)	920±15.5	900±10.02	890±10	30	80	250
COD (mg/L)	3980±29.66	3010±20.2	2980±12.02	250	200	400
Sulphate (mg/L)	4000±14.05	1560±5.56	1500±5.25	1000		-
Chloride (mg/L)	5005±2.5	2080±3.2	1900±2.5	600		-
NH ₄ -N (mg/L)	621.36±2.5	-	-	50		-
NO ₃ -N (mg/L)	77.97±5.5	-	-	10	10	-
NO ₂ -N (mg/L)	111.25±2.5	-	-			-
TN (mg/L)	810.58±.55	-	-	100		-
Ca (mg/L)	785±4.12	-	-		200	-
Mg (mg/L)	981±8.5	-	-	200	50	-
Na (mg/L)	3840±37.42	1330±3.5	1300±2.5	-	-	-
Cr (mg/L)	9.81±0.63	8.5±0.54	8.4±0.55	0.1	0.05	2
Pb (mg/L)	5.35±0.19	1.7±0.20	1.6±0.21	0.2	0.01	-
Cd (mg/L)	3.45±0.15	1.2±0.11	1±0.1	0.1	0.05	-
Mn (mg/L)	3.15±0.63	-	-	0.2	0.05	-
Ni (mg/L)	4.21±0.56	-	-	3.0	1	-
Cu (mg/L)	46.88±5.25	-	-	2	1.5	-
Zn (mg/L)	17.78±1.23	-	-	5	5	-
As (mg/L)	1.35±0.12	0.80±0.05	0.75±0.05	0.1	0.05	

ISI-2000 = Indian Standard Institute-2000, NEQS- 2000 = Nature / National of Environmental quality Standards-2000 (USEPA; United State Environmental Protection Agency), ISW-BDS = Inland Surface Water in Bangladesh (GOB, 1997; ECR, 1997).

The study results show that the mean values of DO in composite tannery effluents at monitoring points P_1 , P_2 and P_3 were in the range 0- 2.7 mg/L (Tables 2.4, 2.5 and 2.6). The analysis results show that DO of composite tannery effluents at monitoring point P_1 was e lower than the other monitoring points P_2 and P_3 value. DO contained in different composite tannery effluents at various monitoring points are in the following order: $P_3 > P_2 > P_1$. These values were below the prescribed limits. These DO values were quite unexpected for fish and other living organisms. DO concentration within 5-8 mg/L is fair for aquatic environment and less than 4 mg/L is critical level (Akan *et al.*, 2007 and Alam *et al.*, 2008). Verma *et al.* (2008) suggested that the low DO levels in (≤ 4 mg/L) all collected effluents were the result of high organic pollution, supporting the presence of high levels of BOD_5 in the effluents.

2.4.5. Sulphate and Chloride

The average sulphate and chloride contained of the effluents at manufacturing units in three selected tannery industries were in the range 1000-27000 mg/L and 300-60000 mg/L, respectively (Tables 2.1, 2.2 and 2.3). The study results reveale that liming and unhairing effluents contained very high concentration of sulphate (around 27000 mg/L) because of hues amount of sodium sulphide were used in liming and unhairing process to produce lime pelt. These high levels of sulphate are found in the tannery effluent may be due to the fact that sulphates are compounds of tannery effluent emanating from the use of sulphuric acid or product with high sodium sulphate content. These high concentrations of sulphate in the tannery effluent may also be attributed to the fact that many auxiliary chemicals used in the industries contain sodium sulphate as a by-product of the manufacturer or chrome tanning powders containing high levels of sodium sulphate (Bosnic *et al.*, 2000). On the other hand, soaking and pickling and chrome tanning effluents contained elevated concentration of chloride, which was about around 46000 and 60000 mg/L, respectively.

These values of chloride in the industries under study were higher than the ISW-BDS standard of 600 mg/L for the discharged of tannery effluent into rivers. The high values of chloride in the effluent might be due to the fact that chloride are introduced into tannery effluents as sodium chloride usually on account of the large quantities of common salt used in hides and skins preservation or the pickling process, being highly soluble and stable, they are unaffected by effluent treatment and nature, thus remaining as a burden on the environment (Bhatia, 2005, Bosnic *et al.*, 2000). The present study indicates that the mean value of sulphate and chloride contained in the composite tannery effluents at monitoring points, P₁, P₂ and P₃ were varied from 1100-4000 mg/L and 1810-5005 mg/L, respectively (Tables 2.4, 2.5 and 2.6). The analysis results show that the sulphate and chloride contained in the composite tannery effluents (P₁) were higher than the other monitoring points. These values were very high compare to all standard limits. Though chloride is required for cell functions in plant and animal life, but high level of chloride (>1000 mg/L) in water can cause human illness and also can affect the plant growth.

2.4.6 Ammonium, Nitrite, Nitrate and Total Nitrogen

The mean concentrations of NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and total nitrogen in the composite tannery effluents at monitoring point (P₁) were 621.38, 111, 78 and 810.58 mg/L respectively (Tables 2.4, 2.5 and 2.6). Similar results were obtained for these parameters at different period of the year as the seasonal variations have no effects inside the industry. These values were very high compare to all standard limits. The concentration of all nitrogen species in the collected tannery effluents was considered to pose a significant risk to surface water and to the environment as well.

2.4.7. Quantity of dissolved metal ions concentration

The analysis results of the average concentration of major metal ions such as Cr, Cd, Pb and As in the effluents at different manufacturing units in selected three leather industries were illustrated in Tables 2.1, 2.2 and 2.3. The concentration levels of these parameters were far above the standard permissible limits for effluents discharged. The mean value of calcium (Ca) and magnesium (Mg) in the composite tannery

effluents at monitoring point (P₁) were 785 and 981 mg/L, respectively (Tables 2.4, 2.5 and 2.6). Similar results were obtained during different time of the year (Tables 5 and 6). The present study indicates that the average value of major cations such as Na, Cr, Pb, Cd, Cu and As of composite tannery effluents at monitoring points P₁, P₂ and P₃ were varied from 1200 – 3840 mg/L, 6.4 - 9.81 mg/L, 2.8- 5.35 mg/L, 0.96 - 3.45 mg/L, 6.05 - 46.88 mg/L; 0.67- 2.15 mg/L, respectively. The concentration of metal ions, Mn, Ni and Zn composite tannery effluents (P₁) are shown in Tables 2.4, 2.5 and 2.6. The concentration of metal ions in the tannery effluents at monitoring points, P₁, P₂ and P₃ are in the following order Na> Mg> Ca> Cu> Zn> Cr> Pb> Ni> Cd> Mn> As. On the basis of above discussion it is clear that the concentration of metal ions in all entire monitored sampling points were much contaminated and exceeding the values prescribed by the Standard limits.

The values of all parameters at monitoring point (P₁) were comparatively higher than the other monitoring points (P₂ and P₃) as the point P₁ was a composite mixture of effluents collected from different manufacturing units inside the tannery industry. The values at points (P₂ and P₃) were decreased due to the sedimentation and dilution from the connecting sewerage system.

2.4.8. Characterization of sludge

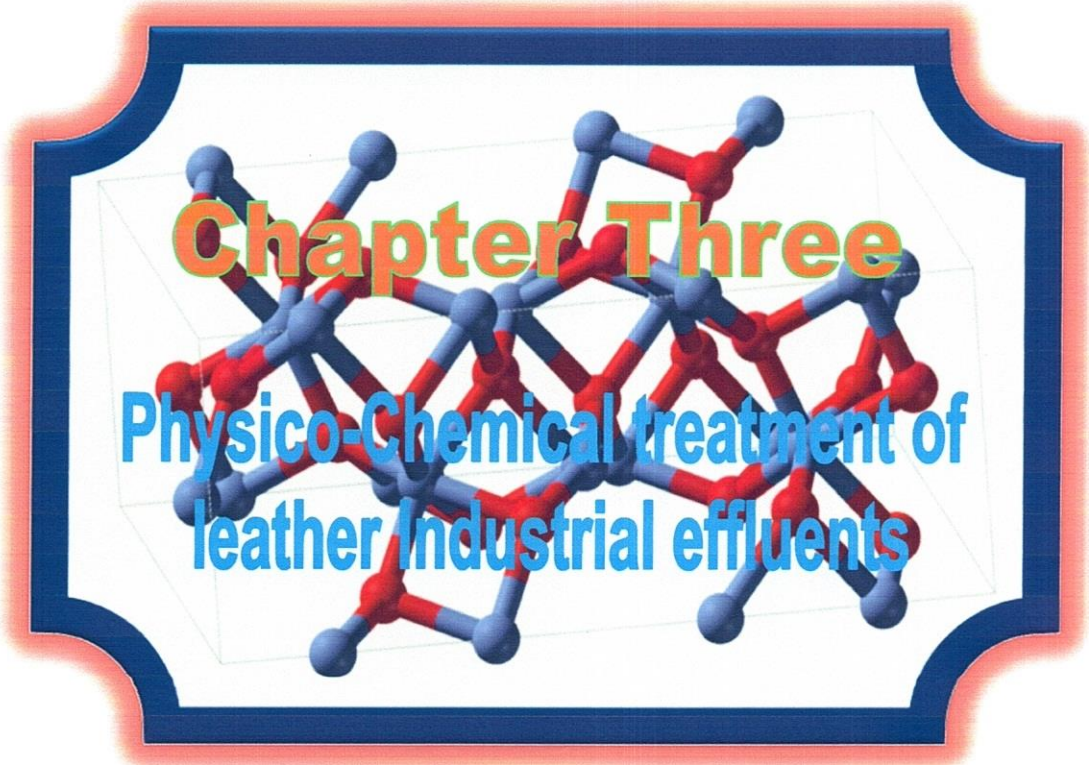
The characterization of collected sludge from sluice gate area (sample 1) and 100 meters from the sluice gate (Sample 2) is shown in Table 2.7.

Heavy metal ions like Zn, Cu, Mg, Cr, Cd, Pb and As were detected in the sludge sample. The percentage of heavy metals in tannery sludge was very higher than that of cultivated soil. Total nitrogen was moderately present in the sludge but the metal ions concentration of Mg, Ca, Pb, Cd and Zn were very high. The average concentrations of ions, Na and Cr in tannery sludge were 6070 to 6050 mg/Kg and 10000 to 9600 mg/Kg respectively which were very higher than the standard limits. Metal ions contained in the sludge are in the following order: Cr > Na > Ca > S > Mg > P > Cu > TN > Zn > Organic compound > Pb > As > Cd.

Table 2.7: Characterization of sludge collected from tannery effluents dumping area at Hazaribagh industrial zone

Parameters (mg/L) except pH and OM	Sample No. 1	Sample No. 2	Standard
pH	7.53	7.8	7.8
Organic matter (%)	9.27	9.02	3.83
Na	6070	6050	0.226-0.30
Ca	234.5	232.5	4.51-6.0
Mg	67.1	65.2	1.126-1.5
TN	22	21	0.271-0.36
P	50.5	49.8	18.1-24.0
S	189	150	27.1-36.0
Cd	0.92	0.9	0.451-0.6
Cu	48.1	39.5	0.451-0.6
Pb	4.7	4.5	0.1-2.0
Cr	10000	9600	0.05- 0.1
Zn	20.02	19.46	0.751-1.125
As	3.5	3.4	0.1

Sample No.1: Rayer- bazer sluice gate area and Sample No. 2: 100 meters from Rayer-bazer sluice gate area OM denoted organic matter.



Chapter Three

Physico-Chemical treatment of
leather Industrial effluents

3. Physico-chemical treatment of leather industrial effluents

3.1. Introduction

Tanning industry is one of the oldest industries in the world. It is typically characterized as pollutants generated industries which produce wide varieties of high strength toxic chemicals. It is recognized as a serious environmental threat due to high chemical levels including salinity, alkalinity, organic load (chemical oxygen load or demand, biological oxygen demand), inorganic matter, dissolved, suspended solids, ammonia, total kjeldahl nitrogen (TKN), specific pollutants (sulphide, chromium, chloride, sodium and other salt residues) and heavy metals etc. (Cooman *et al.*, 2003; Chaudry *et al.*, 1997; Tariq *et al.*, 2005; Apte *et al.*, 2005; Leghouichi *et al.*, 2009; Armienta *et al.*, 2001). Large quantities of water are used in tanning process of which 90% of the water is discharged as effluent. A part of the leather processing, solid and gaseous wastes are also discharged into the environment. During the chrome tanning process, 40% unused chromium salts are usually discharged in the final effluents, causing a serious threat to the environment (van Groenestijn *et al.*, 2002; Leghouichi *et al.*, 2009; Owlad *et al.*, 2008). Exposure to chromium, pentachlorophenol and other toxic pollutants increase the risk of dermatitis, ulcer nasal septum perforation and lung cancer (Carlos *et al.*, 2002). Without any exceptions there is no effluents treatment plant (ETP) in leather tanning industries in the country and moreover, the owners of tannery industries are not much concerned about human health and environmental safety.

The leather industries in Bangladesh are mainly concentrated in Hazaribagh area in the Southwestern part of Dhaka city. More than 200 tannery industries are located in Hazaribagh area, and of them about 90% of tanneries are engaged in chrome tanning process. Daily discharged wastes from all the tanneries in Hazaribagh industrial zone are about 22000 cubic meters of liquid wastes containing 180 mg/L Cr (Shams *et al.*, 2009). Liquid wastes are mostly dumped into the river Buriganga, while parts of these wastes are trapped inside Dhaka flood control embankment. Thus, the treatment of tannery effluents is a matter of great concern for the country. Treatment of tannery effluent is a challenge because of its mixture of biogenic matter of hides, inorganic chemicals and a large variety of organic pollutants with large molecular weights and complex structures (ESCAP, 1982; Elke, 1996; Thorsten, 1997). There are two

different kinds of treatment techniques are being used, i.e., physico-chemical and biological treatments (Repsyte and Simutis, 2004; Bashaar 2004; Wiesmann *et al.*, 2007; Seabloom and Buchanan; 2005; USEPA, 2004). Physico-chemical treatment of tannery effluents are consisted of filtration, coagulation, flocculation, sedimentation, air stripping, chemical precipitation, adsorption, ion exchange, electrochemical (electro-oxidation), and chemical oxidation (Ramesh *et al.*, 2007; Kyung- Sok *et al.*, 2004; Lofrano *et al.*, 2006; Metes *et al.*, 2004; EPA, 2003; UNEP, 2004; Linda and Peter, 1999). Some researchers studied that many toxic chemicals were removed from the tannery effluents using different low-cost adsorbents such as sawdust, silica and iron-oxide wheat shell (Baisali *et al.*, 2005), bagasse fly ash (Gupta and Ali, 2000), fly ash (Bois *et al.*, 2003), spent activated clay (Weng *et al.*, 2007) and modified goethite (Ajmal *et al.*, 1998; Li *et al.*, 2007). Several investigators have used solid supports, including sand, clay, GAC (Fox *et al.*, 1990; Yee *et al.*, 1992), glass beds, rock (Garcia- Calderon *et al.*, 1998) etc for treating the effluents.

Coagulation-flocculation (CF) process has been applied to leather tanning effluents to reduce organic load and suspended solids as well as to remove toxic substances (Ignacio, 1995; Ates *et al.*, 1997; Kabdasli *et al.*, 1999; Lofrano, 2006). In addition, coagulation of chromium can be reduced by chemical precipitation, adsorption, and ion exchange (Rapoport *et al.*, 1995; Tobin *et al.*, 1998; Ramesh *et al.*, 2007). A number of research works on tannery effluent treatment using different technologies, such as flotation, electrochemical treatment, sedimentation, coagulation, filtration, ultra-filtration and reverse osmosis process have been reported (Naumczyk *et al.*, 2005; Ates *et al.*, 1997; Song *et al.*, 2003; Krishnomoorhi *et al.*, 2009).

Coagulation or chemical precipitation has been known for wastewater treatment since the previous century in England where lime was used as coagulant alone and/ or in combination with calcium chloride or magnesium (Genovese and Gonzale, 1998). Different type of chemicals coagulants are added in order to improve and accelerate the settling of suspended solids, especially of fine and colloidal matter. Ayguna *et al.*, (2010) reported that coagulation and/ or flocculation is a commonly used process in water and wastewater treatment, where compounds such as ferric chloride and/or polymer are added to wastewater in order to destabilize the colloidal materials and agglomerate the small particles into larger ones to settle out the flock. Finely

dispersed solids (colloids) suspended in wastewater are stabilized by negative electric charges on their surfaces, causing them to repel each other. Since it prevents these charged particles from colloidizing to form larger masses, called flocks, they do not settle. Coagulation is the destabilization of colloids by neutralizing the forces that keep them apart. Cationic coagulants provide positive electric charges to reduce the negative charge (zeta potential) of the colloids. As a result, the particles collide to form larger particles (flocks). Rapid mixing is required to disperse the coagulant throughout the liquid. The researchers would be taken action for not to be overdose the coagulants as this can cause a complete charge reversal and thus re-stabilize the colloid complex.

Iron-salt is an important coagulant in wastewater treatment and can be used for color removal, organic matter removal and in leachate solid removal from fisheries wastewaters; it is also used for municipal wastewater treatment and surfactant removal from microelectronic plant wastewater. Abdulhassan *et al.* (2006) reported that FeCl_3 was used in coagulation-flocculation process as an effective coagulant for removal of surfactants and COD from microelectronic plant wastewater and the removal efficiencies achieved were 99% and 88% respectively. The report also showed that the rate of COD removal decreased if the pH was lower than 7 or higher than 9. Other researchers also reported that pH is an important parameter for coagulation process since it controls hydrolysis species. When a coagulant such as iron salt is added to water, a series of soluble hydrolysis species are formed. These hydrolysis species have positive or negative charges depending on the water pH. They are positively charged at low pH (< 6) and negatively charged at high pH. The positively charged hydrolysis species can absorb onto the surface of colloidal particles and destabilize the stable colloidal particles or on dissolved natural organic materials(NOM), thus reducing their charge, minimizing the repulsive forces and eventually causing precipitation and particle aggregation, due to stronger van der Waals attraction. This mechanism is called 'charge neutralization'. A precipitate of ferric hydroxide is formed at sufficiently high coagulant doses. These precipitates can physically sweep the colloidal particles from the suspension. This mechanism is called 'sweep-floc coagulation' (Kim *et al.*, 2001; Jian *et al.*, 2001 and Kokila *et al.*, 2011).

Researchers have been searching for alternative methods to reduce chemical usage and cost and use indigenous materials instead of costly chemicals for improving industrial effluent quality. Clay minerals are natural substances used in wastewater treatment and have high adsorption, ion exchange capacity, and catalysis properties as well as low-cost materials. Some researchers reported that clay minerals can be preferable coagulant aid for removal of toxic compounds, pesticide, herbicide, heavy metals and color removal. Some researchers also stated that clay minerals are natural substances used in dairy wastewater treatment for color removal (Hascakir *et al.*, 2008; Errais *et al.*, 2010). Brayant *et al.*, 1992; Zarraa *et al.*, 1995 studied that saw dust was used to remove chromium from tannery effluents. Many researchers reported that in many cases after saw dust filter a chemical pretreatment was needed to activate the sorption sites (Chubar *et al.*, 2004, Batzias, *et al.*, 2004). Hascakir *et al.* (2008) also reported that clay minerals are natural substances used in wastewater treatment for metal ions (Cd and Pb) removal. They also reported that clay minerals are used in coagulant aid for removal of toxic pollutants in waste-water treatment. Rai *et al.* (1998) also reported that non conventional adsorbents such as sand-stone, clay minerals are used for the removal of heavy metals (Cd and Cu) in waste water treatment.

Most of the research reports on tannery effluents in Bangladesh focused on mainly characterization and impact assessment (Das *et al.*, 2006; Mohanta *et al.*, 2010; SEHD, 2002). Only a few studies on tannery effluents treatment so far have been reported in Bangladesh. It is clear that all treatment methods in use have some drawbacks, and there is a need to look for other alternative methods. Leather industrial effluents demonstrates a complicated system containing different components, including pollutants coming from the processed raw materials, chemicals and residues of technological additives used in individual manufacturing operations. The aim of the study was the removal of solids and potentially hazardous substances from tannery effluents using physical and chemical treatment processes. The study used low-cost and indigenous materials such as sand, stone, and sawdust as adsorbent-filtration materials and different type of coagulants such as ferric chloride, ferrous sulphate and ferric oxide to evaluate their performance in tannery effluent treatment. The study also investigated that the cost effective, sustainable and environmental friendly effluent treatment process. The main objective of this part of the study was to

reduce the literature gaps and evaluate the performance before and after each treatment. The other objectives are:

- To achieve better BOD₅, COD, TSS, and TDS removal efficiency
- To achieve better chromium, cadmium, lead, arsenic, sulphate and chloride removal efficiency
- To achieve better nitrogen species removal efficiency
- To produce less sludge
- To consume less chemicals in the treatment process

By achieving the above objectives, the study would definitely be helpful to make more effective efforts to safeguard the environment.

3.2. Materials and methods

3.2.1. Study area and sample collection

The study area and sample collection procedures were the same as stated in the previous chapter.

3.3. Different treatment processes

2 L sample was taken in a beaker and allowed to settle for 24 h. After settling, 1 L supernatant was transfer into another beaker and added 50 mg/L lime to decrease the solubility of cations in the effluent and allowed to settle for a day. Total suspended solids (TSS) were allowed to settle by flock formation. After settling, the supernatant liquids were passed through the three different filtration materials separately to evaluate their filtration efficiency. Filtration process is a primary treatment step to remove organic materials from tannery effluents, including total organic carbon (TOC) and biochemical oxygen demand (BOD₅), and nutrient (ammonia). The efficiency and life span of the process are related to the extent of hydraulic loading and regeneration of the sand-stone and other filters. Four-layer sand-stone filter beds were designed to achieve optimum removal of organic material, BOD₅, COD and nutrients. A report suggested that multiple dosing of sand filters provided superior

performance in the treatment efficiency and filter run time (Kyung *et al.*, 2004). Three different type of filtration materials were used in the filtration process such as (i) sand-stone (ii) saw dust and (iii) combined sand-stone with saw dust. A metallic rack was used for these filtration processes where five earthen pots were placed in different shelf in the rack. The pots in the rack were named as A, B, C, D and E respectively (Fig. 3.1)

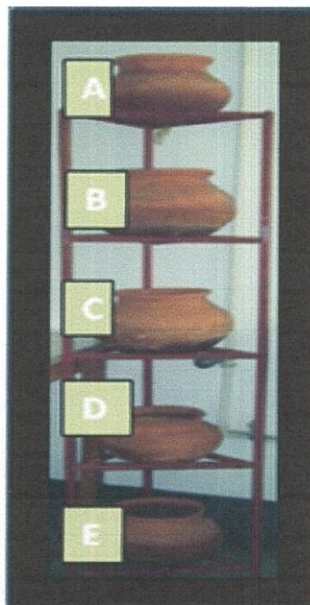


Figure 3.1: Photograph of sand-stone filtration process (SSF)

The filtration materials consisted of layers of sand and stones and each individual layer ranged from 10–12 cm in each pot. For the sand-stone filter, the pots B and C were filled with coarse sands (1 mm diameter) at the top and fine sands (≤ 0.15 mm diameter) at the bottom. The upper layer and lower layer of the pot D were filled with large stone particles (≥ 5 mm diameter) and small stone particles (≤ 5 mm diameter), respectively. A small hole (3mm $\times$ 3 - 4 mm) was drilled at the bottom of pots A to D. A piece of cheese cloth was placed in the hole of each the pots. All the filtering materials were washed three to four times with tap water before putting them in the pot. The decanted waste water from the settling beaker was poured into the top pot (A) on the rack and then allowed to pass successively through pots B, C and D (shown in Fig. 3.1). The flow rate was maintained between 12-14 mL/ min and finally percolated water was collected at the bottom pot, E of the filter (Fig. 3.1). A schematic flow diagram of the effluent treatment process is shown in Fig. 3.2.

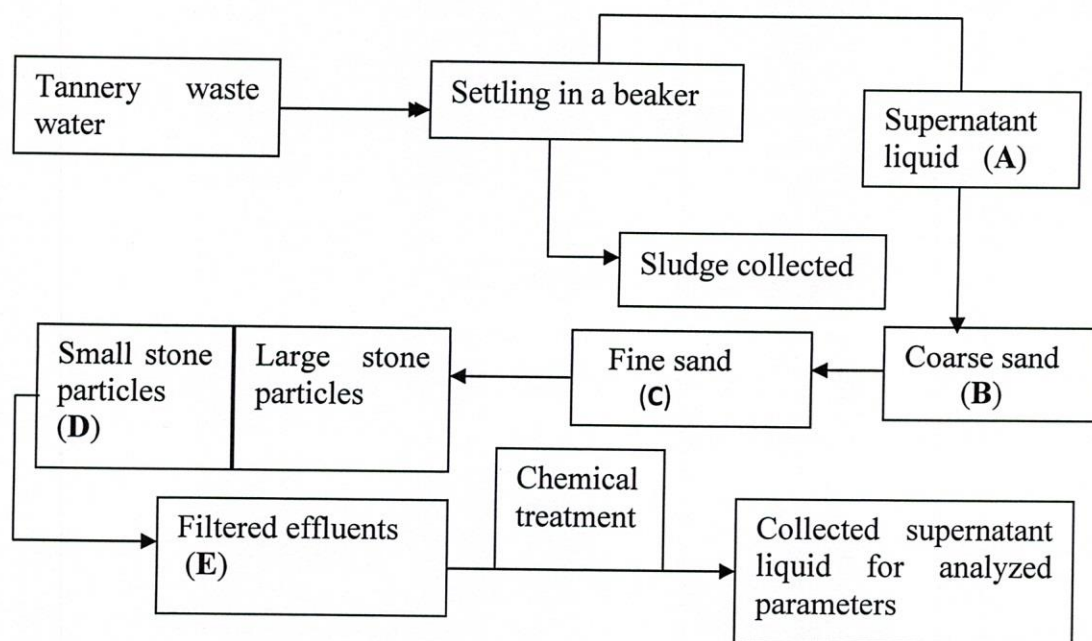


Figure 3.2: Flow chart of physico-chemical treatment process

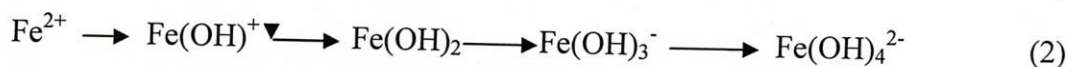
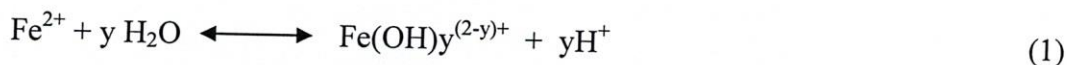
In saw dust filter (SDF) process, white saw dust was used in the pots B, C and D instead of sand and stone in the previous process and carried out similarly as described above.

In combined filter (CF: sand-stone and saw dust) process, the pots B, C and D were filled with saw dust, sand and stones in a definite combination. The upper layer of these pots was filled with saw dust and the lower layers in the pot B, C, and D were filled with coarse sand, fine sand and stones, respectively. In pot D, the lower layer filled with two types of stones, large and small and the small ones remained underneath the large ones. The CF filtration process was carried out similarly as previously described in sand-stone filter (SSF) process.

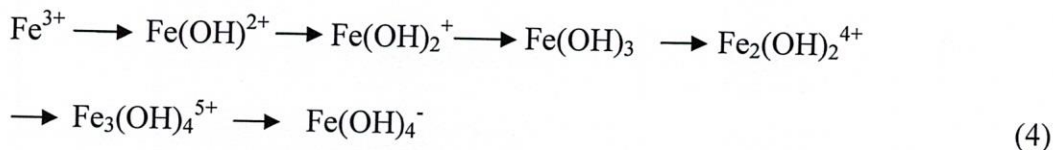
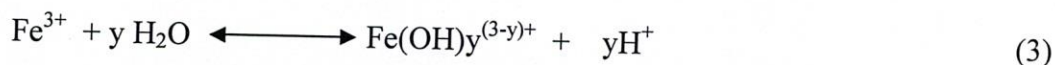
The filtrates obtained from SDF and CF was not considered for chemical treatment as the filtrates were found very dark color and having bad smell. The quality of the filtrates were further deteriorated may be due to the release of organic leachate compounds from SDF and CF filtrate materials during the filtration process. So the results were not good enough to consider as a filtration media. But in case of SSF, the used sand-stone beds were reused and regenerated by cleaning fresh water and boiling

at (100-102°C) with saline water (Basudev *et al.*, 1999). Only the filtrates obtained from SSF were ultimately considered for chemical treatment.

In chemical treatment, batch experiments were carried out involving coagulation, flocculation and sedimentation techniques. The optimization of coagulation process depends on the type of coagulant, its dose, pH and time (Elmaleh *et al.*, 1996; Jiang, 2001; Ali *et al.*, 1992 and Lee and Westerhoff, 2006). The study was conducted using three coagulants namely, FeCl_3 , FeSO_4 and Fe_2O_3 . These coagulants contribute Fe^{2+} and Fe^{3+} ions in aqueous solution. The hydrolysis of Fe^{2+} (in aqueous) was stated by Sweeton and Base (1970) and is shown below



The hydrolysis of Fe^{3+} (in aqueous solution) was illustrated by Zouboulis (proc.) *et al.*, 2007; Base and Mesmer, 1976; Flynn 1984; Jia *et al.*, 1998) and is written as follows:



Equations (1) and (3) are the initial monomeric hydrolysis products of Fe^{2+} and Fe^{3+} can be formed as the hydrolysis proceeds. Equation (2) describes the different monomeric products of Fe^{2+} hydrolysis. Zouboulis *et al.* (2007) described that each step in Equations (2) and (4) involved displacement of a proton with a hydroxyl ion, while an increase of pH value shifted the equilibrium from left to right. Bi *et al.* (2004) also stated that the hydrolysis reactions involved parallelly polymerization reactions which result in a great variety of poly-nuclear species. The hydrolysis products of Fe (II) Fe (III) may adsorb or chemically interact with suspended or dissolved components in the tannery effluents to form complex ions (Zouboulis *et al.*, 2007).

At first, optimizations of coagulant (FeCl_3 , Fe_2O_3 and FeSO_4) doses were determined and then optimization of pH was made. The dependency of pH on coagulation process for the coagulants FeCl_3 and FeSO_4 were examined using pH ranges from 2 to 8 and 3 to 9, respectively. Other coagulants Fe_2O_3 was examined using pH ranges from 4 to 11.5. The pH was adjusted with 1.0 M $\text{Ca}(\text{OH})_2$ or 1.0M HCl . A definite dose of coagulants (50, 70, 100, 150, 200, 250, , 400 and 500 mg/L) was added to the filtered effluents, stirred gently for 15 minutes and then allowed to settle for 1 to 4 days. After settling the supernatants liquid were collected and analyzed for various physico-chemical parameters everyday during the treatment period, and finally the optimization of coagulation period was made. However, this paper presents only the results of 1-day's treatment as the results obtained after two or more days treatment did show any significant difference from those obtained in 1-day. The entire experiments were conducted at room temperature ($25\pm 1^\circ\text{C}$). A schematic diagram of the physico-chemical treatment of tannery effluents is shown in Fig. 3.2.

3.4. Analysis of filtered and chemically treated effluents

The filtrate tannery effluents obtained after passing through different filtration media (filters) and chemically treated effluents were analyzed considering some major physical and chemical parameters. The physical parameters including pH, electrical conductivity (EC), total suspended solids (TSS), and total dissolved solids (TDS) biological oxygen demand in 5 days at 20°C (BOD_5), chemical oxygen demand (COD) of filtered and chemically treated effluents were measured. Other chemical parameters including sulphate (SO_4^{2-}), sodium (Na), calcium (Ca) and heavy metals of filtered and treated effluents were analyzed. Colors (filtered and chemically treated) were measured in Pt-Co color unit. EC and pH values were determined using a portable digital pH meter (Model KRK, KP-5z, Japan) and a digital multi range conductivity meter (Model Hanna HI 9033, Singapore). TSS, TDS and TS were determined gravimetrically (APHA 1998). DO, BOD_5 , COD, SO_4^{2-} , Cl^- , NO_2^- -N, NO_3^- -N, TN, and NH_4^+ -N, were measured as per standard methods of APHA- (2005). Concentrations of Na, Ca and heavy metal ions in the effluent were estimated using AAS (Shimadzu GF-AAS Model AA- 6800) following digestion of the samples with concentrated nitric acid and perchloric acid mixer in the ratio 6:1 (APHA-1998). All chemicals were used of analytical grade reagents and the experiments were performed

at least three times to minimize analytical error. The analysis results were compared with the values of Indian Standard Institute (ISI-2000), Inland Surface Water-Bangladesh Standard (ISW-BDS) and National Environmental Quality Standards (NEQS-United State of Environmental Protection Agency, USEPA-2000) for wastewater discharged to evaluate the efficacy of this work.

Means and standard deviations of triplicate samples were analyzed for physico-chemical parameters of untreated, and physico-chemical treated effluent. Untreated effluents were used as control. Percentage of removal efficiency was calculated as follows:

$$\text{Removal efficiency (\%)} = \frac{C_0 - C_1}{C_0} \times 100$$

where,

C_0 was the concentration of control effluents, and

C_1 was the concentration of treated effluents.

3.5. Results and Discussion

3.5.1. Filtered effluent analysis results

3.5.1.1. Color, appearance and odor

The analysis results (color, odor and appearance) of the filtered with different filtration media; SSF, SDF and CF effluents are shown in Table 3.1. The average color range of the untreated tannery effluent was found to be 1760 Pt-Co units; which was 117 times higher than all the recommended values of standard (15 Pt-Co units).

Table 3.1: Physical characteristics (color, appearance and odor) of filtered effluents

Physical Treatments (different filtration media)	Color units (Pt-Co)	Appearance	Odor
Raw effluent	1760	Yellowish- brown	Disagreeable
Sand-stone filtered	860	Dull-white	Agreeable smell
Saw -dust filtered	1470	Black	Slightly -smell
Combined sand -stone and saw-dust	1400	Dark-brown	Slightly- smell

The color appearance of untreated effluent was found yellowish-brown. The analysis results indicate that the untreated effluents contained highly colored compound which has the potential to pollute the environment. The original color of the effluent (1760 Pt-Co units) was reduced to 860 Pt-Co units after sand-stone filtration, indicating the effectiveness (50%) of the filter media. The color appearance of this filtered effluent was dull-white. The study also used saw dust filter solely and a combination of saw dust with sand-stone to assess the filtration efficiency, but the results of the filtered effluents showed lower efficiencies in removing color and odor than those obtained from the sand-stone filter (Table 3.1). The odor was found very obnoxious smell from the untreated effluent but unobjectionable odor was observed in the filtered effluents. Similar observation was found by Manu *et al.*, 2011. They studied that sand, clay and sand cum clay filtration materials are used in dairy effluents treatment. They also reported that sand filtered effluents color was clear and odor was unobjectionable.

3.5.1.2. pH and EC

The analyses results of untreated (raw effluent) and filtered effluents are shown in Table 3.2. The results indicate that the average pH values of the untreated (control) and treated (filtered) effluents were 7.5, 7.95, 8 and 8.1, respectively.

After sand- stone filtration process, pH slightly increased due to add $\text{Ca}(\text{OH})_2$ during the settling period. In settling, controlled effluents contained pH-8.5 and after filtration, the effluents pH slightly decreased which indicates that the adsorption of minerals during percolation through filtration media. Manu *et al.* (2011) reported that untreated dairy effluents contained pH 8.64 and after sand and clay filtrations, pH of the filtered effluents decreased slightly, which were found to be 7.91, 7.42 respectively. EC (at 25°C) of the untreated tannery effluents (control) observed was 18.65 mS/cm which was about 7 times higher than the prescribed limit for ISI-2000. Reports showed that EC of untreated effluents usually contained about 20 mS/cm (Mandal *et al.*, 2010) supporting the results of this study. The analyses results indicate that the removal efficiency of sand-stone filtered effluent obtained was 33% and the value was 12.68 mS/cm, which was very high compare to ISI (2000) standard limit.

Table 3.2: The physico-chemical characteristics including metal ions of tannery effluents after filtered with different filtration media and compare with untreated effluents

Parameters	Untreated effluents (raw effluents)	Sand-stone filtered effluents (SSF)	Saw –dust filtered effluents (SDF)	Combined sand –stone and saw-dust filtered effluents (CF)
pH	7.5 ± 0.11	7.95 ± 0.11	8 ± 0.01	8.10 ± 0.09
EC (mS/cm)	18.65±0.69	12.68 ±0.56	20 ±1.41	27 ±0.89
TSS (mg/L)	6800 ±35.35	4800 ±28.9	5400 ±49.18	6000 ±31.62
TDS (mg/L)	14000± 0.99	10500 ±60.60	16000 ±61.68	20000 ±70.75
TS (mg/L)	20800±55.01	15300± 45.05	21400± 50.35	26000± 60.37
DO (mg/L)	0.8±0.03	-	-	-
BOD ₅ (mg/L)	920 ±15.81	810 ±9.35	1505±11.94	2400 ±24.49
COD (mg/L)	3980 ±29.66	3500 ±15.81	6800±30.90	14500±100
NH ₄ -N(mg/L)	621.36±2.5	621±0.5	630±1.2	621±1.2
NO ₃ -N(mg/L)	77.97	70±2.5	80±1.05	80±1.1
NO ₂ -N(mg/L)	111.25	111±0.9	114±0.75	112±0.65
TN(mg/L)	810.58	801±1.2	823±1.3	812±1.5
SO ₄ ²⁻ (mg/L)	4000±14.32	3350±30.82	3510 ±13.04	3500 ±15.81
Cl ⁻ (mg/L)	5005±13.05	3720±25.06	1350±7.05	1370±5.06
Na (mg/L)	3840 ±37.42	3050 ±41.23	3000±18.71	3005 ±8.37
Ca (mg/L)	785 ±4.12	750±3.81	730±3.81	720 ±1.58
Cr (mg/L)	9.81±0.63	9.78±0.12	6.8±0.19	7.8 ±0.14
Pb (mg/L)	5.35±0.19	4.55 ±0.07	5.2 ±0.15	4.75 ±0.14
Cd (mg/L)	3.45 ±0.045	3.15±0.10	3.4±0.16	3±0.09
Zn (mg/L)	17.78±1.23	15.5±.06	15.38±2.65	15.17±0.9
Cu (mg/L)	46.66±5.25	44.65±2.25	30.05±2.5	29.05±.85
Ni (mg/L)	4.21±0.56	4±.06	8.53±2.10	8.35±1.2
Mn (mg/L)	3.15±0.63	3±.6	3.1±0.2	3±0.05
As (mg/L)	1.35±0.12	0.85±0.11	1.3 ±0.19	1.29±0.12

This study results show that the other filtration processes were not so effective compare to SSF (Table 3.2). Manu *et al.* (2011) studied on the treatment of dairy effluent using sand, clay and sand cum clay as filtration materials and the results showed that the highest EC removal efficiency of sand filter was found using sand filter and it was about 35%. They also stated that the other two filtration materials could also improved the quality of dairy effluent by reducing EC. The results were almost the same compared to the present study.

3.5.1.3. TSS, TDS and TS

The mean TSS and TDS values of the untreated tannery effluent (control) were 6800 and 14000 mg/L, respectively, which were very high compared to the prescribed limits for ISI-2000 and ISW-BDS. The analysis results indicate that the average TSS values of three filtered effluents were 4800, 5400 and 6000 mg/L, for SSF, SDF and CF, respectively (Table 3.2), which was very high compared to those of prescribed limits for ISI and ISW-BDS. The mean TSS value after filtration was still very high indicating that sand-stone filtration process alone was not sufficient enough to reduce the values below the standard permissible limits. The study results revealed that SSF process has showed the best TSS removal capacity compare to the other filtration media. The mean TDS and TS values of sand-stone filtered effluents obtained were 10500 and 15300 mg/L, respectively. The study results reveal that SSF filters removed substantial amounts of TDS, TSS and TS from the tannery effluents due to adsorption of soluble and suspended materials on the minute sand and stone particles surface (Manu *et al.*, 2011). Previous researchers showed that sand filters significantly removed TS and organic waste present in the dairy effluents. They also illustrated that sand filtered effluents removed 29% TDS from the dairy effluent. The present study also revealed that SDF and CF filtration processes were not effective for TDS and TS removal.

3.5.1.4. DO, BOD₅ and COD

The mean values of DO, BOD₅ and COD of the tannery effluents (control) were 0.8, 920 and 3980 mg/L, respectively (Table 3.2), these values were quite unexpected for fish and other aquatic lives. The analyzed results illustrated that BOD₅ and COD

values were higher than those of prescribed standards for the ISI and ISW-BDS (ECR 1997; GOB 1997).

The average values of BOD₅ and COD for the filtered effluents of three filtration materials, i.e., SSF, SDF and CF are shown in Table 3.2. The study results reveal that the only filtration process was not good enough for BOD₅ and COD removal suggesting the need for further treatment using suitable chemicals.

3.5.1.5. NH₄-N, NO₃-N, NO₂-N and TN

The analyses results indicate that the mean concentration of NH₄-N, NO₃-N, NO₂-N and TN in filtered effluents, i.e., SSF, SDF and CF were found almost the same as were obtained in the untreated effluents (Table 3.2). The study results show that these filtration processes were not so effective for removing nitrogen containing compounds suggesting the need for further treatment using suitable chemicals. Another study carried out on dairy effluent showed that nitrogen compounds were not removed by sand filter (Manu *et al.*, 2011), which supported this study.

3.5.1.6. SO₄²⁻ and Cl⁻

The study observed that the concentration of SO₄²⁻ in the filtered effluents, i.e., SSF, SDF and CF) were found to be 3350, 3510 and 3500 mg/L, respectively (Table 3.2). The study results show that the filtration processes were less effective for SO₄²⁻ removal. The analysis results indicate that the mean chloride contained of SSF, SDF and CF filtered effluents were 3720, 1350 and 1370 mg/L, respectively. The results illustrate that SDF and CF filtration media removed chloride 72 and 73% respectively from the tannery effluents, but SSF process has less effective for chloride removal (26%) compare to the others filtration materials (Table 3.2). Manu *et al.* (2011) stated that Cl⁻ concentration in sand filter treated dairy effluents was 12.27 mg/L and removal efficiency obtained was 38%.

3.5.1.7. Metal ions concentration

The highest metal ions concentrations in the untreated effluent were found for Na and Ca and their mean values were 3840, and 785 mg/L, respectively (Table 3.2). The analysis results of the average concentration of major metal ions of untreated and

filtered effluents were illustrated in Table 3.2. The study results of the filtrated effluents show that all the filtration processes were not suitable to remove Na, Ca and heavy metal ions. The study results reveal that only SDF filter removed Cu and Cr ions were 35 and 31%, respectively, but the other two filters were not achieved any significant removal efficiency. Ajmal *et al.* (1996) studied on industry wastewater to remove Cr ions using phosphate treated saw dust as filtration materials and the result showed that the removal efficiency achieved was 87%. This study results illustrate that saw dust has the better capability to remove Cu and Cr ions compared to sand filtration materials.

3.5.2. Chemically treated effluent

The filtered effluents were then treated separately with three selected coagulants, i.e., FeCl_3 , Fe_2O_3 and FeSO_4 , and the results are discussed below:

3.5.2.1. FeCl_3 treated effluent analysis results

(i) Color, appearance and odor

The study results showed that physical parameters (color, odor and appearance) of FeCl_3 (50-500 mg/L) treated effluents are shown in Table 3.3.

The analysis results revealed that the color value of 150 mg/L FeCl_3 treated effluents was found to be 15 Pt-Co which was within the permissible limit for ISW-BDS and other standards limit. Apparently clean effluents were found after treatment with different doses FeCl_3 (70-250 mg/L).

When the coagulants (FeCl_3) doses increased above 250 mg/L, then the effluent's color turned clear to light brownish and brownish. The study results show that 150 mg/L of FeCl_3 dose was the most effective for color removal which was about 99% (not shown in Table 3.3). Some reports also showed that FeCl_3 is an important coagulant in wastewater treatment and can be used for color removal (Aygün *et al.*, 2011; Manu *et al.*, 2011). Ali *et al.* (2008) studied on industrial wastewater treatment using various steps including screening, coagulation, flocculation, aeration, biological treatment, filtration (sand) and ion exchange and the results obtained after all these treatment steps showed that the effluent was found clear and the color removal efficiency was 98%.

Table 3.3: The physical characteristics (Color, appearance and odor) of different doses of coagulants (FeCl_3 , Fe_2O_3 and FeSO_4) treated effluents in a day 1.

Chemical Treatments (various doses of different chemicals, mg/L)	Color units (Pt-Co)			Appearances			Oder		
	FeCl_3	Fe_2O_3	FeSO_4	FeCl_3	Fe_2O_3	FeSO_4	FeCl_3	Fe_2O_3	FeSO_4
50	400	360	410	DW	DW	DW	AGS	AGS	AGS
70	300	280	315	Clear	Clear	Clear	AGS	AGS	AGS
100	286	5	290	Clear	Clear	Clear	AGS	AGS	AGS
150	15	25	15	Clear	Clear	Clear	AGS	AGS	AGS
200	30	80	85	Clear	Clear	Clear	AGS	AGS	AGS
250	100	120	220	Clear	Clear	Clear	AGS	AGS	AGS
400	450	400	460	LB	LB	LB	AGS	AGS	AGS
500	540	530	640	Br	Br	Br	SS	SS	SS

DW: Dull- white color, AGS: Agreeable smell, LB: Light brownish, Br: Brownish and SS: Slightly smell

Another study was carried out using combine coagulant (alum and FeCl_3) and the results showed that the treated effluent color was clear and color removal efficiency was 87%. The present study results show that the color removal efficiency was found better compare to the previous literature reports. Color responses from all treated effluents were significantly different from those of untreated effluents. The odor was found very obnoxious smell in untreated effluent but unobjectionable odor was observed after treatment with different doses of FeCl_3 .

(ii) pH and EC

pH of the FeCl_3 (50-500 mg/L) treated effluents are illustrated in Table 3.4. This study results observe that pH of the FeCl_3 treated effluents was decreased with increasing FeCl_3 doses as expected (Fig. 3.3).

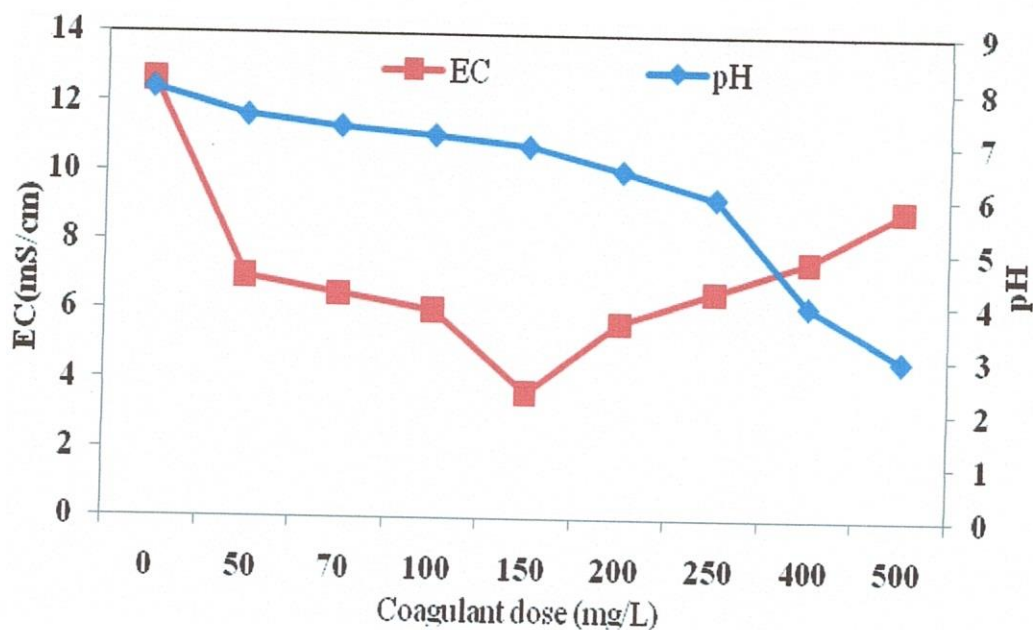


Figure 3.3: Effect of coagulant (FeCl_3) doses on pH and EC

Similar observation was reported by Islam *et al.* (2011). pH is an important parameter since the coagulation occurs within a specific pH for each coagulant (Aygün *et al.*, 2010). EC values of FeCl_3 (50-500 mg/L) treated effluents are shown in Table 3.4. The analysis results observe that the EC value of FeCl_3 treated effluents decreased sharply with increasing FeCl_3 doses until reached at the lowest EC value around 4 and then it slowly increased with FeCl_3 doses (Fig.3.3). The lowest value of EC was observed in 150 mg/L FeCl_3 treated effluents indicating the efficiency of the coagulant dose (Fig. 3.3). The values of EC is directly related with ionic concentrations, hence the results indicate that the coagulant (FeCl_3) was capable of removing ions from the tannery effluents. Lofrano *et al.* (2006) illustrated that 900 mg/L FeCl_3 treated effluents showed maximum removal efficiency for EC at pH 8.5.

The results also showed that the higher coagulant (FeCl_3) dose was unable to remove significant amount of other study results showed comparatively better removal efficiencies in removing pollutant parameters at a dose of 150 mg/L FeCl_3 and near neutral pH.

Table 3.4 Physico-chemical characteristics of FeCl₃ treated tannery effluents

Parameters	Coagulant dose FeCl ₃ (mg/L)									
	50	70	100	150	200	250	400	500		
pH	7.5±0.19	7.3±0.09	7.15.1	6.95±0.19	6.5±0.19	6±0.07	4±0.15	3±0.04		
EC (mS/cm)	7±0.19	6.5±0.05	6.05±0.25	3.68±0.19	5.7±0.25	6.6±0.28	7.5±0.22	9±0.24		
TSS (mg/L)	500±2.39	300±0.5	120±3.54	0±0.07	200±3.61	450±3.08	650±1.41	1050±1.41		
TDS(mg/L)	5661±7.09	5400±1.2	5322±3.03	3575±3.53	5050±3.53	5400±1.41	6071±2.91	8050±1.48		
TS (mg/L)	6161±10.5	5700±2.01	5442±10.0	3575±.09	5250±8.60	5990± 9.61	6721±3.56	9100±9.85		
BOD ₅ (mg/L)	650±1.48	600±1.5	560±3.81	200±1.41	350±3.61	525±3.08	600±3.81	710±3.54		
COD(mg/L)	1230±3.54	1000±0.56	930±7.87	300±1.58	560±1.41	730±1.58	1205±3.08	1450±1.41		
NH ₄ -N(mg/L)	320±2.02	250±0.05	150±1.5	28.29±.5	120±0.1	240±2.05	500±0.4	640±.3		
NO ₃ -N(mg/L)	67±0.02	60±0.8	50±1.03	35.7±0.2	48±0.05	65±2.0	73±0.5	80±0.36		
NO ₂ -N(mg/L)	75±2.0	70±1.25	68±1.0	46±0.3	60±0.03	65±2.1	90±0.2	92±0.52		
TN(mg/L)	462±2.01	380±1.5	268±1.23	109.9±1.1	228±0.1	370±2.03	663±0.5	812±0.6		
SO ₄ ²⁻ (mg/L)	1050±3.08	850±1.32	650±1.58	462±1.41	657±1.82	730±1.58	850±3.7	1260±3.81		
Cl ⁻ (mg/L)	1250±8.05	1180±1.5	1100±10.6	1000±5.35	1580±6.5	1680±5.35	2050±8.5	2350±2.9		
Na (mg/L)	1230±3.81	1170±1.85	1050±3.81	460±1.58	860±3.61	955±5.43	1150±3.54	1450±3.81		
Ca(mg/L)	360±3.08	300±1.75	250±1.58	140±3.81	210±3.08	560±3.81	610±1.41	690±1.58		
Cr(mg/L)	3.65±0.11	2.5±0.5	1.75±0.11	0.35±0.01	0.65±0.04	2.35±0.08	3.5±0.16	6.5±0.14		
Pb(mg/L)	2.5±0.25	1.5±0.08	0.79±0.01	0.67±0.03	1.2±0.11	2.67±0.12	3±0.14	3.45±0.05		
Cd(mg/L)	1.05±0.13	0.89±0.05	0.69±0.03	0.25±0.06	0.75±0.04	2.6 ±0.08	3.05±0.08	3.05±0.06		
Zn(mg/L)	10±.5	5.5±0.05	3.05±0.06	2.5±0.8	4.5±0.09	6.5±0.096	8.66±0.6	15.66±0.2		
Cu(mg/L)	12.5±3.2	10.05±0.05	6.5±0.45	2.5±0.05	3.89±0.5	9.6±0.5	10.69±3.2	17.69±2.6		
Ni(mg/L)	3.6±0.2	2.05±0.08	0.98±0.3	0.85±0.25	1.8± 0.2	2.5±0.05	3.0±0.56	3.5±0.5		
Mn(mg/L)	2.8±0.5	2.01±0.085	1.8±0.6	1±0.06	2±0.06	3±0.05	3.1±0.02	3.15±0.06		
As(mg/L)	0.80±0.04	0.7±0.05	0.65±0.04	0.1±0.04	0.35±0.08	0.5±0.04	1 ±0.01	1.23±0.08		

(iii) TSS, TDS and TS

The treated effluent concentrations of TSS, TDS and TS at different FeCl_3 doses are illustrated in Fig. 3.4. The results show that in the beginning TSS, TDS and TS decreased with increasing FeCl_3 doses until they reached at a certain lowest value and then increased with FeCl_3 . The study results reveal that 150 mg/L FeCl_3 treated effluents showed the lowest TSS concentration which was well below the prescribed standard limits. 150 mg/L of FeCl_3 treated effluents showed the lowest TDS and TS values which were found to be the same, i.e., 3575 mg/L (Fig 3.4).

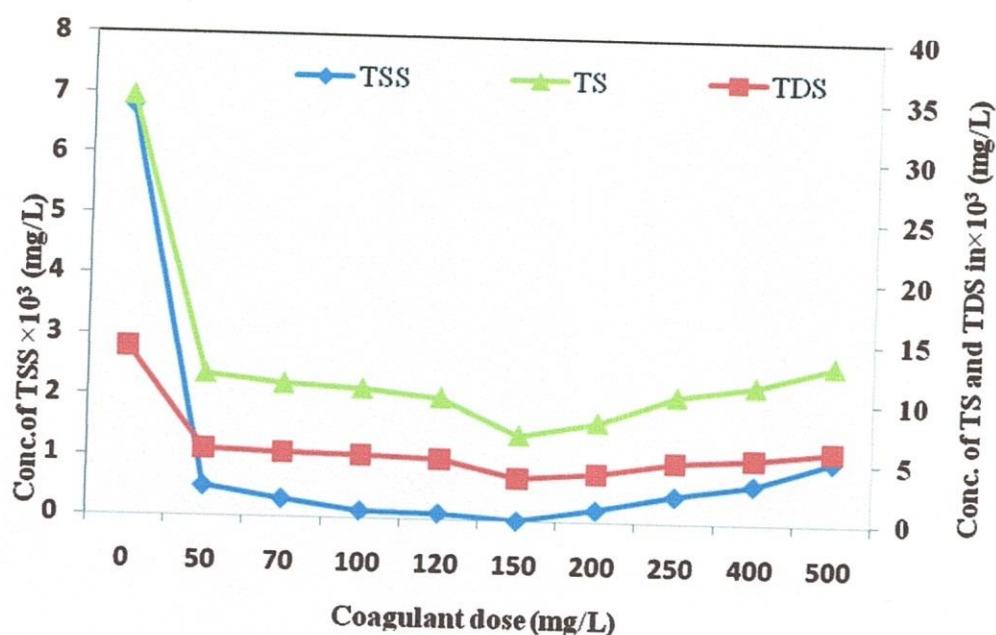


Figure 3.4: Effect of coagulant (FeCl_3) doses on TSS, TS and TDS

The lowest values of these parameters were found in 150 mg/L of FeCl_3 treated effluents. But TDS and TS values were still far above the standard limits for all treatment doses. The study results reveal that the TSS concentration was almost “zero” (0) mg/L and the removal efficiency was 100% at a coagulant (FeCl_3) dose of 150 mg/L indication of the suitability of the coagulant dose. Manu *et al.* (2011) indicated that concentration of TSS, TDS and TS for sand filtered dairy effluent treated with 200 mg/L FeCl_3 was found to be 100.83, 141.20 and 242.03 mg/L and the removal efficiencies were 78, 80 and 79 %, respectively. Islam *et al.*, 2011 also reported that alum and FeCl_3 treated effluents contained lowest TDS value which was found to be 4200 mg/L. In comparison with above literature reports, this study results

show comparatively better removal efficiencies in removing the parameters at a dose of 150 mg/L FeCl_3 and near neutral pH.

(iv) BOD_5 and COD

The mean values of BOD_5 and COD in FeCl_3 (50-500 mg/L) treated effluents are shown in Table 3.4. The effluent concentrations of BOD_5 and COD at different FeCl_3 doses are illustrated in Fig. 3.5. The results show that in the beginning BOD_5 , COD decreased with increasing FeCl_3 doses until they reached at certain the lowest values and then increased with FeCl_3 doses. The lowest values of BOD_5 (200 mg/L) and COD (300 mg/L) were found in 150 mg/L FeCl_3 treated effluents which were within the prescribed limits for ISW- BDS.

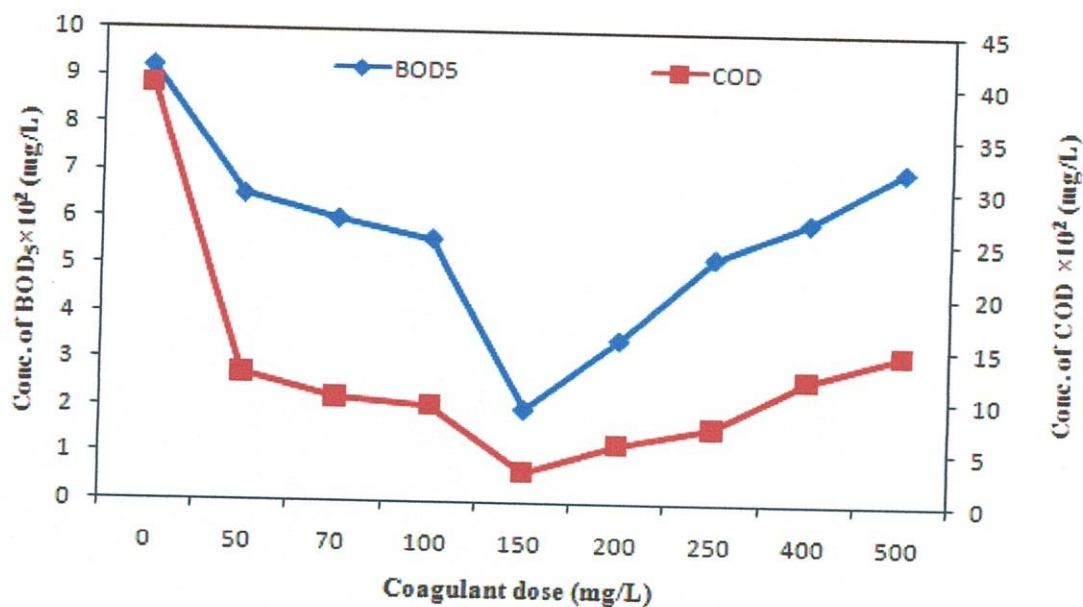


Figure 3.5: Effect of coagulant (FeCl_3) doses on BOD_5 and COD

Aboulhassan, *et al.* (2006) reported that FeCl_3 is an important coagulant for COD removal. They also found that the rate of COD removal decreased if the pH was lower than 7 or higher than 9. Many researchers observed that ferric chloride is a better coagulant in removal of BOD_5 , and COD from the tannery effluents in an optimum condition (Islam *et al.*, 2011). Manu *et al.* (2011) also studied that sand + 200 mg/L ferric chloride treated effluents were removed 82, 83 %, for BOD_5 and COD respectively, from the dairy effluents. Mahvi *et al.* (2004) also stated that FeCl_3 were used as a coagulant, and COD removal efficiency was 89%. The analyses results

revealed that 150 mg/L coagulant dose showed the best removing efficiency for BOD₅ (78%), and COD (92%). This study results observed comparatively better removal efficiencies in removing the parameters at an optimum dose chemicals (150 mg/L of FeCl₃ at near neutral pH).

(v) NH₄-N, NO₃-N, NO₂-N and TN

The mean values of NH₄-N, NO₃-N, NO₂-N and TN in FeCl₃ (50-500 mg/L) treated effluents are illustrated in Table 3.4. The effluents concentrations of NH₄-N, NO₃-N, NO₂-N and TN at different FeCl₃ doses were accumulated in Fig. 3.6. The study results reveal that in the beginning NH₄-N and TN were decreased sharply with increasing FeCl₃ doses until they reached at a certain peak values of NH₄-N and TN and then slowly increased with FeCl₃ doses.

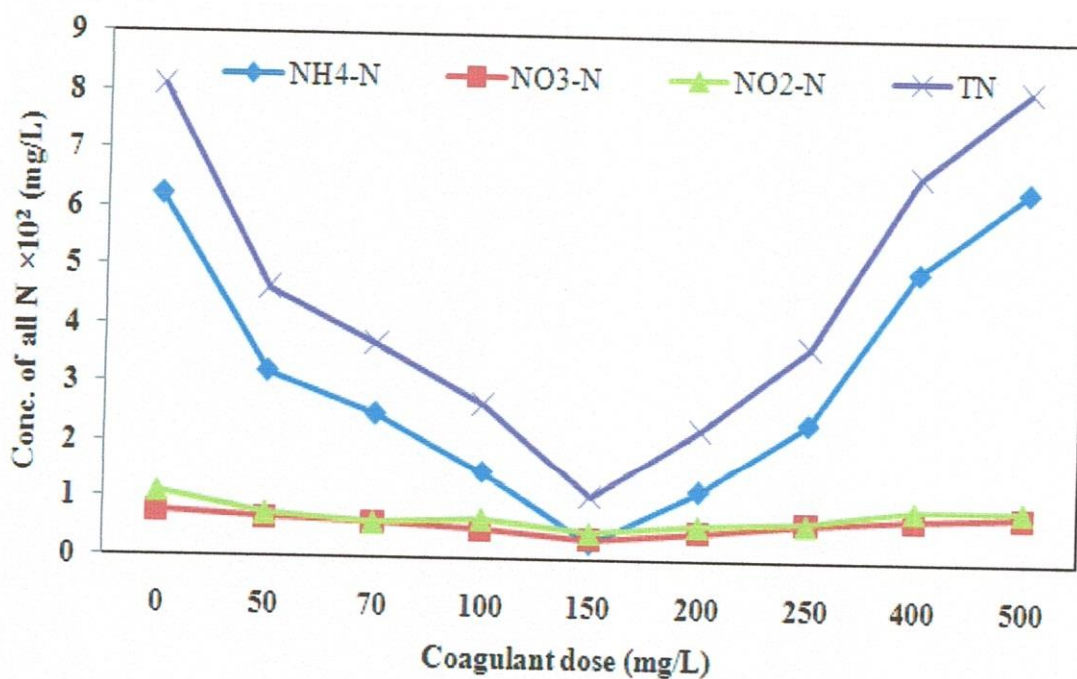


Figure 3.6: Effect of coagulant (FeCl₃) doses on NH₄-N, NO₃-N, NO₂-N and TN

The lowest values of all nitrogen compounds were found in treatment with 150 mg/L FeCl₃ doses (Fig. 3.6). So the study results indicate that FeCl₃ is capable of removing excess nutrients (ammonium nitrogen compounds) from the effluents. The analysis results showed that the concentration of NH₄-N and TN in 150 mg/L FeCl₃ treated effluents were found to be 28.29, 109.9 mg/L which were removed 95, and 86 %, respectively.

respectively. But the values of all N containing compounds (except $\text{NH}_4\text{-N}$) were still far above the standard limits for all treatment doses suggesting the need for further treatment before discharged the effluents into the environment. Manu *et al.*, (2011) demonstrated that 200 mg/L FeCl_3 coagulant is better in removal of nutrients from the effluents. They also demonstrated that ferric chloride treated effluents were removed 74 % for N from the dairy effluents. Mahvi *et al.*, (2008) also stated that high N removal in sequencing batch reactor could be achieved.

(vi) SO_4^{2-} and Cl^-

The mean values of SO_4^{2-} and Cl^- in FeCl_3 treated effluents are shown in Table 3.4 which was found in relation between FeCl_3 doses and contained values of SO_4^{2-} and Cl^- . The analyses results reveal that 150 mg/L FeCl_3 treated effluent showed the lowest Cl^- value (1000mg/L). But the value of Cl^- contained still far above the standard limits for all treatment doses. Manu *et al.*, 2011 reported that 200 mg/L ferric chloride treated effluents contained Cl^- was found to be 10.83 mg/L which was Cl^- removal efficiency 49% from the dairy effluents. Islam *et al.*, 2011 also stated that alum and FeCl_3 treated effluents could be removed Cl^- only for 11%. Coagulant FeCl_3 removed SO_4^{2-} efficiently from effluents and the lowest SO_4^{2-} concentration (462 mg/L) in the effluent was observed in treatment with 150 mg/L FeCl_3 dose (Table 3.4). The results showed that maximum sulfate removal efficiency achieved was 88.45% and the value was well below the standards permissible limits of ISW-BDS and other International standards.

(vii) Metal ions concentration

The mean concentration of Na, Ca, Cr, Pb, Cd, Zn, Cu, Ni, Mn and As ions in FeCl_3 (50-500 mg/L) treated effluents are illustrated in Table 3.4. The study results observe that 150 mg/L FeCl_3 treated effluents contained the lowest Na value which was found to be 460 mg/L. The value was still far above the standards limits, suggesting the need for further treatment. The analysis results also observed that 150 mg/L FeCl_3 treated effluents contained the lowest value of major ions: Ca, Cr, Zn, Ni and As which were found to be 140, 0.35, 2.5, 0.85 and 0.1 mg/L, respectively (Table 3.4). These values were within the standards permissible limit of ISW-BDS. The other metal ions, e.g., Pb, Cd Cu and Mn concentration were found to be 0.67, 0.25, 2.5 and 1mg/L,

respectively after treating with 150 mg/L FeCl_3 (Table 3.4). Many researchers stated that FeCl_3 coagulants have higher treatment efficiency for heavy metal ions at a specific condition from tannery effluents (Mikami *et al.*, 1984; He *et al.*, 1987; Wu and Liu, 1989; Du *et al.*, 1990). Ajmal *et al.*, (1993 and 2001) reported that heavy metal ions such as Cr, Ni, Cu, Cd, and Zn had been removed from the electroplating wastes using Fe (III) salt as adsorbent. They also reported that the maximum removal efficiency of these metal ions (79%) at definite pH 8-9.

(viii) Influencing factors on treatment processes

(a) Effect of coagulant dose

Various amount of FeCl_3 (50-500 mg/L) doses were applied to observe the optimum coagulant dose at definite pH (7) and room temperature ($25 \pm 1^\circ\text{C}$) for the treatment of wastewater. The effect of coagulant (FeCl_3) dose on the removal efficiency of different parameters (BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd) are shown in Fig. 3.7. The figure illustrates that the graphs show a common trend, where the removal efficiency (%) for different parameters initially increased with doses until the efficiencies got maximum and then decreased with doses.

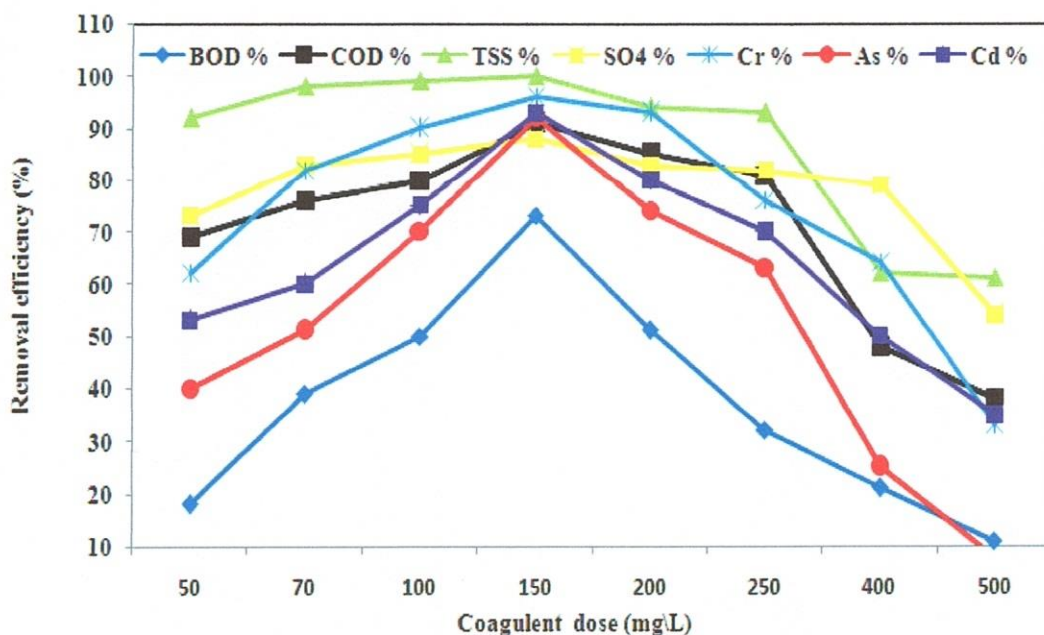


Figure 3.7: Effect of coagulant (FeCl_3) doses on the removal efficiency of different parameters (BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd)

The highest removal efficiencies for different chemical parameters achieved were 78% (BOD₅), 92% (COD), 100% (TSS), 88% (SO₄²⁻), 96% (Cr), 93% (As), and 93% (Cd). The results of this study reveal that the poor coagulation obtained at lower coagulant doses as expected and also at higher (>150 mg/L) coagulant dose. This phenomenon could be happened as the coagulant was not adequate to form flocks or to bind the colloid particles to form bridging. At higher doses (more than 150 mg/L) of the coagulant, the colloid particles become suspended due to electrostatics repulsion between colloid particles and coagulant and in low flock appearance.

Islam *et al.*, (2011) described that the effluents particles were surrounded by a large quantity of coagulant particles, when coagulant dose was very high and as a result their surfaces were saturated resulting less possibility to combine with each other and thus reach another state of stability. Some researcher's reports showed that the inorganic sludge production was increased with increasing FeCl₃ concentration (Mandal *et al.*, 2010; Naumczyk *et al.*, 2005). The present study observed that 150 mg/L coagulant (FeCl₃) dose showed maximum removal efficiency. A high coagulant dose in effluent treatment process increase operational costs and produce a large amount of sludge. In this study, the coagulant amount used was relatively smaller than used in the previous studies (Manu *et al.*, 2011; Amuda *et al.*, 2006, Lofrano *et al.*, 2006), suggesting minimum sludge production in one hand and reduce cost and environmental burden on the other hand.

(b) Effect of pH

The study carried out in the pH range from 2 to 8 to determine the optimum pH value at a definite coagulant dose (150 mg/L FeCl₃) and room temperature (25±1°C). The effect of pH on removal efficiency (%) is shown in Fig. 3.8. The maximum efficiencies for different parameters were found at neutral pH area and they gradually reduced on both sides of the pH. The colloid substances in the leather industrial effluents usually carry negative electrical charges, whereas coagulant (FeCl₃) possesses a lot of Fe³⁺ ions. Around neutral pH, Fe(III) are hydrolyzed into mononuclear and multi nuclear hydroxyl complex ions, which have the capability of adsorbing particles and formed electric double layer for destabilization of the particles (Ratnaweera *et al.*, 1992).

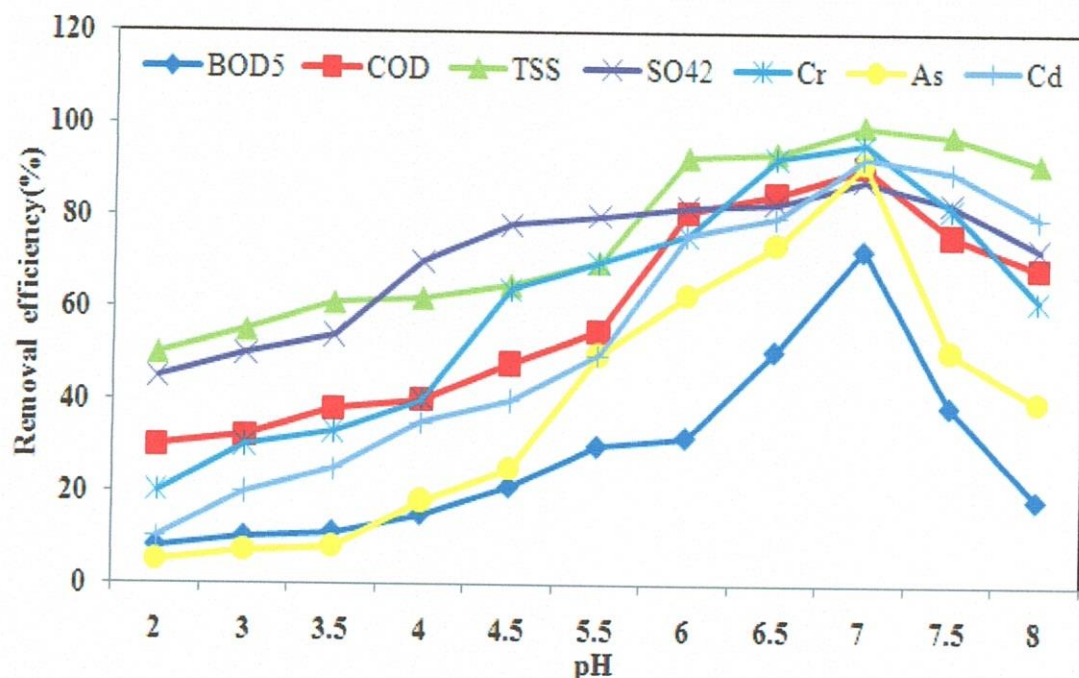


Figure 3.8: Effect of pH on removal efficiency of BOD₅, COD, TSS, SO₄²⁻, Cr, As and Cd for coagulant FeCl₃

The study results illustrate that the maximum removal efficiencies were 78, 92, 100, 88, 96, 92 and 92 % for BOD₅, COD, TSS, SO₄²⁻, Cr, As and Cd, respectively. The study suggests that the lower coagulation efficiencies for the parameters observed at low pH which may be attributed to the competition between protons and cations for common binding sites and also colloidal re-stabilization caused by charge reversal at the colloidal surface. Jia *et al.*, (1998) stated that Fe³⁺ ions did not exist in solutions around neutral pH and a range of hydrolysis products [Fe(OH)₂⁺ and Fe(OH)₂⁺] are responsible for the de-stabilizing effects on colloid impurities. Different hydrolysis products can cause different treatment performances. The result of the present study agreed to the above observation and may be illustrated that at high pH (> 6.95) and coagulant dose (>150 mg/L), amorphous ferric hydroxide precipitates were formed which enmeshed and co-precipitated the colloidal impurities and settle together and thus reduced the coagulation efficiency. pH has an important role in coagulation process since it controls hydrolysis species (Kim *et al.*, 2001). When a coagulant such as iron salt is added to water, a series of soluble hydrolysis species are formed. These hydrolysis species have positive or negative charges depending on the water pH. They are positively charged at low pH (< 6) and negatively charged at high pH. Kim *et al.*,

(2008) stated that the positively charged hydrolysis species can absorb onto the surface of colloidal particles and destabilize the stable colloidal particles or on dissolved natural organic materials (NOM), thus reducing their charge, minimizing the repulsive forces and eventually causing precipitation and particle aggregation, due to stronger van-der Waals attraction. A precipitate of ferric hydroxide is formed at sufficiently high coagulant doses. These precipitates can physically sweep the colloidal particles from the suspension. Another report illustrated that the removal efficiency decreased at pH lower than 7 or higher than 9 (Abdullahassan *et al.*, 2006). The present study attributes from the above discussion that pH has a great influence on coagulation process in removing different parameters including metal cations from tannery effluents.

(c) Effect of time

The dependency of removal efficiency of some physico-chemical parameters on reaction time is shown in Fig. 3.9.

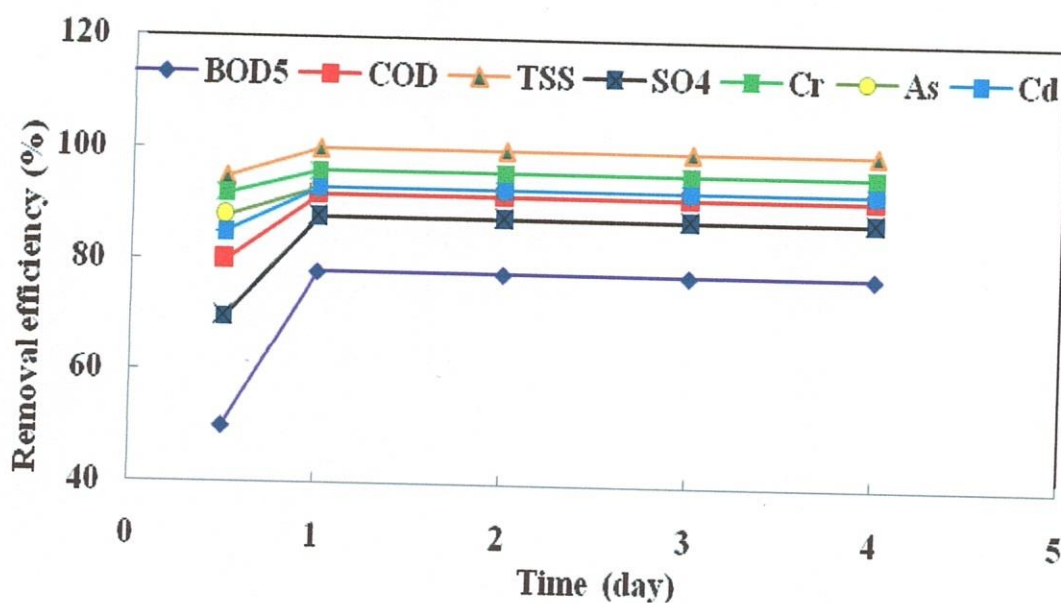


Figure 3.9: The dependency of removal efficiency of some physico-chemical parameters (TSS, COD, SO_4^{2-} , Cl, Cr, As and Cd) on reaction time for coagulant, FeCl_3

The results of the chemical treatment and subsequent settling after a half day (12 hours) were not enough for getting maximum removal efficiency for any pollutant, but the removal efficiencies for all parameters showed maximum after one day treatment and

they remained unchanged after two or more days treatment period (Fig. 3.9). The study results indicate that the time of coagulation reaction and subsequent settling has a large effect on the removal efficiency for various parameters. Stephenson and Duff, (1996) stated that the inorganic coagulant hydrolyzes quickly and the coagulation process was accelerated. This observation is in agreement to this study. Thus the study suggests that one day reaction and settling time would be enough to complete the hydrolysis and sedimentation processes for the coagulant FeCl_3 .

3.5.2.2. Fe_2O_3 treated effluent analysis results

(i) Color, appearances and odor

The study results showed that physical parameters (color, appearances and odor) of Fe_2O_3 (50-500 mg/L) treated effluents are illustrated in Table 3.3. The analyses results revealed that the color value of Fe_2O_3 (100 mg/L) treated effluents was found to be 5 Pt-Co, which was well below the permissible recommended level for ISW- BDS and other standards limit. Apparently clean effluents were found after treatment with different doses Fe_2O_3 (70-250 mg/L). When the coagulant (Fe_2O_3) doses increased above 250 mg/L, then the effluents' color turned clear to light brownish and brownish. The study results show that 100 mg/L of Fe_2O_3 dose was the most effective for color removal which was about 100% (not shown in Table 3.3). Some reports showed that FeCl_3 salt is an important coagulant for wastewater treatment and can be used for color removal (Aygun *et al.*, 2011; Manu *et al.*, 2011; Ali *et al.*, 2008; Islam *et al.*, 2011; Adil *et al.*, 2007 and Smita *et al.*, 2012). This study considered Fe_2O_3 as coagulant and the results show that the color removal efficiency was found to be about 100% at an optimum condition. The study results show that the color removal efficiency was found better compare to the previous literature reports. Color responses from all treated effluents were significantly different from those of untreated effluents. The odor was found very obnoxious smell in untreated effluent but unobjectionable odor was observed after treatment with different doses of Fe_2O_3 .

(ii) pH and EC

pH of Fe_2O_3 (50-500 mg/L) treated effluents are accumulated in Table 3.5. The study results observed that the pH of the treated effluents was decreased with increasing Fe_2O_3 doses as expected (Fig. 3.10). Similar pH trend was observed by Rajat Ameta *et al.* (2006). EC values of Fe_2O_3 (50-500 mg/L) treated effluents are shown in Table 3.5.

Table 3.5: Physico-chemical characteristics of Fe₂O₃ treated tannery effluents

Parameters	Coagulant dose Fe ₂ O ₃ (mg/L)									
	50	70	100	150	200	250	400	500		
pH	10±0.5	9.6±0.56	9±0.2	8.5±0.25	8±0.26	7.5±0.24	6±0.25	5±0.2		
EC (mS/cm)	6±0.12	5.6±0.21	1.5±0.5	2.5±0.4	4.65±1.0	5.6±0.35	6.8±0.5	8.8±0.5		
TSS (mg/L)	400±2	200±1	0±1.5	100±2.5	200±0.2	600±2.2	1000±1.2	2000±5		
TDS(mg/L)	5520±0.06	4505±0.98	3062±2.5	3260±2.5	4075±2.6	4420±2.7	5320±3.5	6280±5.64		
TS (mg/L)	5920±2.3	4705±0.69	3062±2.5	3360±0.95	4275±1.5	5120±1.6	6320±1.7	8280±1.56		
BOD ₅ (mg/L)	730±1.23	540±2.4	200±2.6	450±2.8	500±2.5	625±2.13	700±2.6	750±1.5		
COD(mg/L)	1230±2.3	880±2.1	300±1.3	400±1.3	850±4.0	1050±1.6	1680±0.07	2020±0.05		
NH ₄ -N(mg/L)	230±1.2	110±1.3	85±1.3	150±0.09	200±0.07	300±0.06	500±1.2	560±0.65		
NO ₃ -N(mg/L)	50±1	46±2.5	40±2.3	65±1.05	67±0.07	70±0.06	71±0.08	75±1.3		
NO ₂ -N(mg/L)	68±1.3	46±4.5	20±3.2	25±2.0	40±1.0	60±1.2	78±1.06	90±1.05		
TN(mg/L)	348±1.3	202±1.2	145±1.4	240±0.09	307±0.08	430±0.08	649±1.6	725±1.2		
SO ₄ ²⁻ (mg/L)	1550±1.5	1050±1.6	600±1.6	750±1.4	1060±0.09	1530±1.0	1850±1.03	2350±1.5		
Cl ⁻ (mg/L)	1040±1.8	850±0.09	480±1	580±1.5	880±1.63	1070±4	1250±2	2050±1.3		
Na (mg/L)	1830±2.4	1020±1.3	460±1.63	860±2.5	950±1.8	1020±1.45	1300±1.56	1540±1.5		
Ca((mg/L)	280±2.7	200±1.8	140±1.8	240±1.02	360±1.4	450±1.2	650±2.06	750±0.41		
Cr(mg/L)	5.2±0.07	3.5±0.5	0.1±0.05	0.65±0.06	0.95±0.06	2.5±1.3	3.5±.52	4.5±0.08		
Pb(mg/L)	2.5±0.05	0.67±0.6	0.2±0.03	0.45±0.09	0.70±0.04	1.5±0.5	2.5±0.06	3.5±0.21		
Cd(mg/L)	1.75±0.06	0.5±0.4	0.1±0.05	0.65±0.06	0.95±0.06	1.85±0.07	2.9±0.08	3.2±0.05		
Zn(mg/L)	10±0.04	2.89±0.5	2.1±0.6	4±0.05	5.2±0.5	6.5±0.6	8.5±0.41	10.5±0.15		
Cu(mg/L)	0.95±0.04	0.03±0.4	0±0.06	0.6±0.05	0.5±0.02	0.95±0.08	3.5±0.51	10±1.02		
Ni(mg/L)	2.6±0.05	1.5±0.6	0.95±0.06	1±0.05	1.5±0.05	2±0.52	2.36±0.06	3±0.06		
Mn(mg/L)	2.5±0.06	1.4±0.7	0.2±0.05	0.56±0.06	0.65±0.03	1.5±0.06	2.2±0.08	3±0.05		
As(mg/L)	0.65±0.04	0.35±0.05	0.1±0.05	0.2±0.04	0.3±0.02	0.5±0.05	0.65±0.09	0.95±0.06		

The study results show that EC of Fe_2O_3 treated effluents was decreased sharply with increasing Fe_2O_3 doses until reached at the lowest EC value around 1.5 and then it slowly increased with Fe_2O_3 concentrations (Fig. 3.10).

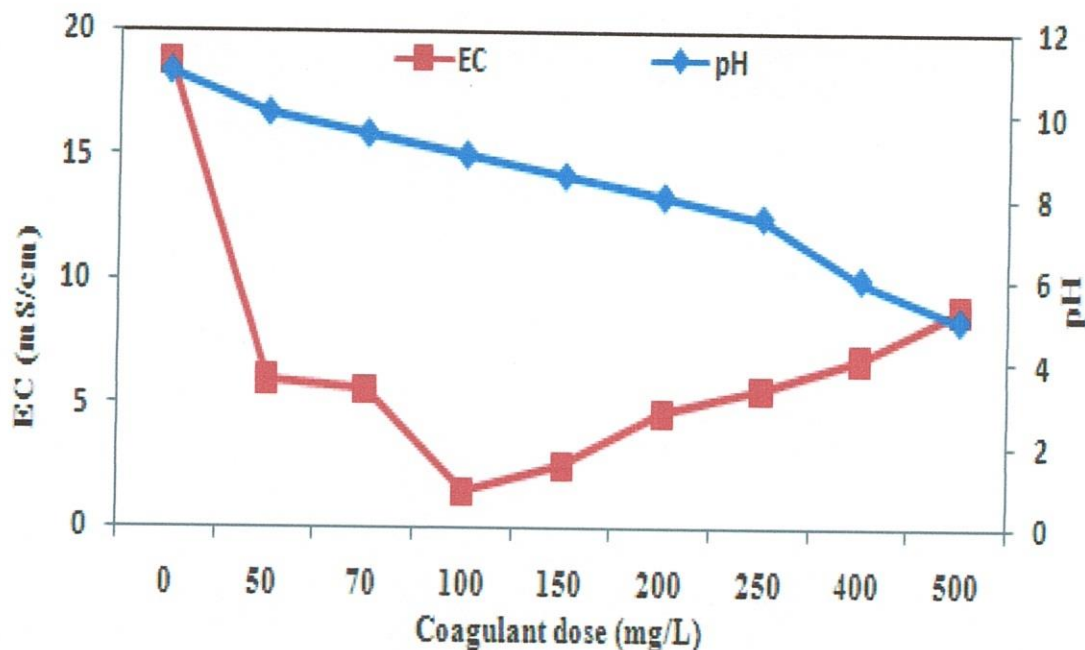


Figure 3.10: Effect of coagulant (Fe_2O_3) doses on EC and pH

The lowest value of EC was observed in 100 mg/L Fe_2O_3 treated effluents indicating the efficiency of the coagulant dose (Fig. 3.10). The values of EC is directly related to the ionic concentrations, hence the results indicate that coagulant (Fe_2O_3) was also capable of removing ions from the tannery effluents. This study results showed comparatively better removal efficiencies (92%) in removing the parameters at a dose of 100mg/L Fe_2O_3 and pH 9.

(iii) TSS, TDS and TS

The treated effluent mean concentrations of TSS, TDS and TS at different Fe_2O_3 doses are stated in Table 3.5. The study results show that in the beginning TSS, TDS and TS decreased with increasing Fe_2O_3 doses until they reached at a certain lowest value and then increased with

Fe_2O_3 . The study results reveal that 100mg/L Fe_2O_3 treated effluents showed the lowest TSS concentration which was well below the prescribed standard limits. 100mg/L of Fe_2O_3 treated effluents showed the lowest TDS and TS values which were found to be the same i.e., 3062 mg/L, (Fig. 3.11). The lowest values of these parameters were found in 100 mg/L of Fe_2O_3 treated effluents. But TDS and TS values were still far above the standard limits for all treatment doses. The study results reveal that the TSS concentration was almost “zero” (0) mg/L and the removal efficiency was 100% at a coagulant (Fe_2O_3) dose of 100mg/L indication of the suitability of the coagulant dose. Some researchers stated that FeCl_3 salt is a better coagulant in removal of TSS, TDS and TS at an optimum condition (Amuda *et al.*, 2006; Claudia *et al.*, 1998; Manu *et al.*, 2011; Islam *et al.*, 2011).

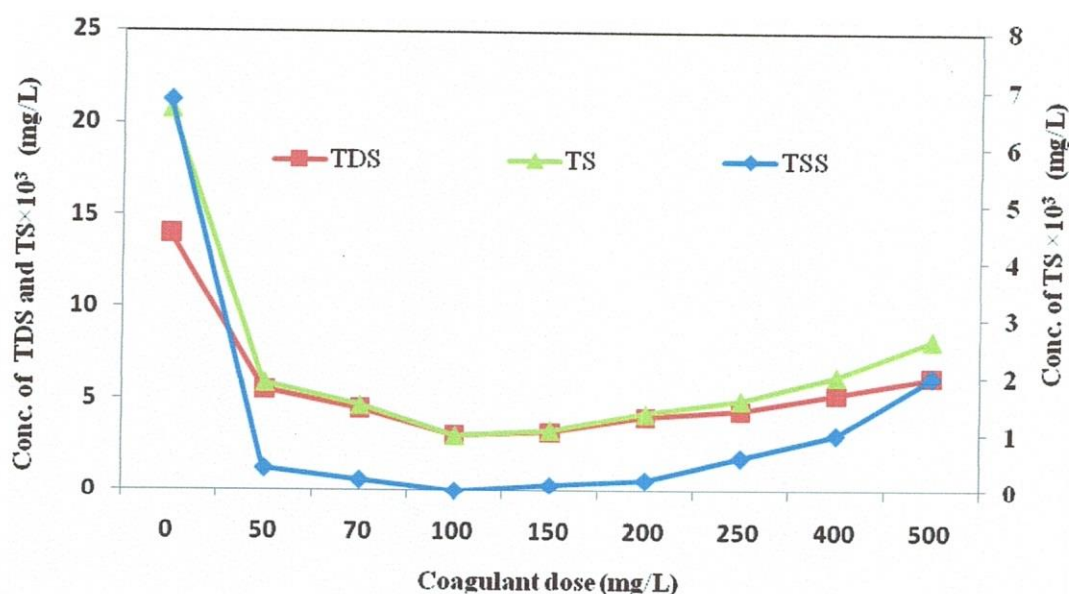


Figure 3.11: Effect of coagulant (Fe_2O_3) doses on TSS, TDS and TS

Amuda *et al.*, (2006) indicated that 300 mg/L FeCl_3 treated beverage effluents contained TSS was found to be 0 mg/L and removal efficiencies was 100%. Islam *et al.*, 2011 also reported that alum and FeCl_3 treated effluents contained lowest TDS value which was found to be 4200mg/L. In comparison with above literature reports, this study results show comparatively better removal efficiencies in removing the parameters at a dose of 100mg/L Fe_2O_3 and pH 9.

(iv) BOD₅ and COD

The mean values of BOD₅ and COD in Fe₂O₃ (50-500 mg/L) treated effluents are shown in Table 3.5. The effluent concentrations of BOD₅ and COD at different FeCl₃ doses are illustrated in Fig. 3.12. The results show that in the beginning BOD₅, COD decreased with increasing Fe₂O₃ doses until they reached at certain the lowest values and then increased with Fe₂O₃ doses. The lowest values of BOD₅ (200 mg/L) and COD (300 mg/L) were found in 100 mg/L Fe₂O₃ treated effluents which were within the prescribed limits for ISW-BDS. Aboulhassan *et al.*, (2006) reported that Fe³⁺ salt is an important coagulant for COD removal. They also found that the rate of COD removal decreased if the pH was lower than 7 or higher than 9. Many researchers observed that ferric chloride is a better coagulant in removal of BOD₅, and COD from the tannery effluents in an optimum condition (Islam *et al.*, 2011).

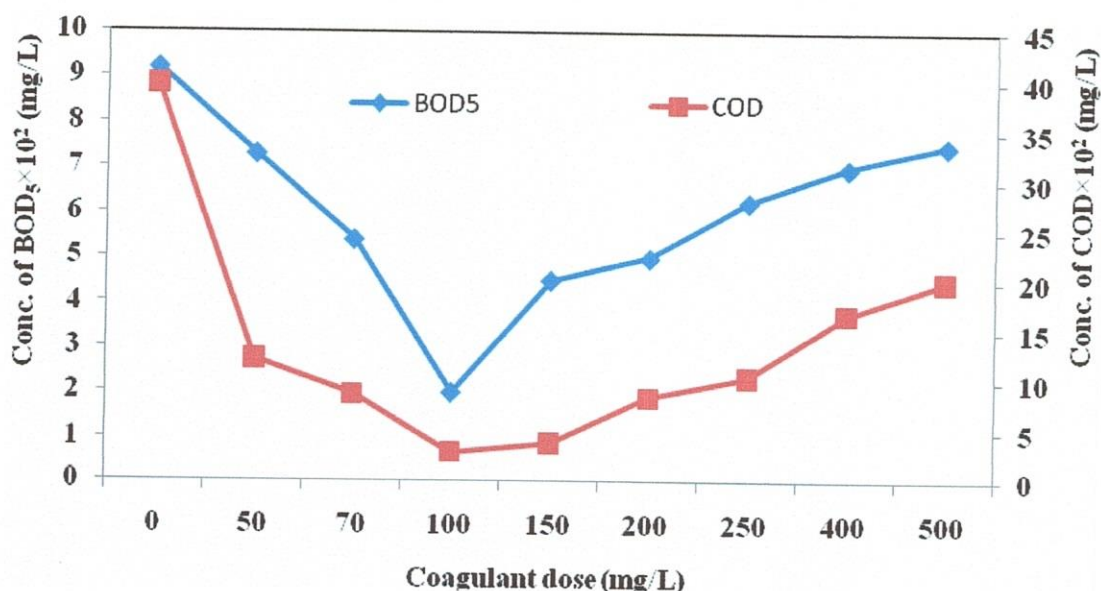


Figure 3.12: Effect of coagulant (Fe₂O₃) doses on BOD₅ and COD

Lofrano *et al.*, (2006) also studied that 2 mg/L polyelectrolyte and 1000 mg/L ferric chloride treated tannery effluents removed were 97 and 77 %, for BOD₅ and COD, respectively. Mahvi, *et al.*, 2004 also stated that FeCl₃ were used as a coagulant, and COD removal efficiency of FeCl₃ treated effluents was 89%. The analyses results revealed that 100 mg/L coagulant dose showed the best removing efficiency for BOD₅ (78%), and COD (92%). This study results observed comparatively better removal efficiencies in removing the parameters at a dose of 100mg/L Fe₂O₃ and at pH 9.

(v) $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN

The mean values of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN in Fe_2O_3 (50-500 mg/L) treated effluents are illustrated in Table 3.5. The effluents concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN at different Fe_2O_3 doses were accumulated in Fig. 3.13. The study results reveal that in the beginning $\text{NH}_4\text{-N}$ and TN were decreased sharply with increasing Fe_2O_3 doses until it reached at a certain peak values of $\text{NH}_4\text{-N}$ and TN and then slowly increased with Fe_2O_3 doses. The lowest values of all nitrogen compounds were found in treatment with 100mg/L Fe_2O_3 doses (Fig. 3.13). So the study results indicate that Fe_2O_3 is capable of removing excess nutrients (ammonium nitrogen compounds) from the effluents. The analysis results showed that the concentration of $\text{NH}_4\text{-N}$ and TN in 100 mg/L Fe_2O_3 treated effluents were found to be 85, 145 mg/L which were removed 86 and 82 %, respectively. But the values of all N containing compounds were still far above the standard limits for all treatment doses suggesting the need for further treatment before discharged the effluents into the environment.

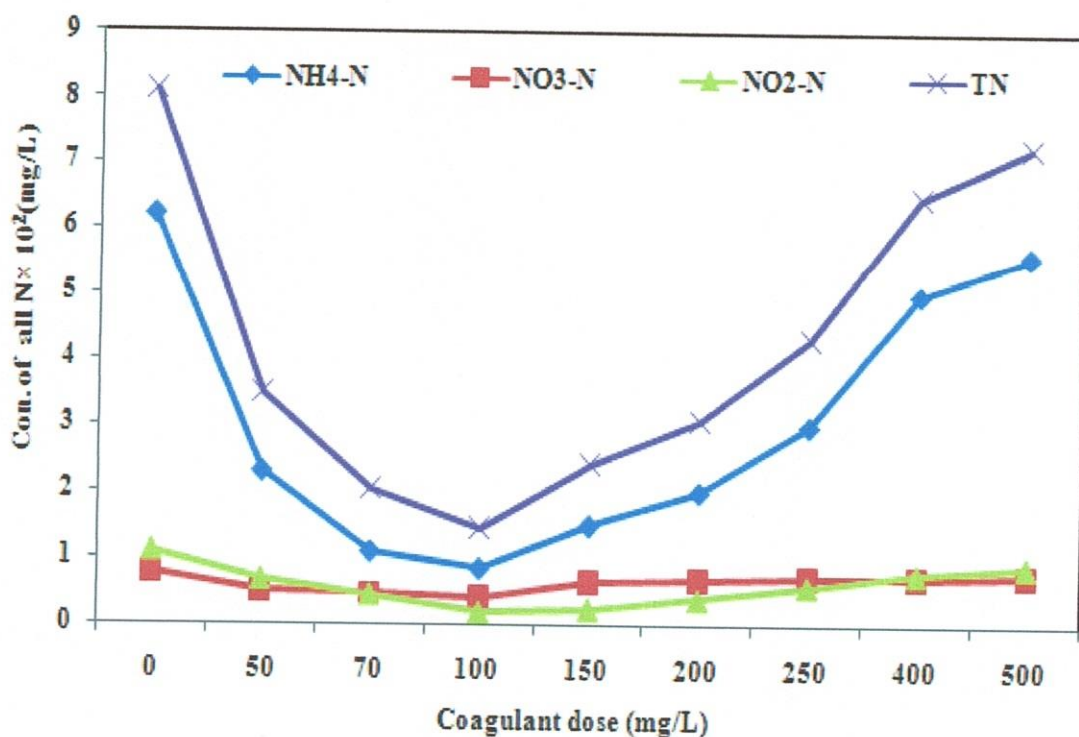


Figure 3.13: Effect of coagulant (Fe_2O_3) doses on $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN

Lofrano *et al.*, (2006) demonstrated that 1000 mg/L FeCl_3 coagulant and 2 mg/L polyelectrolyte was not sufficient for N removal from the effluents. Mahvi *et al.*, 2008 also stated that high N removal in sequencing batch reactor could be achieved. The study results reveal that coagulation-fluctuation processes are not good enough to remove nitrogen species for the effluent treatment.

(vi) SO_4^{2-} and Cl^-

The mean values of SO_4^{2-} and Cl^- in Fe_2O_3 treated effluents are shown in Table 3.5 which was found in relation between Fe_2O_3 doses and contained values of SO_4^{2-} and Cl^- . The analyses results reveal that 100 mg/L Fe_2O_3 treated effluent showed the highest removal efficiency for Cl^- and SO_4^{2-} removal and the values were found to be 480 and 600 mg/L, respectively. The study results showed that maximum SO_4^{2-} and Cl^- removal efficiency achieved were 85 and 90%, respectively and the value was well below the standards permissible limits of ISW-BDS and other International standards. Manu *et al.*, 2011 reported that chloride concentration of 200 mg/L ferric chloride treated dairy effluents was 10.83 mg/L and the removal efficiency was 49%. Islam *et al.*, 2011 also stated that alum and FeCl_3 treated tannery effluents removed Cl^- only 11%. This study results illustrate that removal efficiencies showed comparatively better removal efficiencies the parameters at an optimum dose (100 mg/L) of Fe_2O_3 at pH 9.

(vii) Metal ions concentration

The mean concentration of Na, Ca, Cr, Pb, Cd, Zn, Cu, Ni, Mn and As ions in FeCl_3 (50-500 mg/L) treated effluents are illustrated in Table 3.5. The study results observe that 100mg/L Fe_2O_3 treated effluents contained the lowest Na value which was found to be 460 mg/L. The value was still far above the standards limits, suggesting the need for further treatment. The analysis results also observed that 100mg/L Fe_2O_3 treated effluents showed the lowest concentration of major metal ions and the values were: 140, 0.1, 2.1, 0.95, 0.1, 0.2, 0.1 and 0.2 mg/L for Ca, Cr, Zn, Ni, As, Pb, Cd and Mn, respectively (Table 3.5). These values were within the standards permissible limit of ISW-BDS. The study results reveal that other metal ion, e.g., Cu concentration was

almost “zero” (0) mg/L and removal efficiency was found to be 100% at a coagulant (Fe_2O_3) dose of 100 mg/L indication of the efficiency of the dose. The study results reveal that 100 mg/L Fe_2O_3 dose showed the best removal efficiency for all of major metal ions. Many researchers stated that FeCl_3 coagulants have higher treatment efficiency for heavy metal ions at a specific condition from tannery effluents (Mikami *et al.*, 1984; He *et al.*, 1987; Wu and Liu, 1989; Du *et al.*, 1990). Ajmal *et al.*, (1993 and 2001) reported that heavy metal ions such as Cr, Ni, Cu, Cd, and Zn had been removed from the electroplating wastes using Fe (III) salt as adsorbent and the maximum removal efficiency of these metal ions were almost the same and it was about 79% at pH 8-9. In comparison with above literature reports, this study results show comparatively better removal efficiencies in removing the parameters at a dose of 100mg/L Fe_2O_3 and pH 9.

(viii) Influencing factors on treatment processes

(a) Effect of coagulant dose

Various amount of Fe_2O_3 (50-500 mg/L) doses were applied to observe the optimum coagulant dose at definite pH (9) and room temperature ($25 \pm 1^\circ\text{C}$) for the treatment of wastewater. The effect of coagulant (Fe_2O_3) dose on the removal efficiency of different parameters (EC, TSS COD, SO_4^{2-} , Cl^- Cr, As and Cd) are shown in Fig. 3.14. The figure illustrates that the graphs show a common trend, where the removal efficiency (%) for different parameters initially increased with dose until the efficiencies got maximum and then decreased with doses. The highest removal efficiencies for different chemical parameters achieved were 92% (EC), 92% (COD), 100% (TSS), 85% (SO_4^{2-}), 99% (Cr), 93% (As), and 97% (Cd). The results of this study reveal that the poor coagulation obtained at lower coagulant doses as expected and also at higher (>100 mg/L) coagulant dose. The coagulation effect mechanism discussed in above of FeCl_3 can be Fe^{3+} .

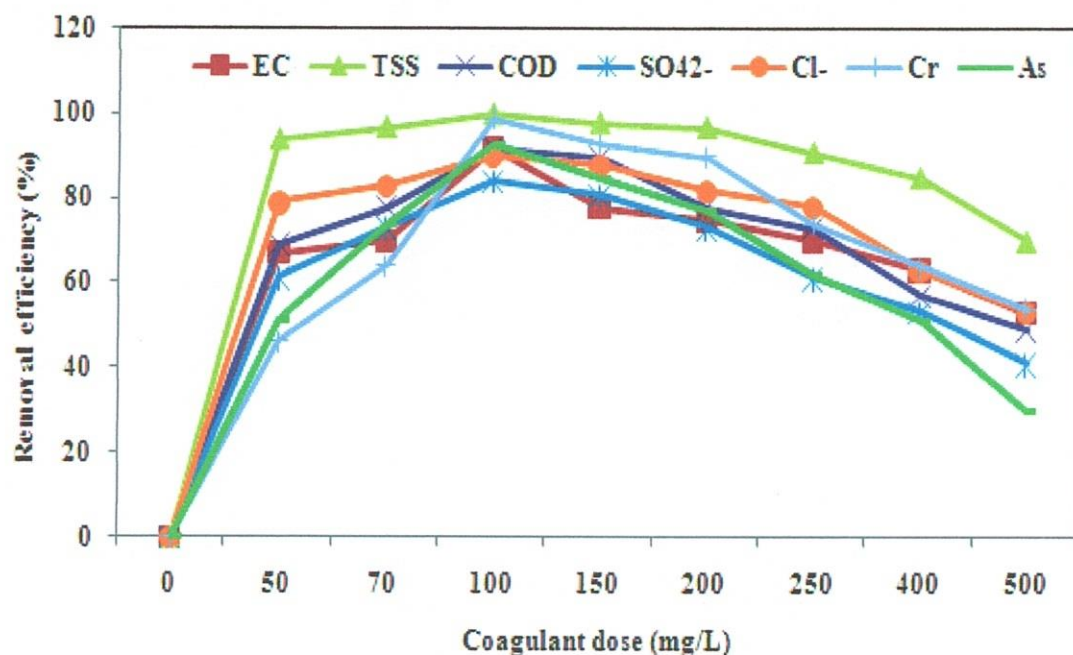


Figure 3.14: Effect of coagulant (Fe_2O_3) doses on the removal efficiency of different parameters (EC, TSS, COD, SO_4^{2-} , Cl^- , Cr, As and Cd)

The results of this study reveal that the poor coagulation obtained at lower coagulant doses as expected and also at higher (>100 mg/L) coagulant dose. This phenomenon could be happened as the coagulant was not adequate to form flocks or to bind the colloid particles to form bridging. At higher doses (more than 100 mg/L) of the coagulant, the colloid particles become suspended due to electrostatics repulsion between colloid particles and coagulant and in low flock appearance.

Islam *et al.* (2011) described that the effluents particles were surrounded by a large quantity of coagulant particles, when coagulant dose was very high and as a result their surfaces were saturated resulting less possibility to combine with each other and thus reach another state of stability. Some researcher's reports showed that the inorganic sludge production was increased with increasing Fe^{3+} concentration (Mandal *et al.*, 2010; Naumczyk *et al.*, 2005). The present study observed that 100 mg/L coagulant (Fe_2O_3) dose showed maximum removal efficiency. A high coagulant dose in effluent treatment process increase operational costs and produce a large amount of sludge. In this study, the coagulant amount used was relatively smaller than used in the previous studies (Manu *et al.*, 2011; Amuda *et al.*, 2006, Lofrano *et al.*, 2006), suggesting minimum sludge production in one hand and reduce cost and environmental burden on the other hand.

(b) Effect of pH

The study carried out in the pH range from 4 to 11.5 to determine the optimum pH value at a definite coagulant dose (100 mg/L Fe_2O_3) and room temperature ($25 \pm 1^\circ\text{C}$). The effect of pH on removal efficiency (%) is shown in Fig. 3.15. The maximum efficiencies for different parameters were found at pH 9 area and they gradually reduced on both sides of the pH.

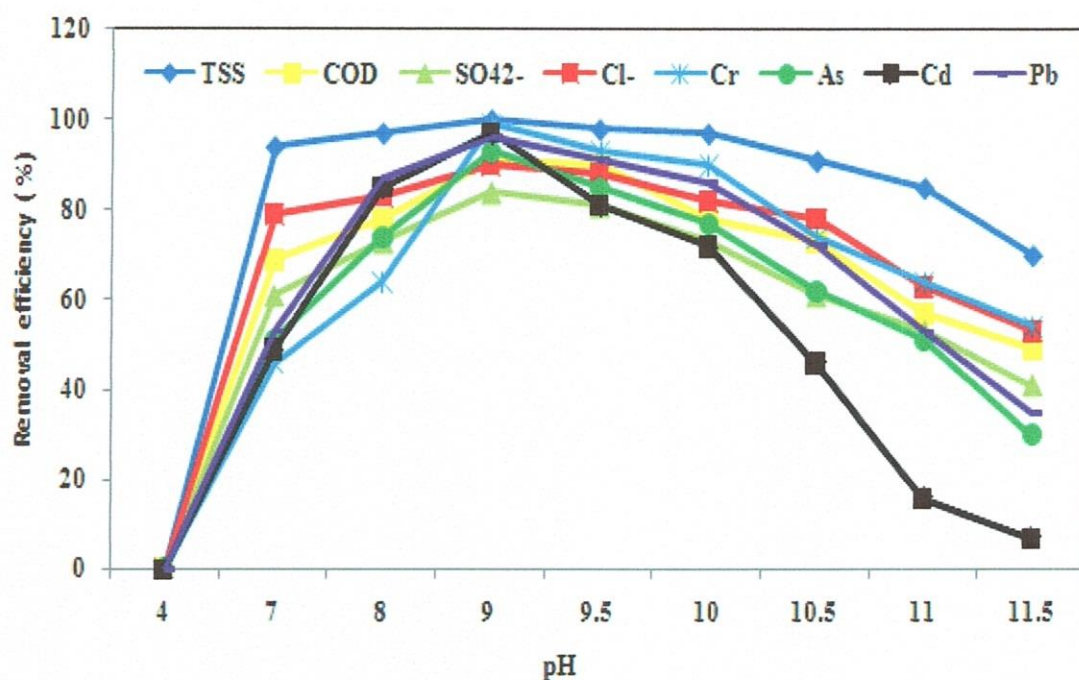


Figure 3.15: Effect of pH on TSS, COD , SO_4^{2-} , Cl^- , Cr, As and Cd for coagulant Fe_2O_3

The colloid substances in the leather industrial effluents usually carry negative electrical charges, whereas coagulant (Fe_2O_3) possesses a lot of Fe^{3+} ions. Around pH 9, Fe(III) are hydrolyzed into mononuclear and multi nuclear hydroxyl complex ions, which have the capability of adsorbing particles and formed electric double layer for destabilization of the particles (Ratnaweera *et al.*, 1992). The study results illustrate that the maximum removal efficiencies were 100, 92, 85, 90, 99, 92 and 97 % for TSS, COD, SO_4^{2-} , Cl^- , Cr, As and Cd, respectively. The study suggests that the lower coagulation efficiencies for the parameters observed at low pH which may be attributed to the competition between protons and cations for common binding sites

and also colloidal re-stabilization caused by charge reversal at the colloidal surface. Jia *et al.* (1998) stated that Fe^{3+} ions did not exist in solutions around above neutral pH and a range of hydrolysis products $[\text{Fe}(\text{OH})_2^+ \text{ and } \text{Fe}(\text{OH})_2^+]$ are responsible for the de-stabilizing effects on colloid impurities. Different hydrolysis products can cause different treatment performances. The result of the present study agreed to the above observation and may be illustrated that at high pH (> 9) and coagulant dose ($>100 \text{ mg/L}$), amorphous ferric hydroxide precipitates were formed which enmeshed and co-precipitated the colloidal impurities and settle together and thus reduced the coagulation efficiency. pH has an important role in coagulation process since it controls hydrolysis species (Kim *et al.*, 2001). When a coagulant such as iron salt is added to water, a series of soluble hydrolysis species are formed. These hydrolysis species have positive or negative charges depending on the water pH. They are positively charged at low pH (< 6) and negatively charged at high pH. The positively charged hydrolysis species can absorb onto the surface of colloidal particles and destabilize the stable colloidal particles or on dissolved natural organic materials (NOM), thus reducing their charge, minimizing the repulsive forces and eventually causing precipitation and particle aggregation, due to stronger van der Waals attraction. A precipitate of ferric hydroxide is formed at sufficiently high coagulant doses. These precipitates can physically sweep the colloidal particles from the suspension. Another report illustrated that the removal efficiency decreased at pH lower than 7 or higher than 9 (Abdullahassan *et al.*, 2006). The present study attributes from the above discussion that pH has a great influence on coagulation process in removing different parameters including metal cations from tannery effluents.

(c) Effect of time

The dependency of removal efficiency of some physico-chemical parameters on reaction time is shown in Fig. 3.16.

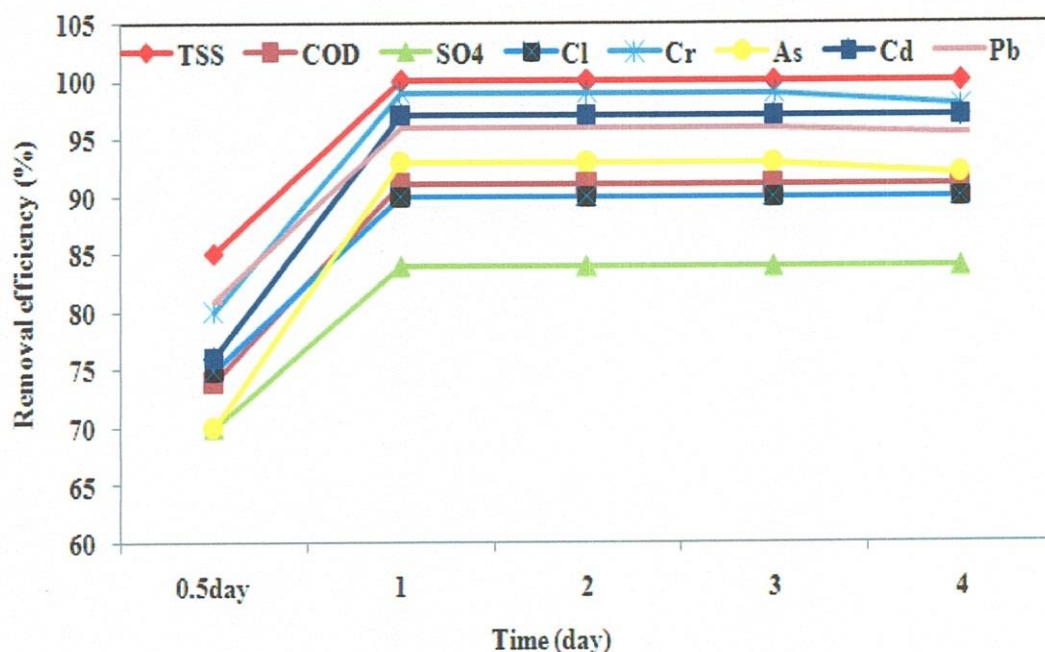


Figure 3.16: The dependency of removal efficiency of some physico-chemical parameters (TSS, COD, SO_4^{2-} , Cl, Cr, As, Cd and Pb) on reaction time for coagulant Fe_2O_3

The results of the chemical treatment and subsequent settling after a half day (12 hours) were not enough for getting maximum removal efficiency for any pollutant, but the removal efficiencies for all parameters showed maximum after one day treatment and they remained unchanged after two or more days treatment period (Fig. 3.16). The study results indicate that the time of coagulation reaction and subsequent settling has a large effect on the removal efficiency for various parameters. Stephenson and Duff, (1996) stated that the inorganic coagulant hydrolyzes quickly and the coagulation process was accelerated. This observation is in agreement to this study. Thus the study suggests that one day reaction and settling time would be enough to complete the hydrolysis and sedimentation processes for the coagulant Fe_2O_3 .

3.5.2.3. FeSO_4 treated effluent analysis results

(i) Color, appearance and odor

The study results showed that physical parameters (color, odor and appearance) of FeSO_4 (50-500 mg/L) treated effluents are shown in Table 3.3. The analysis results revealed that the color value of 150mg/L FeSO_4 treated effluents was found to be 15 Pt-Co which was within the permissible limit for ISW-BDS and other standards limit.

Apparently clean effluents were found after treatment with different doses FeSO_4 (70-250 mg/L). When the coagulants (FeSO_4) doses increased above 250 mg/L, then the effluent's color turned clear to light brownish and brownish. The study results show that 150 mg/L of FeSO_4 dose was the most effective for color removal which was about 99% (not shown in Table 3.3). Some reports also showed that FeSO_4 was an important coagulant in wastewater treatment and could be used for color removal (Adil *et al.*, 2007; Nabi *et al.*, 2006). Ali *et al.*, (2008) studied on industrial wastewater treatment using various steps including screening, coagulation, flocculation, aeration, biological treatment, filtration (sand) and ion exchange and the results obtained after all these treatment steps showed that the effluent was found clear and the color removal efficiency was 98%. Another study was carried out using combine coagulant (50 mg/L lime and 1500 mg/L FeSO_4) and the results showed that the treated effluent color was clear and color removal efficiency was 93%. The present study results show that the color removal efficiency was found better compare to the previous literature reports. Color responses from all treated effluents were significantly different from those of untreated effluents. The odor was found very obnoxious smell in untreated effluent but unobjectionable odor was observed after treatment with different doses of FeSO_4 .

(ii) pH and EC

pH of the FeSO_4 (50-500 mg/L) treated effluents are illustrated in Table 3.6. This study results observe that pH of the FeSO_4 treated effluents was decreased with increasing FeSO_4 doses as expected (Fig. 3.17). pH is an important parameter since the coagulation occurs within a specific pH for each coagulant (Aygün *et al.*, 2010). EC values of FeSO_4 (50-500 mg/L) treated effluents are shown in Table 3.6. The analysis results observe that the EC value of FeSO_4 treated effluents decreased sharply with increasing FeSO_4 doses until reached at the lowest EC value around 5 and then it slowly increased with FeSO_4 doses (Fig. 3.17).

The lowest value of EC was observed in 150 mg/L FeSO_4 treated effluents indicating the efficiency of the coagulant dose (Fig. 3.17). The values of EC is directly related with ionic concentrations, hence the results indicate that the coagulant (FeSO_4) was capable of removing ions from the tannery effluents.

Table 3.6: Physico-chemical characteristics of FeSO₄ treated tannery effluents

Parameters	Coagulant dose FeSO ₄ (mg/L)							
	50	70	100	150	200	250	400	500
pH	8.5±0.05	8±0.06	7.6±0.03	7.05±0.02	6.5±0.05	6±0.06	5±0.04	4±0.06
EC (mS/cm)	8±0.5	7±0.08	6±0.04	4.8±0.07	5±0.06	6.8±0.09	8±0.06	10±0.05
TSS (mg/L)	1000±1.5	750±1.56	500±1.65	0±0.5	100±1.2	500±1.5	850±1.65	1550±1.35
TDS(mg/L)	6020±5.2	5720±4.5	5050±4.65	4085±4.85	4180±4.67	5450±5.4	6500±2.5	7500±2.65
TS (mg/L)	7020±2.5	6470±3.5	4085±5.8	4085±5.4	4280±4.6	5950±4.75	7350±3.56	9050±0.5
BOD ₅ (mg/L)	750±2.4	620±1.5	525±1.5	250±1.6	400±1.7	480±1.8	680±1.75	850±1.65
COD(mg/L)	2250±5.0	1560±4.1	1350±2.2	400±2.3	680±2.1	900±2.4	1060±2.3	2050±1.6
NH ₄ -N(mg/L)	350±1.3	250±1.4	200±1.6	140±1.5	200±1.4	240±1.7	300±1.4	600±1.6
NO ₃ -N(mg/L)	50±1.3	47±1.5	40±1.6	15±1.9	25±2.3	30±2.4	40±2.8	60±4.2
NO ₂ -N(mg/L)	78±1.6	60±2.4	55±2.6	30±2.5	45±2.7	53±2.4	65±2.4	80±1.4
TN(mg/L)	478±0.5	357±0.64	295±0.75	185±1	270±1.2	323±1.23	405±1.5	740±1.4
SO ₄ ²⁻ (mg/L)	1860±1.6	1550±2.4	1350±2.6	1100±3.5	1150±3.1	1290±3.4	1450±3.2	1890±3.1
Cl ⁻ (mg/L)	1250±1.2	1120±1.1	1050±1.02	560±1.3	600±1.3	850±1.36	970±1.4	1280±2.1
Na (mg/L)	2230±1.5	2060±1.3	1860±1.3	850±1.5	900±1.6	1090±1.5	1250±1.4	1520±1.43
Ca(mg/L)	460±1.1	340±1.3	270±1.4	160±1.6	200±1.4	300±1.6	450±1.5	580±1.4
Cr(mg/L)	4.65±0.5	4±0.6	3.2±0.2	0.5±0.05	0.65±0.04	1.8±0.03	2.5±0.02	3.5±0.06
Pb(mg/L)	2.5±0.08	1.67±0.09	1±0.05	0.5±0.05	0.8±0.04	1.8±0.04	2.5±0.08	3.6±0.08
Cd(mg/L)	1.05±0.05	0.85±0.04	0.75±0.06	0.45±0.04	0.90±0.05	1.5±0.06	2.5±0.04	3.5±0.04
Zn(mg/L)	10.05±0.5	8.89±0.03	4.5±0.03	3±0.3	3.6±0.4	4.5±0.05	6.5±0.06	10±0.4
Cu(mg/L)	20±0.5	12±0.09	10±0.45	0.9±0.07	1.8±0.06	5.8±0.05	15±0.06	40±0.04
Ni(mg/L)	3.6±0.04	2.85±0.06	1.96±0.08	0.6±0.04	0.85±0.07	1.2±0.07	2.5±0.06	3.2±0.05
Mn(mg/L)	2.8±	2.45±0.07	2±0.06	0.9±0.05	2±0.07	2.8±0.09	3±0.04	3.15±0.045
As(mg/L)	0.65±0.05	0.5±0.04	0.35±0.04	0.15±0.03	0.2±0.05	0.54±0.05	0.89±0.025	1±0.06

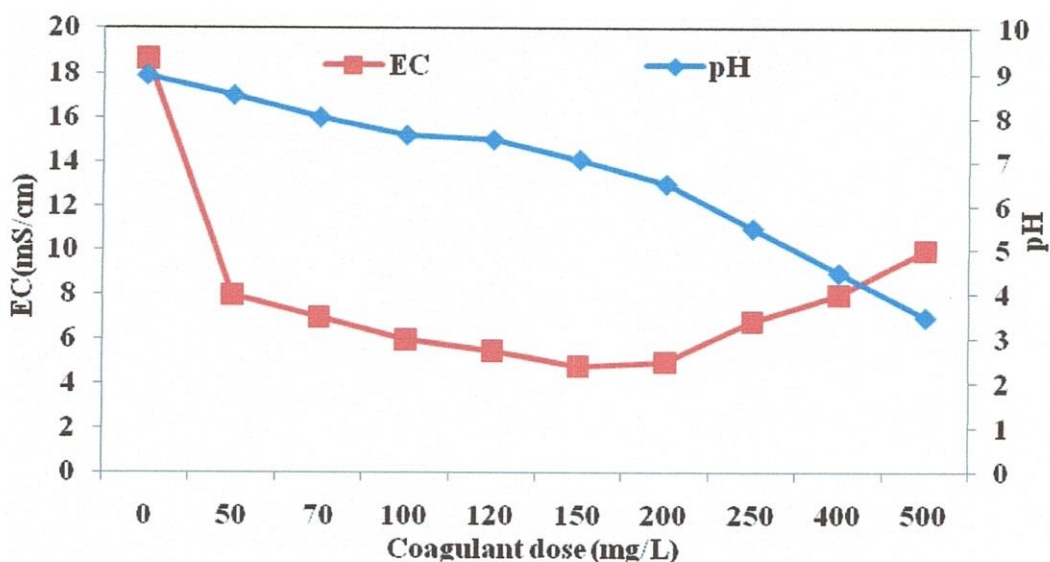


Figure 3.17: Effect of coagulant (FeSO_4) doses on EC and pH

Lofrano *et al.*, (2006) illustrated that 900 mg/L FeCl_3 treated effluents showed maximum removal efficiency for EC at pH 8.5. The results also showed that the higher coagulant (FeCl_3) dose was unable to remove significant amount of other pollutants. Similar observation was made by Naumczyk *et al.*, (2005) reported that 600 mg/L dose of FeCl_3 showed the best removal efficiency for majority of parameters at pH 6.35. In comparison with the above literature reports, the study results show comparatively better removal efficiencies in removing the parameters at a dose of 150 mg/L FeSO_4 and pH 7.05.

(iii) TSS, TDS and TS

The treated effluent concentrations of TSS, TDS and TS at different FeSO_4 doses are illustrated in Table 3.6. The results show that in the beginning TSS, TDS and TS decreased with increasing FeSO_4 doses until they reached at a certain lowest value and then increased with FeSO_4 . The study results reveal that 150mg/L FeSO_4 treated effluents showed the lowest TSS concentration which was well below the prescribed standard limits. 150mg/L of FeSO_4 treated effluents showed the lowest TDS and TS values which were found to be the same, i.e., 4085 mg/L (Fig. 3.18).

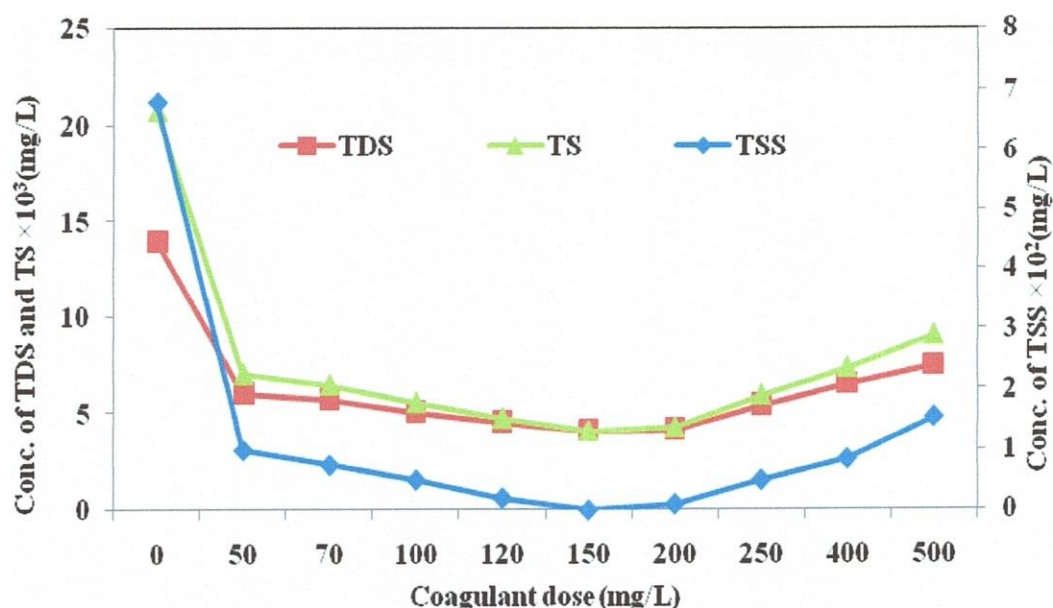


Figure 3.18: Effect of coagulant (FeSO_4) doses on TDS, TS and TSS

The lowest values of these parameters were found in 150 mg/L of FeSO_4 treated effluents. But TDS and TS values were still far above the standard limits for all treatment doses. The study results reveal that the TSS concentration was almost “zero” (0) mg/L and the removal efficiency was 100% at a coagulant (FeSO_4) dose of 150mg/L indication of the suitability of the coagulant dose. Manu *et al.* (2011) indicated that concentration of TSS, TDS and TS for sand filtered dairy effluent treated with 200 mg/L FeCl_3 was found to be 100.83, 141.20 and 242.03 mg/L and the removal efficiencies were 78, 80 and 79 %, respectively. Smita *et al.* (2012) also reported that 600 mg/L poly aluminum chloride (PACl) and 65 mg/L polyelectrolyte treated effluents showed that the maximum removal efficiency was 80% for TSS at pH 4. Amuda *et al.* (2006) stated that 500 mg/L $\text{Fe}_2(\text{SO}_4)_3 \cdot 3\text{H}_2\text{O}$ and 25 mg/L polyelectrolyte treated effluents indicated maximum removal efficiency was 94% for TSS at pH 7. The present study results show better removal efficiencies at a dose of 150 mg/L FeSO_4 and neutral pH compare to the literature reports.

(iv) BOD_5 and COD

The mean values of BOD_5 and COD in FeSO_4 (50-500 mg/L) treated effluents are shown in Table 3.6. The effluent concentrations of BOD_5 and COD at different FeCl_3 doses are illustrated in Fig. 3 19. The results show that in the beginning BOD_5 , COD decreased with increasing FeSO_4 doses until they reached at certain the lowest values and then increased with FeSO_4 doses.

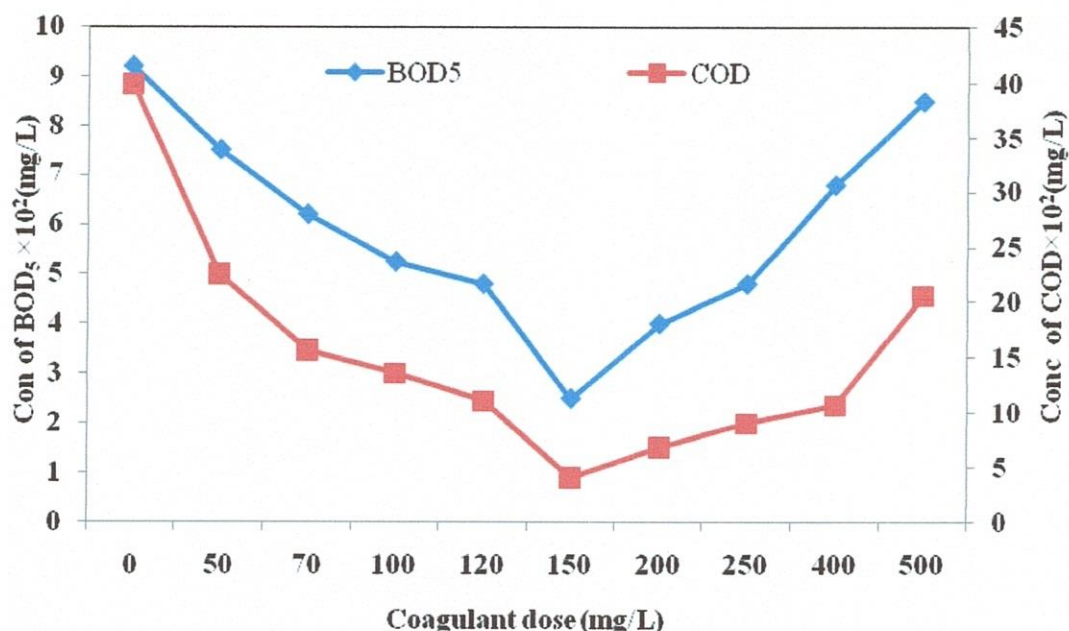


Figure 3.19: Effect of coagulant (FeSO_4) doses on BOD_5 , and COD

The lowest values of BOD_5 (250 mg/L) and COD (400 mg/L) were found in 150 mg/L FeSO_4 treated effluents which were within the prescribed limits for ISW- BDS. Mausavi *et al.* (2011) stated that 600 mg/L H_2O_2 and 130 mg/L FeSO_4 treated effluents removed 60% for COD. Kokila *et al.* (2011) also studied that 200 mg/L FeSO_4 treated dairy effluents removed 59%, for COD. Amuda *et al.* (2006) also stated that 500mg/L $\text{Fe}_2(\text{SO}_4)_3 \cdot 3\text{H}_2\text{O}$ and 85 mg/L polyelectrolyte treated beverage effluents showed the maximum removal efficiency (89%) for COD. The analyses results revealed that 150 mg/L coagulant dose showed the best removing efficiency for BOD_5 (73%), and COD (90%). This study results observed comparatively better removal efficiencies in removing the parameters at an optimum dose of 150mg/L FeSO_4 at neutral pH.

(v) $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN

The mean values of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN in FeSO_4 (50-500mg/L) treated effluents are illustrated in Table 3.6. The effluents concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN at different FeSO_4 doses were accumulated in Fig. 3.20. The study results reveal that in the beginning $\text{NH}_4\text{-N}$ and TN were decreased sharply with increasing FeSO_4 doses until they reached at a certain peak values of $\text{NH}_4\text{-N}$ and TN and then slowly increased with FeSO_4 doses. The lowest values of all nitrogen

compounds were found in treatment with 150mg/L FeSO_4 doses (Fig. 3.20). The study results indicate that FeSO_4 was capable of removing excess nutrients (ammonium and nitrate nitrogen compounds) from the effluents.

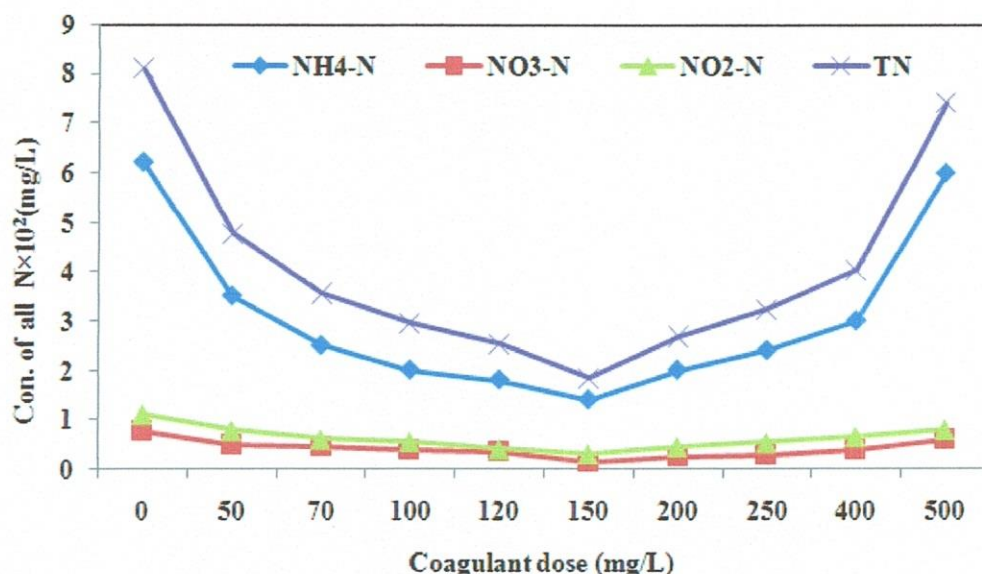


Figure 3.20: Effect of coagulant (FeSO_4) doses on $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and TN

The analysis results show that the concentration of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and TN in 150 mg/L FeSO_4 treated effluents were found to be 140, 15 and 185 mg/L and removal efficiencies were 77, 81 and 82 %, respectively. But the values of all N containing compounds were still far above the standard limits for all doses suggesting the need for further treatment before discharged the effluents into the environment. Manu *et al.* (2011) demonstrated that 200 mg/L FeCl_3 coagulant was better in removal of nutrients from the effluents and it removed 74 % for TN from the dairy effluents. Mahvi *et al.* (2008) also stated that high N removal in sequencing batch reactor could be achieved. Thus the study results reveal that coagulation-fluctuation processes are not good enough to remove nitrogen species for the effluent treatment.

(vi) SO_4^{2-} and Cl^-

The mean values of SO_4^{2-} and Cl^- in FeCl_3 treated effluents are shown in Table 3.6 which was found in relation between FeSO_4 doses and contained values of SO_4^{2-} and Cl^- . The analyses results reveal that 150 mg/L FeSO_4 treated effluent showed the lowest SO_4^{2-} value (1100 mg/L) indicate the highest removal efficiency (78%). The present study observes that the value of SO_4^{2-} was still far above the standard limits

for all treatment doses. The lowest Cl^- concentration in the effluent observed was 560 mg/L in treatment with 150 mg/L FeSO_4 dose (Table 3.6) and the maximum Cl^- removal efficiency achieved was 86%. The value was well below the standards permissible limits of ISW-BDS and other International standards. Manu *et al.* (2011) reported that 200 mg/L ferric chloride treated dairy effluents Cl^- concentration was 10.83 mg/L and the removal efficiency was 49%. Islam *et al.* (2011) also stated that alum and FeCl_3 treated effluents removed Cl^- only 11%. In comparison with above literature reports, this study results show comparatively better removal efficiencies in removing for Cl^- at a dose of 150 mg/L FeSO_4 and neutral pH.

(vii) Metal ions concentration

The mean concentration of Na, Ca, Cr, Pb, Cd, Zn, Cu, Ni, Mn and As ions in FeSO_4 (50-500 mg/L) treated effluents are illustrated in Table 3.6. The study results observe that 150 mg/L FeSO_4 treated effluents contained the lowest Na value which was found to be 850 mg/L. The value was still far above the standards limits, suggesting the need for further treatment. The analysis results also observed that 150 mg/L FeSO_4 treated effluents contained the lowest value of major ions: Ca, Cr, Zn, Ni and Cu which were found to be 160, 0.5, 3, 0.9, 0.6 and 0.9 mg/L, respectively (Table 3.6). These values were within the standards permissible limit of ISW-BDS. The other metal ions, e.g., Pb, Cd As and Mn concentration were found to be 0.5, 0.45, 0.15 and 0.9 mg/L, respectively after treating with 150 mg/L FeSO_4 (Table 3.6). Many researchers stated that polymeric coagulants iron salt have higher treatment efficiency for heavy metal ions at a specific condition from tannery effluents (Mikami *et al.*, 1984; He *et al.*, 1987; Wu and Liu, 1989; Du *et al.*, 1990). Ajmal *et al.* (1993 and 2001) reported that heavy metal ions such as Cr, Ni, Cu, Cd, and Zn were removed from the electroplating wastes using Fe (III) salt as adsorbent. They also reported that the maximum removal efficiency of these metal ions (79%) at definite pH 8-9. The overall removal efficiency of heavy metal ions were found better compare to the literature reports.

(viii) Influencing factors on treatment processes

(a) Effect of coagulant dose

Various amount of FeSO_4 (50-500 mg/L) doses were applied to observe the optimum coagulant dose at definite pH (7) and room temperature ($25 \pm 1^\circ\text{C}$) for the treatment of wastewater. The effect of coagulant (FeSO_4) dose on the removal efficiency of

different parameters (BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd) are shown in Fig. 3.21. The figure illustrates that the graphs show a common trend, where the removal efficiency (%) for different parameters initially increased with dose until the efficiencies got maximum and then decreased with doses. The highest removal efficiencies for different chemical parameters achieved were 73% (BOD_5), 90% (COD), 100% (TSS), 90% (Cl^-), 70% (SO_4^{2-}), 95% (Cr), 89% (As), and 87% (Cd). The results of this study reveal that the poor coagulation obtained at lower coagulant doses as expected and also at higher (>150 mg/L) coagulant dose.

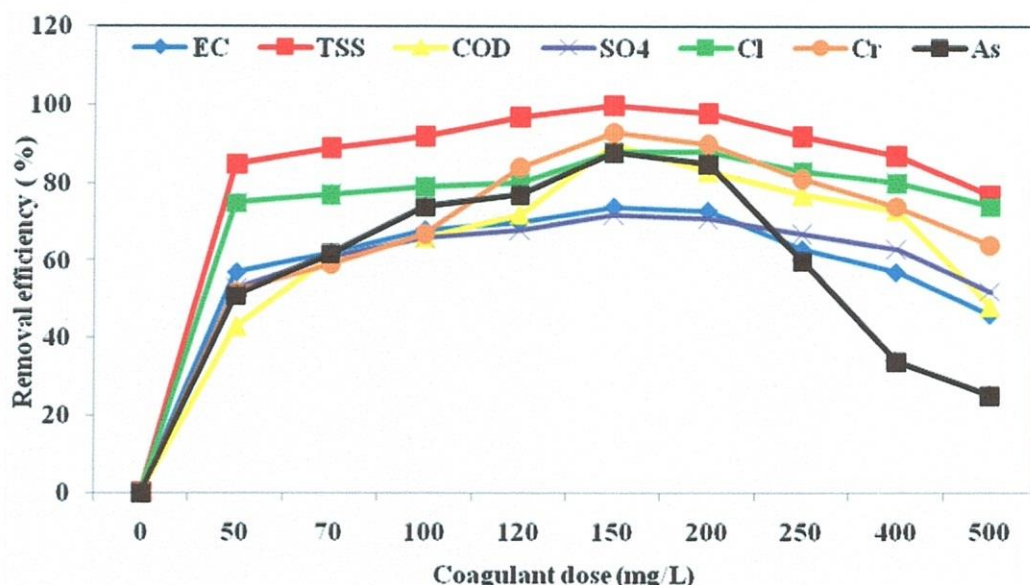


Figure 3.21: Effect of coagulant (FeSO_4) doses on the removal efficiency of different parameters (BOD_5 , COD, TSS, SO_4^{2-} , Cr, As and Cd)

This phenomenon could be happened as the coagulant was not adequate to form flocks or to bind the colloid particles to form bridging. At higher doses (more than 150 mg/L) of the coagulant, the colloid particles become suspended due to electrostatics repulsion between colloid particles and coagulant and in low flock appearance.

Islam *et al.* (2011) described that the effluents particles were surrounded by a large quantity of coagulant particles, when coagulant dose was very high and as a result their surfaces were saturated resulting less possibility to combine with each other and thus reach another state of stability. Some researcher's reports showed that the inorganic sludge production was increased with increasing FeSO_4 concentration (Mandal *et al.*, 2010; Jia *et al.*, 1998). The present study observed that 150 mg/L

coagulant (FeSO_4) dose showed maximum removal efficiency. A high coagulant dose in effluent treatment process increase operational costs and produce a large amount of sludge. In this study, the coagulant amount used was relatively smaller than used in the previous studies (Manu *et al.*, 2011; Amuda *et al.*, 2006, Lofrano *et al.*, 2006), suggesting minimum sludge production in one hand and reduce cost and environmental burden on the other hand.

(b) Effect of pH

The study carried out in the pH range from 3 to 9 to determine the optimum pH value at a definite coagulant dose (150 mg/L FeSO_4) and room temperature ($25 \pm 1^\circ\text{C}$). The effect of pH on removal efficiency (%) is shown in Fig. 3.22. The maximum efficiencies for different parameters were found at neutral pH area and they gradually reduced on both sides of the pH.

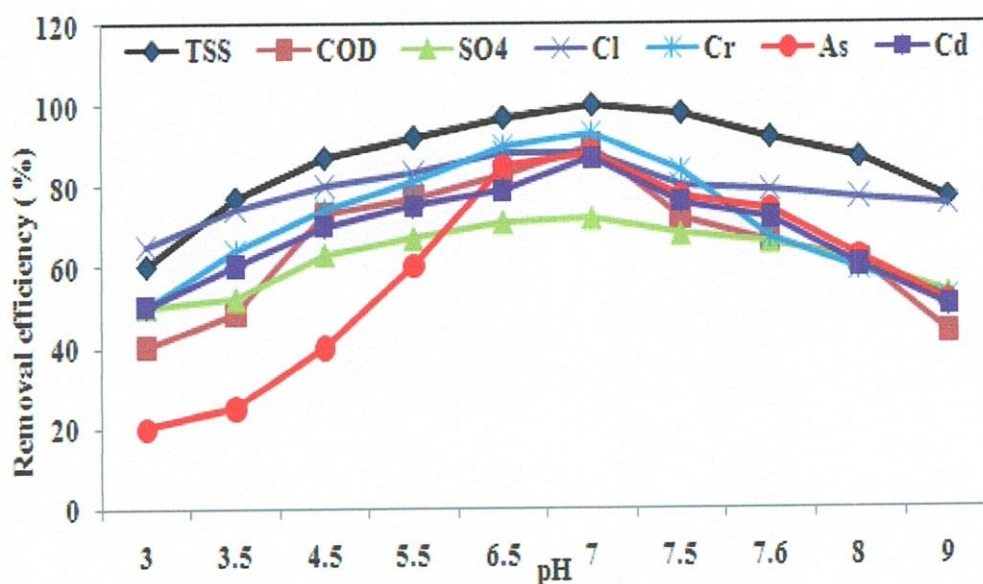


Figure 3.22: Effect of pH on TSS, COD, SO_4^{2-} , Cl, Cr, As and Cd for coagulant (FeSO_4)

The colloid substances in the leather industrial effluents usually carry negative electrical charges, whereas coagulant (FeSO_4) possesses a lot of Fe^{3+} and Fe^{2+} ions. Around neutral pH, Fe(II) are hydrolyzed into mononuclear species [$\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})^{2+}$] which have the capability of adsorbing particles and formed electric double layer for destabilization of the particles (Ratnaweera *et al.*, 1992). The study

results illustrate that the maximum removal efficiencies were 72, 90, 100, 70, 90, 95, 89 and 87 % for BOD₅, COD, TSS, SO₄²⁻, Cl⁻, Cr, As and Cd, respectively. The study suggests that the lower coagulation efficiencies for the parameters observed at low pH which may be attributed to the competition between protons and cations for common binding sites and also colloidal re-stabilization caused by charge reversal at the colloidal surface. Jia *et al.*, (1998) stated that Fe²⁺ ions did not exist in solutions around neutral pH and a range of hydrolysis products [Fe(OH)₂⁺ and Fe(OH)₂⁺] are responsible for the de-stabilizing effects on colloid impurities. Different hydrolysis products can cause different treatment performances. The result of the present study agreed to the above observation and may be illustrated that at high pH (>7.05) and coagulant dose (>150mg/L), amorphous ferric hydroxide precipitates were formed which enmeshed and co-precipitated the colloidal impurities and settle together and thus reduced the coagulation efficiency. pH has an important role in coagulation process since it controls hydrolysis species (Kim *et al.*, 2001). When a coagulant such as iron salt is added to water, a series of soluble hydrolysis species are formed. These hydrolysis species have positive or negative charges depending on the water pH. They are positively charged at low pH (< 6) and negatively charged at high pH. The positively charged hydrolysis species can absorb onto the surface of colloidal particles and destabilize the stable colloidal particles or on dissolved natural organic materials (NOM), thus reducing their charge, minimizing the repulsive forces and eventually causing precipitation and particle aggregation, due to stronger van der Waals attraction. A precipitate of ferric hydroxide is formed at sufficiently high coagulant doses. These precipitates can physically sweep the colloidal particles from the suspension. Another report illustrated that the removal efficiency decreased at pH lower than 7 or higher than 9 (Abdullahassan *et al.*, 2006). The present study attributes from the above discussion that pH has a great influence on coagulation process in removing different parameters including metal cations from tannery effluents.

(c) Effect of time

The dependency of removal efficiency of some physico-chemical parameters on reaction time is shown in Fig. 3.23. The results of the chemical treatment and subsequent settling after a half day (12 hours) were not enough for getting maximum removal efficiency for any pollutant, but the removal efficiencies for all parameters showed maximum after one day treatment and they remained unchanged after two or more day's treatment period (Fig. 3.23).

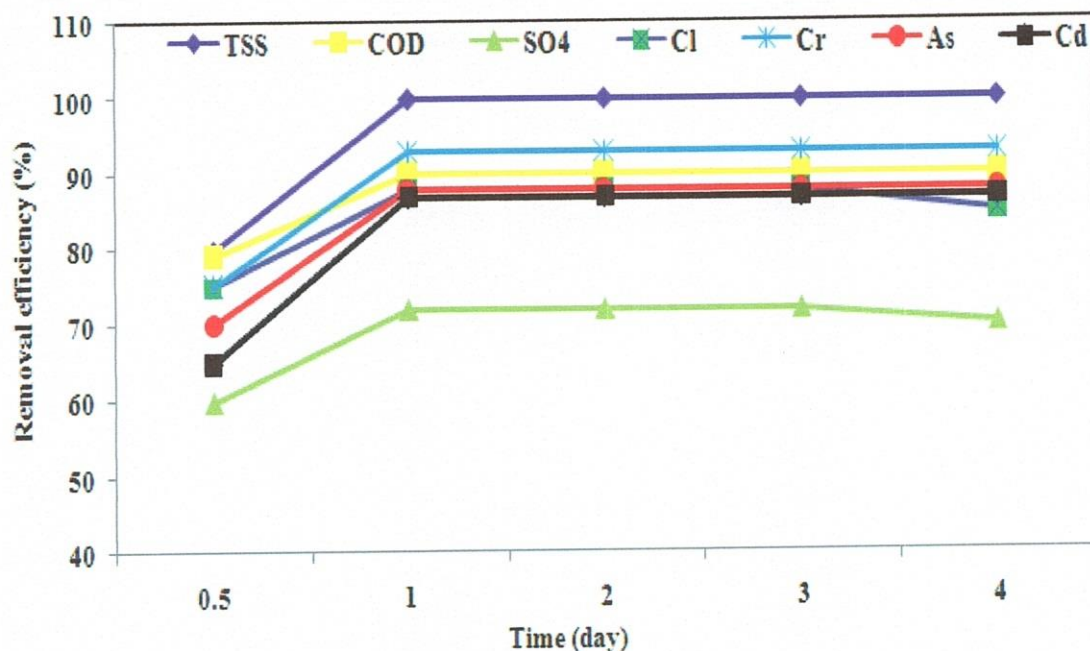


Figure 3.23: The dependency of removal efficiency of some physico-chemical parameters (TSS, COD, SO_4^{2-} , Cl, Cr, As and Cd) on reaction time for FeSO_4

The study results indicate that the time of coagulation reaction and subsequent settling has a large effect on the removal efficiency for various parameters. Stephenson and Duff, (1996) stated that the inorganic coagulant hydrolyzes quickly and the coagulation process was accelerated. This observation is in agreement to this study. Thus the study suggests that one day reaction and settling time would be enough to complete the hydrolysis and sedimentation processes for the coagulant FeSO_4 .

3.6. Comparison with different treatment processes

The optimization of coagulant dose and pH were made for each coagulant and their values were 150 mg/L dose for FeCl_3 and FeSO_4 and 100 mg/L for Fe_2O_3 (Figs. 3.7, 3.21 and 3.14). The study results illustrate that optimum pH for the coagulants FeCl_3 and FeSO_4 was near neutral pH (7) and for the coagulant Fe_2O_3 , it was 9 (Figs. 3.8, 3.22 and 3.15). Comparison of removal efficiencies for different coagulants, FeCl_3 , Fe_2O_3 and FeSO_4 are illustrated in Table 3.7.

Table 3.7: Comparison of among coagulants, FeCl_3 , Fe_2O_3 and FeSO_4 for different parameters removal efficiencies

Parameters	Removing Efficiency (%)		
	FeCl_3 An optimum condition: dose- 150mg/L, pH--6.95	Fe_2O_3 An optimum condition: dose- 100mg/L, pH--9	FeSO_4 An optimum condition: dose--150mg/L, pH—7.05
Color	99	99.7	99
EC	80	92	74
TSS	100	100	100
TDS	74	78	70.82
BOD ₅	78	78	72.82
COD	92.46	92.46	90
SO_4^{2-}	88.45	85	70
Cl^-	80	90	89
$\text{NH}_4\text{-N}$	95	86	77
TN	86	82	77
Cr	96	99	95
Cd	93	97	87
Pb	87	96	91
As	93	93	89
Cu	95	100	98
Zn	86	88	83
Mean removal efficiency	89	91	85

Removal efficiency of color and TSS achieved were about 100% for all coagulants. The results also reveal that coagulant Fe_2O_3 removed Cu and Cr almost 100% and the mean removal efficiency for the coagulants Fe_2O_3 , FeCl_3 and FeSO_4 were was 91%, 89% and 85%, respectively, indicating that Fe_2O_3 was the best removal performance among the coagulants. It may be mentioned that Fe_2O_3 showed lower removal efficiency only for $\text{NH}_4\text{-N}$ and TN compared to coagulant FeCl_3 . In consideration of removal performance of different parameters, the coagulants can follow the order $\text{Fe}_2\text{O}_3 > \text{FeCl}_3 > \text{FeSO}_4$.

The other processes mentioned in Table 3.8 have used huge amount of chemicals compare to the present study. It may be suggested that few amount of coagulant could reduce sludge production and thus reducing the environmental burden.

Table 3.8: Comparison of removal percentage (present and others research findings)

Coagulants	Dose mg/L	Removal efficiency for considerable parameters (%)							References
		COD	TSS	TDS	EC	Cr	Pb	As	
Alum+ polyelectrolyte	600+2	82	85	-	-	97	-	-	Lofrano <i>et al.</i> , 2006
Alum+ polyelectrolyte	400+2	77	85	-	-	98	-	-	Lofrano <i>et al.</i> , 2006
FeCl ₃ + polyelectrolyte	1000+2	77	83	-	-	99	-	-	Lofrano <i>et al.</i> , 2006
Alum	800	30-37	38-46	-	-	74-99	-	-	Song <i>et al.</i> , 2004
SBR	-	81.9	-	-	-	-	88.6	-	Sivianuntapiboon <i>et al.</i> , 2007
Fenton-photo Fenton oxidation (ferrous ions and H ₂ O ₂)	1000+1500	90	-	-	-	76	-	-	Dantas <i>et al.</i> , 2003
Alum + ferric chloride	70+ 70	81	100	57	-	99	-	-	Islam <i>et al.</i> , 2011
Ferric chloride	300	63.5	98	-	60.86	-	-	-	Naumczyk <i>et al.</i> , 2005
Fenton oxidation (H ₂ O ₂ and FeSO ₄)	111000+ 6000	77	-	-	-	23	-	-	Mandal T. <i>et al.</i> , 2010
TiO ₂ /UV oxidation	O ₃ /h dose 2600	3	-	-	-	-	-	-	Schrank <i>et al.</i> , 2004
FeCl ₃ (after sand-stone filtered)	150	92	100	74	80	96	87.49	93	The present findings
Fe ₂ O ₃ (after sand-stone filtered)	100	92	100	78	92	99	96	93	The present findings
FeSO ₄ (after sand-stone filtered)	150	90	100	71	74	95	91	89	The present findings

Moreover, FeCl₃, Fe₂O₃ and FeSO₄ salt are inexpensive compare to TiO₂, alum salts, and copper salt and other commonly used coagulants and compare to used coagulants removal % for different parameters in the present study (Table 3.7). The removals efficiencies of some major parameters of the study are compared to other treatment processes reported in the literatures are shown in Table 3.8. Various chemicals and processes have been practicing for removing different inorganic and organic pollutants from tannery effluents (Mesdaghinia *et al.*, 1991; Dantas *et al.*, 2003; Yajun *et al.*, 2006). Most commonly used chemicals are FeCl₃, FeSO₄, Fe₂(SO₄)₃, alums, and TiO₂ as coagulants, MgSO₄ and NiSO₄ as oxidizing agent, FeSO₄, and H₂O₂ in Fenton-photo process for COD, Cr and other cations removal. The coagulants

used in the study showed the best performance in terms of removal efficiencies of different parameters and amount of coagulant doses (Table 3.8). Amuda *et al.* (2006) conducted a research using FeCl_3 as coagulant showed that the optimum dose of FeCl_3 was 300 mg/L which was almost double to the amount used in this study. It may be suggested that few amount of coagulant could reduce sludge production and thus reducing the environmental burden. Moreover, FeCl_3 , Fe_2O_3 and FeSO_4 salt are inexpensive compare to TiO_2 , alum, copper salt and other commonly used coagulants. The study observes that the coagulants FeCl_3 , Fe_2O_3 and FeSO_4 were potentially suitable for removing major chemical parameters from the tannery effluent and thus suggests that the treatment process could be applied to tannery industries effluent treatment.



Chapter Four

**Microbial approach for removal of toxic
metal ions from tannery effluents**

4. Microbial approach for removal of toxic metal ions from tannery effluents

4.1 Introduction

Water is one of the most important natural resources, essential for all forms of life. This natural resource is being contaminated every day through various anthropogenic activities such as rapid growth of populations, urbanization and industrialization that ultimately make the environment polluted. Most of the industrial activities generate pollutants which was totally unsuitable for the surrounding environment. These pollutants are also major sources of water pollution. Water pollution is caused due to the discharge of harmful toxic chemicals and compounds in water, which leaves the water unsuitable for drinking and other purposes. This renders the water useless for humans, and also endangers the aquatic life. The characteristics and the amount of pollutants emissions are the result of technology and specific processes used in each industrial activity. Tanneries are responsible for great part of chemical pollution. Along their productive process, tanneries generate residues, which are mainly released to water, causing an excessive demand for oxygen, as well as an increase of toxicity levels. Tanning is a leather making process from skin and hides. The process involves a complex combination of mechanical and chemical practices as a result of which organic and inorganic materials become chemically bound to the protein component of the hides and preserve it from deterioration. A significant number of operations within a tannery industry are wet operations consuming large amounts of water, chemicals and energy leading to large amounts of polluted water. The industrial effluents contain several types of chemicals such as dispersion agents, leveling agents, acids, alkalis, phenols, carbonates, alcohols, heavy metals and different type of dyes, pigments, resins, lacquers and solvents etc (Cooper, 1995). Majority of tanneries worldwide use chromium salts (33% basic Cr salt) as tanning materials which are highly toxic and possess a serious threat to the environment upon improper disposal of their wastewaters. Even in low concentrations, these salts have a toxic effect upon *Daphnia*, thus disrupting the food chain for fish life and possibly inhibiting photosynthesis of aquatic plants (Bosnic *et al.*, 2000). Effluents from raw hide processing tanneries contain compounds of Cr and sulphides in most cases with a major proportion of dyes. Release of these effluents into aquatic ecosystems alters the pH, increases the BOD₅ and COD and gives the water intense colorations (Ajayi and

Osibanjo, 1981). The effluents of tanning industry are mostly comprised of chemical impurities including various inorganic, organic compounds. The most common metal ions are Cr, Cd, Pb, Zn, Cu, Ca, Na, Mn and Ni etc and anions such as Cl^- , SO_4^{2-} , NO_3^- , PO_4^{3-} (Bosnic *et al.*, 2000). The excessive amount of these ions creates major problems for the tannery effluent management. The nature of industrial wastes depends upon the industrial processes in which they originated. The problem of adequately handling industrial wastewater is more complex and much more difficult than sewage. The rapid industrialization has affected the ecosystem to a very large extent due to the pollutants discharged from their operations.

Metal resistance trait in microorganisms is regulated by genes which are organized in operons. Generally, the genes for heavy metal resistance are found on extra-chromosomal circular DNA i.e. is called plasmid which carried out by metal resistant bacteria (Munees Ahemad, 2012). The heavy metal resistance genes are expressed and induced in the presence of specific metals in the vicinity of bacterial niche. Since the regulation of the metal resistant gene expression is specific for each heavy metal and is dependent upon metal species concentration, the promoters and regulatory genes from the bacterial operons responsible for resistance attribute can be used to create metal-specific biosensors (promoter-reporter gene fusions). The metal specific bacterial sensors coupled with tools for chemical analyses can be used to differentiate the bio-available metal concentration from the total metal concentration of the samples (Tauriainen, S *et al.*, 2000; Turpeinen, 2002).

Plasmids seem to play a major role in the adaptation of bacteria to xenobiotic and in the acquisition of new genetic traits due to pollution. A particularly important aspect is the occurrence of some broad host range plasmids specialized in the degradation of synthetic chemicals. Modern ecological biotechnology attempts to solve the problems of pollution by screening for and molecularly breeding microbial strains that are capable of degrading recalcitrant. Research in this direction is in good shape. This investigation aimed to apply microbial genetic technique to induce recombinants from bacteria and yeast to be testing for maximal accumulation of heavy metals from tannery effluents to improve the quality of irrigated water in the industrial regions. This enhancement of bio-sorption which would be resulting in a decrease of

environmental loading pollutants such as contamination of groundwater and also receiving surface waters would be less threatened.

Plasmids are extra chromosomal elements, which are wide spread in bacteria. Plasmids are ideal for the study of DNA replication, its control and gene expression. They mediate the transfer of properties, such as drug resistance, between individuals belonging to different genera. This may be an important factor for bacterial evolution (Broda, 1979). Lederberg (1952) also stated that, the behaviors and the properties of some bacteria suggested that its genetic control is not governed by the chromosome but the extra chromosomal structures known as plasmids.

Plasmids are duplex, super coiled circles, ranging in size from 2 to over 100 kb. Generally the small plasmids are maintained at a level of about twenty copies per host chromosome. The numerous, remarkable features of plasmids have been appreciated for many years, but the recent intensity of interest in them is based particularly on their transposability, their self transmissibility, their use as vehicles for cloning foreign DNA and their attractiveness as models for replication control (Appendix 4.1).

Biodegradation of soluble hexavalent chromium can be obtained by utilizing microbes in wastewater treatment. Several reports indicated that biological reduction of Cr (VI) by bacteria, both by aerobes and anaerobes (Wang and Shen, 1995). Other researchers stated that biological reduction of Cr (VI) using indigenous microorganism offer a new cost-effective and environmentally compatible technology (Camargo *et al.*, 2003, 2005; Cervantes *et al.*, 2001). Many researchers have explored the potential of microorganisms such as *Aerococcus* sp. isolated from tannery effluents in Kanpur by Ramteke *et al.* (2001), *Arthrobacter* sp. and *Bacillus* sp. from Tannery effluents in Australia by Megharaj *et al.* (2003), *Bacillus sphaericus* from Serpentine Soil by Paul *et al.* (2004), *Acinetobacter haemolyticus* from heavy metal contaminated waste water by Zakaria *et al.* (2007) and *Sphaerillus natans* from activated sludges by Caravelli *et al.* (2008). In view of the above investigations, it is clear that all treatment methods in use have some drawbacks, and there is a need to look for further alternative methods. Leather industrial effluents demonstrates a complicated system containing different components, including pollutants coming from the processed raw materials, chemicals and residues of technological additives used in individual manufacturing operations.

Most of the research reports on tannery effluents in Bangladesh focused on mainly characterization and impact assessment (Das *et al.*, 2006; Mohanta *et al.*, 2010; SEHD, 2002; Azom *et al.*, 2012). Only a few studies on tannery effluents chemical and microbial treatment so far have been reported in Bangladesh. Biological reduction of hexavalent chromium usually occurs at a neutral pH- range and generates an insignificant quantity of chemical sludge as well as offers potential cost-effective remediation strategy (Mahesh *et al.* 1997). Most of these have been investigated using Cr, Cd and Pb containing aqueous solution and have not shown significant or complete removal of Cr (VI), Cd and Pb. All the reported microbial strains have lower MIC for Cr, Cd and Pb. There is only limited investigation for tannery effluent treatment particularly Cr, Cd and Pb contaminants using microbial strains. Still now there is no literature cited on any molecularly identified and sequenced microbial species for chromium, cadmium and lead resistant and removal from Hazaribagh Industrial zone. The present investigation have isolated an indigenous bacterial strain and characterized it by 16 S RNA sequencing as *Enterobacter species* (GenBank Accession Number: EU781952) for remediation of Cr, Cd and Pb containing leather industrial effluent. Thus our present study uses the potential indigenous microbial strains for treatment of leather industrial effluent which may be suitable for biological treatment of heavy metal ions contaminated waste water. So, the objective of this study was to screen the chromium, cadmium and lead resistant bacteria for their ability to reduce heavy metals biologically, for environment cleanup and bioremediation of heavy metal ions contaminated tannery effluents.

The present research was undertaken to satisfy the following objectives:

- Heavy metal ions (Cr, Cd and Pb) resistant bacterial species would be isolated from the tannery effluents which are able to metabolize the metal ions.
- Microscopic and AFM view; 16SrRNA gene sequencing and biochemical tests would be studied for the identified microbial species.
- Determination of physiological and biochemical characteristics of the microorganisms.
- The antibiotic disc resistance test for isolated bacteria might be studied.

- MIC of the resistant antibiotic disc would be studied.
- The plasmid DNA of bacteria also would be studied by gel electrophoresis technique.
- The Plasmid DNA could be eliminated from the isolated bacteria using different chemicals.
- Metal ions removal rate might be determined by Atomic Absorption Spectrophotometer.

4.2. Materials and methods

This chapter described materials are used and outlines the experimental design for bioremediation of effluents containing Cr, Cd and Pb from the leather industrial zone through the batch process. It was also given an overview of the isolation and characterization of bacterial strains from effluents. Optimization of culture and process parameters for the biomass growth was studied. 16SrRNA identification, phylogenetic analysis, anti-microbial sensitivity test, biochemical test, minimum inhibitory concentration of resistant antibiotic disc and plasmid DNA sequence of the enrichment bacterial culture and removal percentage (%) of Cr, Cd and Pb was also studied using indigenous strain.

4.2.1. Study area and sample collection

The study was conducted during the year 2010-2011 and the tannery effluents were collected from the outlet of some selected leather industries and dumping zone located in Hazaribagh industrial zone Dhaka, Bangladesh. The present study deals with the isolation of heavy metals degrading bacterium which were used considered such as Cr, Cd and Pb as a source of nutrients and energy. Isolated bacteria were able to degrade or utilize the toxic chemicals thereby clean up the environment.

4.3. Apparatus and chemicals

The following instruments were used during the present experimental work.

- All of the microbial routine work such as preparation of different type plate, culture, subculture etc. was done under the laminar airflow cabinet (environmental air control Inc, USA). The bacterial culture was incubated at 37°C for 4 days in a Thermostat Incubator (Gallenkamp, England and Sanyo Growth Cabinet, Japan). The optical density of liquid culture media (bacterial growth) was measured by a Photo Electric Colorimeter (Model no.AE-11M & AE-11D, japan) at 660 nm wave length. The liquid culture media of bacterial growth was taken in a conical flask and shaken in a Orbital shaker (New Brunswick scientific company, UK). Different type of chemicals was measured by Electric balance (AR 3130 Ohasu Crop, Pine Book, NJ, and USA).
- The liquid culture media (bacterial growth) was centrifuged in a Centrifuge machine (Gallenkamp, England) at 10000rpm (revolution per minute). The liquid culture media (bacterial growth) was centrifuged by the Centrifuge (H-800, china) at 4000rpm.
- The culture media (bacterial growth) was diluted with de-ionized water and made homogeneous solution in a vortex Mixture (VBRIOFIX VFI, Germany).
- A water bath (Gallenkamp, England) was used to control temperature of buffers and culture media during the DNA sequence techniques.
- A Gallenkamp autoclave (Model no. 7B 1053F England) was sterilized all media and instrument before used in bacterial culture medium.
- Agar medium was homogeneously dissolved in an oven (Model no.NDO-600 ND, Manufactured by Tokio Rikakikai Co. Ltd, Japan).
- A pH meter (Model no. IOK, TOA Electronics ltd. Japan) was used to measure the pH.

- The plasmid DNAs were extracted from isolated bacteria using a Gel Electrophoresis (E-C Apparatus Corporation, Petersburg, U.S.A) and Gel documentary instruments.
- Stock solutions of Cr, Cd and Pb were prepared following the standard procedure (EPA Methods 7196A).

4.4. Culture media and isolation of heavy metal degrading bacteria

Mineral salt medium or minimum salt (MS) medium enriched with heavy metals (Cr, Cd and Pb) were used for isolation and characterization of heavy metals degrading bacteria. The carbon source in MS medium was replaced with heavy metals, a little amount glucose and industrial effluents containing total organic compound (TOC). Effect of heavy metals on the growth of bacterial isolates was determined in MS media which contained (gm/L): di-potassium hydrogen phosphate (K_2HPO_4), 2.0; ammonium sulphate $(NH_4)_2SO_4$, 0.5; Potassium dihydrogen phosphate (KH_2PO_4), 0.02; magnesium sulphate $(MgSO_4)7H_2O$, 0.05 gm/L, respectively and trace elements solution 1ml/L. Above these chemicals were taken in a 1-liter beaker and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up with de-ionized water up to the mark. The solution was added with 2M HCl or NaOH for adjust to pH 7.2. The trace elements solution which were comprised the following chemicals (mg/L): $FeSO_4 4H_2O$, 400; $MnSO_4 4H_2O$, 400 ; $ZnSO_4 7H_2O$, 200 ; $CuSO_4 7H_2O$, 40; $CoCl_2 6H_2O$, 40 ; KI, 300; $Na_2MoO_4 2H_2O$, 50 mg/L, respectively. The chemicals were taken in a 1-liter beaker and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up with de-ionized water up to the mark. Luria-Bertani (LB) medium or Nutrient broth media which was comprised the following chemicals (gm/100mL); yeast extracts, 0.5; Peptone, 1 and NaCl, 0.5 gm respectively. The three chemicals were taken in a 100 ml volumetric flask and gradually added de-ionized water and mixed thoroughly with a magnetic stirrer. Finally the solution was made up with de-ionized water up to the mark. The solution was added with 2M HCl or NaOH for adjust to pH 7.2. When the solid media were prepared 3% agar was added to liquid media. All the media were autoclaved at 121-126°C and 15 lb pressure for 30 min. The culture media, MS was containing the heavy metals (5-100 mg/L Cd, Cr

and Pb). The heavy metals were added after the steam sterilization of the bulk liquid media. But the culture media, LB was containing the heavy metals (100-1600 mg/L Cd, Cr and Pb). The heavy metals were also added after the steam sterilization of the bulk liquid media. For isolation of Cr, Cd and Pb resistant bacteria, 1 ml of the tannery effluent samples was spread on LB medium (peptone, yeast-extract and NaCl) agar plates were supplemented with metal ions (100 µg/mL of Cr, Cd and Pb, respectively) of the medium. The LB plate was incubated at 37°C for 4 days in the incubator, but sufficient growth of the bacterial colonies was observed after 24 hours. The study also determined the effect of Cr, Cd and Pb on the growth of bacterial isolates in MS media, supplemented with metals ions (10µg/mL of Cr, Cd and Pb, respectively) at 37°C for 4 days. This process was repeated with successively higher concentrations of heavy metals (Cr, Cd and Pb) until the minimum inhibitory concentration (MIC) of bacterial isolates was obtained. However, the metal ion concentration was selected 20 µg/mL as the composite tannery effluents contained maximum 10 µg/mL Cr ions. The single colonies from these plates were sub cultured and then produced replicate plates and the colonies were eventually transferred into mineral salts (MS) medium containing 20 µg/mL of Cr/Cd/Pb. The single colonies were picked and streaked on MS agar plates with the help of calibrated loop and incubated at 37°C for 24 hours for recovery of potent isolates. Pure cultures were also grown on slants or stab and streak method for storage purpose and subsequently for identification, biochemical tests, morphological characterization, plasmid isolation, curing experiment and further experiments by standard identification methods (Holt *et al.*, 1994).

4.5. Identification and characterization of the effluents bacteria

The shape and colors of the colonies were examined under the microscope, after Gram staining. Isolates were biochemically analyzed for the activities of oxidase, catalase, V-P test, MR-VP test, starch hydrolysis and gelatin hydrolysis, motility, indole production and citrate utilization. The tests were used to identify the isolated bacteria according to Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1986).

4.6. Microscopic examination of bacterial cells

The staining technique was used for the observation of morphological characteristics of bacterium.

4.6.1. Staining technique

4.6.1.1. The slide preparation

The clean slide was taken and three drops of sterilized distilled water were drowpped on three different places of the slide. A loopful of isolate bacterial colonies was transferred with a sterilized loop on one of the drop of distilled water on the slide and a very thin film was prepared by spreading uniformly. The loop was then sterilized. A loopful of bacterium was transferred from the middle of the smear to the next water drop and thin film was prepared. In the same way bacterium was transferred from the second to the third drop and thin film was prepared. This dilution technique was applied for better observation of each isolated bacterium. The films were fixed by passing it over the gentle flame for two or three times.

4.6.1.2. Gram staining

The three stains (0.5% crystal violet solution, Lugol's iodine solution, 1% iodine solution and 1% safranin solution) were prepared freshly and used for gram staining technique.

Procedure

- The clean slide was taken for staining technique. A drop of sterilized distilled water put on the middle of the clean slide. Then a loopful bacterial suspension was transferred to the sterilized drop of water and very thin film was prepared on the slide by spreading uniformly.
- The slide with fixed bacterial film was flooded with crystal violet solution for 30 seconds and washed thoroughly with gentle stream of tap water.
- The slide was flooded with iodine solution for 1 minute and washed thoroughly with gentle stream of tap water.
- The slide was washed with alcohol (95%) for 10-15 seconds.
- The bacterial slide was washed thoroughly with gentle stream of tap water.
- The slide was thoroughly washed with safranin for 1 minute.
- The bacterial slide was also washed with tap water and bloated dry.

At last the bacterial slide was examined under microscope.

4.6.2. Motility Test

4.6.2.1. Hanging drop method

Three materials (liquid bacterial culture, microscopic grove slide with a central depression and cover slip) were used for the technique.

Procedure

Freshly prepared broth cultures of bacteria were taken in a test tube.

The cultures were centrifuged and supernatant was removed carefully and the cell pallet was suspended by 1% saline water.

A small drop of suspended bacterial solution was placed into the central depression of a slide.

The slide was covered with cover slip and it was prepared for examined microscopically.

A darting, zigzag, tumbling or other organized movement indicated positive results and negative results indicated no movement or Brownian motion only.

4.6.3. Atomic force microscopic analysis

Tannery effluents bacterial cells were cultured in LB medium and incubated at 37°C with agitation for 24 h. Cells were harvested in a e centrifuge machine at 4000 rpm for 15 min and washed twice with sterile milli Q water. Samples for AFM analysis were mounted on the cover glass and mounted directly on the specimen metal disc using a double adhesive tape. Samples were scanned at different areas using AFM (Shimadzu SPM 9500-2j). For high resolution, contact mode micro cantilever was used for the analysis. Digital images were stored in the computer and processed.

4.7. Determination of optimal growth conditions

pH and temperature is an important factor which was influenced on the bacterial growth. The optimal growth conditions with reference to pH and temperature were determined. The isolates were grown in LB medium with different pH values (5, 6, 7.2, 8, and 9) and incubation was carried out at temperature 25°C, 28°C, 37° C and 40°C. The optical density of the log phase growing cultures conditions was noted at 660 nm wavelengths with photoelectric colorimeter (AE-11M, ERMAINC, TOKYO) to determine the bacterial growth.

4.8. Biochemical studies

4.8.1. Carbohydrate utilization

In order to find out the ability of the isolates to utilize different carbohydrates, the cultures were inoculated in MS media containing different carbohydrates viz. glucose, galactose, mannose, arabinose, maltose, sucrose, lactose, manitol and cellulose. The final concentration of the carbohydrates was 1%. The tubes were incubated at 37°C for 1-2 days and observed for any growth.

4.8.2. Catalase tests

Catalase is an enzyme that splits hydrogen peroxide into water and oxygen. Hydrogen peroxide was produced as a byproduct of respiration and was lethal if it accumulated in the cell. All respiring organisms therefore must have some mechanism for detoxification. Catalase is one of the common methods. When one drop of 30% hydrogen peroxide was added over a colony of catalase- producing bacteria, it was broken down and the oxygen that was produced could be seen as bubbles.

4.8.3. Citrate utilization tests

Some bacteria may be able to use organic compounds other than sugars as their sole source of carbon. Simons Citrate agar is a medium containing citrate as the sole carbon sources and ammonium salts as the sole nitrogen sources. Organisms that metabolize citrate utilize the ammonium salts releasing ammonia and increasing the pH of the medium. Bromo thymal blue was present in the medium as the indicator dye.

For the Citrate utilization test, slope culture with a 1 inch butt of Simon's citrate agar was inoculated by streaking over surface with a wire needle and incubated at 37°C for 1-2 days. The color of the medium changed from green to bright blue due to the utilization of citrate and when citrate was not utilized, the color of the medium was remains unchanged.

4.8.4. Methyl Red test

Methyl Red (MR) tests detects acid production to a sufficient degree (below pH 4.5) from glucose. One ml of fresh bacterial culture grown in glucose phosphate medium was taken in a test tube. Five drops of methyl red reagent was added and read immediately.

4.8.5. Oxidase test

Oxidase test was performed to detect the presence of cyto-chrome oxidase in the organism. This test was carried out by wet paper method. A strip of what man filter paper no.1 was soaked with 2-3 drops of dyes. A single colony was picked up with a sterile toothpick and rubbed on to the paper.

4.8.5.1. MacConkey agar plate

For the selective isolation, cultivation and differentiation of coli formed enteric pathogens based on the ability to ferment lactose. Lactose- fermenting organisms appear as red to pink colonies. Lactose non-fermenting organisms appear as colorless or transparent colonies.

4.8.5.2. Triple sugar iron agar slant

To test, the selective isolation base on their fermentation of three sugars (lactose, sucrose, dextrose) and the production of H_2S . The inoculated tubes incubated at $35^{\circ}C$ in aerobic condition for 24 hrs. Bacteria that ferment sugars turn the medium orange red.

4.8.6. Indole tests

For the Indole, one loopful fresh bacterial culture was inoculated in SIM (Simmon's citrate media) broth medium and incubated at $37^{\circ}C$ for 1-2 days, after incubation, Kovac's solution was added and shaken vigorously for one minute.

4.8.7. Urea hydrolysis test

Sterilize the glucose and urea solutions by filtration prepare the basal medium without glucose or urea. Adjust the pH and sterilize by autoclaving in a flask at $121^{\circ}C$ for 15 min. Cool to about $50^{\circ}C$ add the glucose and urea and tube the medium as slant. The medium used to detect urea's production by the bacteria.

4.8.8. Production of hydrogen sulphide

Hydrogen sulphide reacts with lead acetate to form lead sulphide (black in color). This property of hydrogen sulphide was utilized in the demonstration of its production

in the peptone iron agar medium (ISP-VI) by microbes (Tresner & Danga, 1958). There are two major fermentative pathways by which some microorganisms become able to produce hydrogen sulphide.

Three inches long filter paper strip was taken in lead acetate solution and then dried. Peptone iron agar medium was inoculated and lead acetate paper strips were introduced in each of tubes by holding them between the plug and the wall of the test tube. The tubes were then incubated at 37°C for 24 to 48 hours.

4.8.9. Voges-Proskauer (V.P.) test

For this test, V.P. medium in each was inoculated with 48-72 hours old culture and incubated at 37°C for 24-48 hours. After incubation 3 ml of 5% alcoholic α -naphthol solution was added to each tube followed by 1 ml of potassium hydroxide creatine solution. The tubes were then shaken vigorously for 1-2 minutes.

4.9. Identification of the Bacterial Isolates

For molecular identification, genomic DNA was isolated and analyzed from tannery effluents bacteria by the method of Sambrook *et al.* (1989). Bacterial 16S rDNA was amplified by using the universal bacterial 16S rDNA primers, F (5' - AGA GTT TGA TCC TGG CTC AG - 3') and R (5' - GGT GTT TGA TTG TTA CGA CTT - 3'). PCR was performed with a 50- μ l reaction mixture containing 1- μ l(10 ng) of DNA extract as a template, each primer at a concentration of 5 mM, 25 mM $MgCl_2$ and dNTPs at a concentration of 2 mM, as well as 1.5 U of *Taq* polymerase and buffer used as recommended by the manufacturer(Applied Biosystem 9700, USA). After the initial denaturation for 5 min at 96°C, there was 35 cycles consisting of de-naturation at 96°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. PCR was carried out in a gene AMP PCR system 2700 (Applied Bio-systems, California, USA). PCR products were analyzed by 1.5% (w/v) agarose gel electrophoresis in 1x TAE buffer with ethidium bromide (0.5 μ g/mL).

4.9.1. Nucleotide sequencing and phylogeny

A DNA fragment was eluted by using QIAGEN Gel Extraction Kit. PCR product was sequenced by 3730x1DNA synthesizer (Applied Biosystems, California, and USA).

Sequences were matched with previously published bacterial 16S rDNA sequences in the NCBI databases using ADVANCED BLAST (Altschul *et al.*, 1997). Based on the scoring index the most similar sequences were aligned with the sequences of other representative bacterial 16S rDNA regions by using ClutalX software version 1.83 (Jeanmougin *et al.*, 1998). Further phylogenetic tree, similarity index was generated and compared with known sequences. 16S rDNA sequences of tannery effluent bacteria have been deposited in GenBank (<http://www.ncbi.nih.gov/BLAST> algorithm).

4.9.2. PCR amplification of the 16s rRNA Gene

Gene fragments specific for the highly variable region of the bacterial 16S rRNA gene was amplified by PCR as described by Loffler *et al.* (2000). This reaction was carried out using the universal primer 16S F & 16S R (Sigma, USA). The primer sequences were as follows:

Table 4.1: Primer of the detection of 16s small subunit ribosomal RNA genes (Loffler *et al.* 2000)

Gene	Primer	Oligonucleotide sequence(5'-3')	Annealing Temperature	Size of amplifying Product(bp)
16S rRNA	Forward	GAGTTTGATCCTGGCTCAG	55 °C	1500
	Reverse	GAAAGGAGGTGATCCAGCC		

All PCR reagents were used from the invitrogen. PCR was performed in a 50 µl reaction mixture containing 5µl of 10X PCR Buffer, 4µl of 2.5mM dNTPS, 2µl of 50mM MgCl₂, 2.5µl of each primer (10µM), 0.2µl of 5U/µl Taq DNA polymerase, 3 µl genomic DNA and rest of the PCR water. PCR program was as follows: an initial denaturation step at 95°C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min, 1 min of primer extension at 72 °C and 10 min of final extension at 72 °C for one cycle. Amplicons were separated by agarose gel electrophoresis (1 %) in 0.5X Tris/borate/EDTA buffer. Products were

stained with ethidium bromide, destained with distilled water, and visualized under UV light and photographed on a gel-documentation system. 1500 bp PCR products were obtained. A 1 kb+ DNA size marker (Invitrogen, USA) was used to determine the size of the PCR product.

4.9.3. Purification of the PCR products

The PCR product was cut from the gel and purified by using commercially available Microcon Centrifugal Filter device, USA according to the following way:

The desired band was cut from the gel and purified by the Freeze 'N Squeeze DNA Gel Extraction Spin Columns. Then partial purified sample reservoir was inserted into screw cap vial. The entire solution (PCR product) was pipetted into sample reservoir (0.5 ml maximum volume), without touching the membrane with the pipette tip. The attached cap of screw cap vial was then sealed. The assembly (screw cap vial with sample reservoir) was placed in a centrifuge machine. When placing the assembled device into the centrifuge rotor, the cap strap was aligned toward the centre of the rotor. The assembly was spin at 14000 rcf (relative centrifugal force) for 20 mins. The assembly was then removed from the machine and the vial was separated from the sample reservoir. The sample reservoirs was placed upside down in a new vial, and then spin at 1000 rcf for 3 min to transfer the concentrate to vial. The sample reservoir was then separated from vial and discarded. 100 μ L Tris EDTA (TE) buffer was added to vial containing the purified PCR product. Concentration of PCR product was measured using UV spectrophotometer and diluted to 10ng/ μ L with TE buffer.

Cycle sequencing

The primer was diluted to 3.2 μ M using PCR water. The reagents were added to a separate tube for each reaction which was shown in Table 4.2.

Table 4.2: The reagents used in cycle sequencing

Reagent	Quantity (μ L)
Big dye reaction mix	4
Template 10 ng/ μ L	1
Primer 3.2 μ M	1
PCR water	14
Total volume	20

The contents of the tubes were mixed well and spin briefly. The tubes were placed in the thermal cycler (Applied Biosystem 9700, USA) and the following cycle sequencing program employed is summarized in the following table.

Table 4.3: Cycle sequencing program

1. Place the tubes in a thermal cycler and set to the correct volume.
2. Perform an initial de-naturation. a. Rapid thermal ramp to 96 ° C b. 96 °C for 4 min
3. Repeat the following for 25 cycles: Rapid thermal ramp to 96 °C 96 °C for 10 seconds. Rapid thermal ramp to 50 °C 50° C for 5 seconds. Rapid thermal ramp to 60°C 60 °C for 4 minutes
4. Rapid thermal ramp to 4 °C and hold ready to purify.
5. Spin down the contents of the tubes low spin in a micro-centrifuge.
6. Next step is purification of extension products.

4.9.4. Rapid thermal ramp:

Applied Bio-System 9700 Series or Similar machine was used in a rapid thermal ramp at 1 °C /second. Purification of the Cycle Sequence Product and Detection of Nucleotides by ABI- PRISM 310

Table-4.4: Purification of the cycle sequence product and Detection of Nucleotides by ABI- PRISM 310

Step	Temp(°C)	Time	No. of cycle
Initial de-naturation	96	5 min	1
De-naturation	96	10 sec	25
Annealing	50	5 sec	25
Extention	60	4 min	25
Final Extention	60	1 min	1

2 µl of 3M sodium acetate (pH 4.6) and 50 µl of 95% ethanol were placed in a 1.5 ml eppendorf tube. The entire content of each reaction was transferred to a tube where sodium acetate and ethanol mixture was placed. Then they were mixed thoroughly. The tubes were then incubated at room temperature for more than 15 min to precipitate the extension products. The tubes were then centrifuged at 13,000 rpm for 20 min. The supernatant was discarded carefully using a micropipette. The pellet was washed with 250 µl of 70% ethanol by washing the walls of the tubes. The tubes were spin at 13,000 rpm for 10 min. The supernatant was discarded very carefully using a micropipette and tips were used to remove the supernatant from the bottom of the tubes. The washing steps were repeated twice. The pellet was dried by keeping the caps of the tubes opened for 2-3 hours at room temperature. The opened tubes were covered by parafilm. Then the pellet was resuspended into formamaide and mixed well. Then it was heated at 95°C for 2 min & immediately transferred into ice and kept for 5 min. Then the products were transferred to a septal tube.

4.9.5. Detection of the nucleotides by ABI- PRISM 310

The purified cycle sequence products were analyzed by electrophoresis in the genetic analyzer (Prism 310, USA). DNA was separated through the POP6 contained in the capillary and detected by the laser beam. When the nucleotides reach a detector window in capillary electrophoresis, the fluorescent labeled fragments were excited

by the laser beam of the machine. The laser excites the fluorescent dye labels and emitted fluorescence was collected by CCD camera. The fluorescence intensity data was interpreted into sequence data by specific bioinformatics software.

4.9.6. Sequence analysis

The sequences were then edited by bioinformatics software Chromas. The homology of the 16S rRNA gene sequences was checked with the 16S rRNA gene sequences of other organisms that had already been submitted to GenBank database using the BLASTN (<http://www.ncbi.nih.gov/BLAST>) algorithm.

4.10. Antibiotics disk sensitivity test

To determine the antibiotic sensitivity of the bacterial strain, antibiotic – impregnated discs were placed on freshly prepared agar plates and the plate was incubated at 37°C for 24 hrs. The diameter of the inhibition zones was measured to the nearest cm and the isolate were classified as resistant, intermediate and susceptible following the standard antibiotic disc sensitivity testing method. Bauer *et al.* (1996) also stated that isolated heavy metal resistant isolates were tested for antibiotic sensitivity and resistance according to the Kirby Bauer disc diffusion method. After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to diameter of inhibition zone given in standard antibiotic disc chart. The heavy metal (Cr, Cd and Pb) resistance strains were tested for their sensitivity to 10 different antibiotics and as followed. Gentamycin (10 µg), Cefuroxime sodium (30µg), Nitrofurantoin (300µg), Azithromycin (15µg), Penicillin (10µg), Ceftazidime (30µg), Vancomycin (30µg), Ciprofloxacin (5µg), Pefloxacin (5µg) and Amoxycillin (10µg).

4.11. Preservation of Bacterial Isolates

The preservation media of isolated bacterial cell was prepared in a stock vial using 1% tryptone, 1% NaCl, and 0.6 % agar. Bacteria was taken with a needle from isolated colonies and stabbed in the media. The vials were then filled with sterile paraffin oil, tightly capped and sealed with paraffin and preserved at room temperature.

4.12. Extraction of plasmid DNA

Plasmid DNA isolation of bacterial cells was carried out according to Brinboim and Doly method (1979), and Kudo and Liu method (1981) using alkaline lysis procedures.

4.13. Determination of Minimum Inhibitory Concentration (MIC) of Gentamycin

Minimum Inhibitory Concentration (MIC) may be defined as the lowest concentration of an antimicrobial drug to inhibit the visible growth of a microorganism after overnight incubation. The MIC value of *Gentamycin* was determined by serial tube dilution technique or turbid metric assay. Serial tube dilution or turbidimetric technique was followed using MS broth medium to determine the MIC value of *Gentamycin* against the isolated bacteria. MIC of Cr/ Cd /Pb isolate was also determined by colony counting method. Plates containing nutrient agar medium supplemented with different concentrations (50 -8000 µg/mg) of antibiotic, were inoculated with 100µl of a fresh overnight culture grown in nutrient broth medium. All plates were incubated at 37°C for 48hours. The growth of bacteria was monitored by colony counting method.

4.14. Preparation of inoculums

Fresh cultures of the test organisms were grown at 37°C for 24 hours on MS medium. The log phase bacterial suspensions were then prepared in sterile MS broth medium in such a manner so that the suspension contains 10^6 cfu/mL. These suspensions were used as inoculums. The bacterial suspensions were centrifuged at 12000 rpm for 2 minutes. The supernatant was removed and the cell pellet was thoroughly dissolved in 1% saline water and the measured optical density should be 1 at 660 nm. The dissolve pellet was used as inoculums.

Procedure

Seven (7) pairs of autoclave test tubes were taken and they were marked as 1, 2, 3, 4, 5, 6 and 7 and the two were assigned as C_m (medium) and C_i (medium + inoculum). 10 ml of sterile MS broth medium was added to each of the seven pairs' test tubes. 50, 100, 200, 300, 500, 600 and 800 µg/mL concentration of Gentamycin were added to the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th test tubes and mixed well.

The diluted pellet inoculums of 100 μ l was added to each of seven test tubes and mixed well.

100 μ l of inoculum was also added to the control test tube Ci, to observe the growth of the organism in the medium used.

The control test tube Cm, containing only medium was used to confirm the sterility of the medium.

All the test tubes were incubated at 37°C for 72 hours.

4.15. Curing experiments

The involvements of naturally occurring plasmids in the degradation of synthetic organic compounds were extensively documented (Sayler, Hooper, Layton & Henryking, 1990). Catabolic plasmids are thought to play an important role in the evolution of metal ions-degrading ability in microorganisms (Chaudhry et al., 1988). Curing of plasmid DNA was carried out with ethidium bromide, acriflavin, SDS (sodium dodecyl sulphate) and temperature at 40°C. Plasmid DNA curing was achieved from the growing strain treatment with the above three chemicals at 40°C. In addition, repeated subcultures in nutrient broth in the absence of appropriate carbon source were used as a curing strategy. Autoclave test tubes were taken and 10 ml sterile nutrient broth medium was added and supplemented with the respective curing agents over a range of concentration which was accumulated in Table 4.9. Inoculated cultures (2% by volume i.e. 1 ml from a late exponential phase culture of the pure strain) was taken and incubated at 37°C with shaking at 120 rpm. The test tubes (containing curing agents and medium) were used to confirm the sterility of the medium. All test tubes were incubated at 37°C for 5-7 days. After incubation, culture tubes with highest concentration of curing agents and showing visible growth (very little turbidity) was selected. 500 μ l of the cultures were spread on to nutrient agar plates containing antibiotic, Gentamycin (200 μ g/mL) and without antibiotics treated as control. After overnight incubation at 37°C, a large number of colonies were found without antibiotics nutrient agar plate and a few colonies were found in nutrient agar plates containing antibiotics (Gentamycin). 25 colonies from the nutrient agar plate, were transferred to antibiotics (Gentamycin, 200 μ g/mL) plates (replica-plated) and heavy metals (20 μ g/ml) containing MS medium plates (replica-plated) incubated for

24 hr at 37°C colonies from the master plate which failed to grow on the antibiotic plates. Streaked out on nutrient agar and further tested for their inability to grow on MS broth medium containing heavy metal ions (Cr, Cd and Pb, 20 µg/mL) and for their loss of plasmid DNA content. The extracted plasmid DNA was run in another agarose gel submerged in TBE buffer at 50 volt for 2 hours.

4.16. Degradation rate of metal ions (Cr, Cd and Pb) containing effluents by bacteria

4.16.1. Experimental description

(i) Sample preparation

For biodegradation studies, fresh cultures of the test organism was grown at 37⁰ C for 24 hours on nutrient broth supplemented with heavy metal ions (Cr, Cd and Pd). The cultures were used as inoculums. Four ml of late exponential phase culture of the pure strain (OD at 660nm-1) was added in a 500-ml Erlenmeyer flask with 400 ml of MSM media containing Cr, Cd and Pb (20 µg/mL, respectively) and 0.01% glucose. 20µg/ml of Cr/ Cd/ Pb respectively contained media was inoculated with bacteria and media containing Cr, Cd and Pb (20µg/ml) without bacteria served as control. All the bacterial culture media including control cultures were incubated for 24 hours at 37°C and shaken at 120 rpm on an orbital shaker. Before incubation (time 0) 10ml sample were taken and centrifuged at 6000 rpm for 10 minutes. After centrifugation the supernatant layer was carefully analyzed for Cr, Cd and Pb by AAS. Simultaneously, after incubation at 37⁰C, the cultures were found to be turbid and each 10ml samples were taken from the flask and centrifuged at 6000 rpm for 10 minutes. After centrifugation the required aqueous layer were preserved in a freez at 4oC. Similarly after 2, 3, 4, 5 and up to 24 days of incubation each 10ml samples were taken from the flasks using a micro pipette and same centrifugal procedure was maintained before analysis. For composite raw tannery effluents, the pH of the effluent was adjusted to 7.2 with NaOH and/ or HCl and then taken 100 ml to each three of 250 ml conical flasks. 0.01% glucose was added and 3ml of overnight grown culture (containing OD 1) was initiated in each conical flask. The media contained Cr/ Cd/ Pb (20µg/mL). The flasks were kept on rotary shaker at 120 rpm for *Entero bacter species* at 37°C. *Entero bacter species* were maintained at 37°C for a period of 21 days. Cr, Cd and Pb were estimated everyday and the process was continued for 30days to calculate the

Cr, Cd and Pb depletion from the effluent by the indigenous organisms. After treatment, same centrifugal procedure was maintained to analyze the metal ion concentrations and controls were analyzed for Cr, Cd and Pb by AAS (AA-6800, and SHIMADZU, JAPAN).

4.16.2. Preparation of standard solution

Cr, Cd and Pb salts were used for the standard solution to determined Cr, Cd and Pb and calibration curve was stated in the Appendix 2.11, 2.12 and 2.13.

All the instruments were standardized or calibrated with standard solution before use and all the chemicals were used in analytical grade supplied by Sigma Aldrich Company Ltd., Germany, and BDH Ltd., England. The experiments were performed at least three times to minimize analytical error. Each time three readings were taken, their mean value and standard error of the mean were used.

4.17. Results and Discussion

4.17.1. Isolation of bacteria

The present study reveals that the identified and characterized heavy metal ions resistant bacteria were isolated from tannery effluents. About three hundred plates (isolated colonies containing) in each medium were screened from initial level of heavy metal supplemented LB and MS agar medium. Isolated colonies contained about 36 plates were selected in the secondary screening from tannery effluents. Finally three strains in each medium were selected based on high degree of heavy metals. The three isolates were examined the tolerance test of each metal ion i.e., Cr, Cd and Pb individually with different concentrations (5-100 µg/mL, respectively) in MS agar medium and these isolates were also examined the tolerance test of each metal ion, i.e., Cr, Cd and Pb individually with different concentrations (100-1600 µg/mL) in nutrient LB agar medium. All the three bacterial strains showed resistance to 1500, 1300, 1200 µg/ml for Cr, Cd and Pb, respectively in nutrient (LB) and 60, 55 and 50 µg/mL for Cr, Cd and Pb, respectively in MS agar media. The study results observed that the order of tolerance of metal ions to bacterial strains was found to be Cr > Cd > Pb on the MS and LB agar plates. It indicates that the bacterial species could be used to degrade the metal ions, i.e., Cr, Cd and Pb at optimum conditions. Previous reports showed that *Pseudomonas species* were resistant to cadmium up to 7mM in

TY agar plates and only 2mM Cd resistance was observed in sewage of Morocco ((Pal *et al.*, 2004; Edward Raja *et al.*, 2006; Filali *et al.*, 2000). Edward Raja *et al.* (2006) also stated that *Proteus* and *Acinetobacter species* were highly resistant to As (16, 13mM) and Ni (8mM). But Ni resistant isolates tolerating were screened from West Bengal, India (Bhadra *et al.*, 2006) and from serpentine outcrops of central Italy (Mengoni *et al.*, 2001). Roane T. M. (1999) stated that Pb resistant isolates were resistant to 0.6 mM and 2.5 mM in minimal medium. It may be suggested that bacteria exposed to high levels of heavy metals in their environment have adapted to different stress by developing various resistance mechanism.

The morphological and physiological characteristics of tannery effluents bacteria are illustrated in Table 4.5. The analyses results were accumulated in the Table from the Plates 4.1, 4.2. Microscopic examination and AFM of bacterial cells were observed after gram staining and the images are shown in Figures 4.2 and 4.3.

Table 4.5: Morphological and physiological characteristics and Microscopic observation of the isolated Cr-tolerance bacterial strain

Agar plate	Characteristics	Results
MSM- Agar plate	Size	<1-1.5 mm
	Shape	Circular
	Colour	Creamy - white
	Consistency	Sticky –water
	Opacity	Translucent
	Mucoid	++
	Margin	Entire
	Elevation	Raised
Nutrient agar slant	Abundance of growth	Moderate
	Colour	Creamy white
	Form	Filiform
	Optical characteristics	Opaque
Nutrient broth culture	Turbidity	Uniform with fine turbidity
Microscopic observation	Gram - character	--
	Motility	Motile

The three isolated bacterial strains gave the same results for morphological and physiological characteristics.

The present study observed that all the three bacterial strains were Gram-negative, rod shaped, motile bacteria. The study also stated that bacterial isolates from the effluents showed optimum growth conditions at 37°C for 1 day incubation period at pH 7.2.



Plate-4.1: Isolated bacterial colonies (nutrient: Cr 20µg/mL)

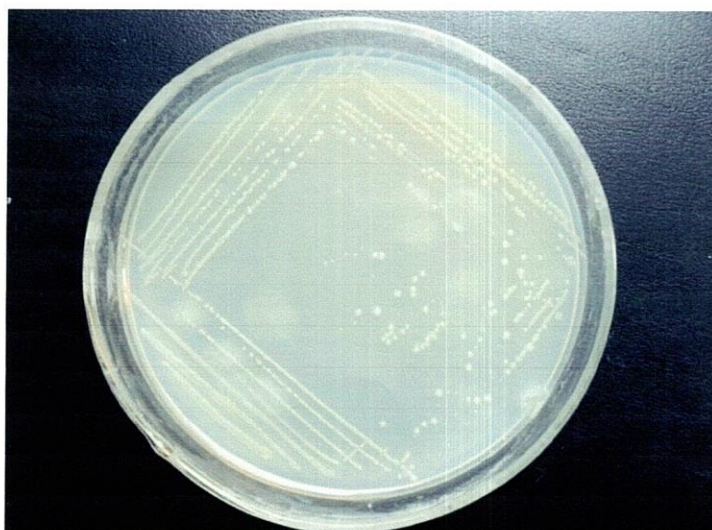


Plate-4.2: Isolated bacterial colonies (neutrient : Cr 50 µg/mL)



Figure 4.2: Microscopic imaged of isolated bacteria.

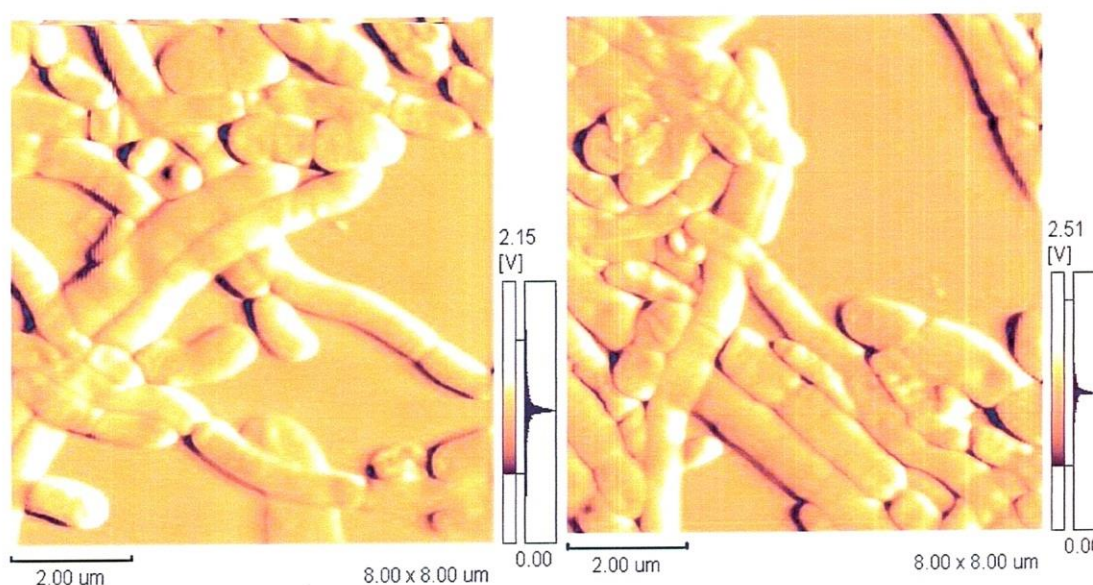


Figure 4.3: Atomic force microscopic imaged of isolated bacteria.

Isolated bacterial cells were imaged with AFM operated in contact mode in liquid condition (Fig.4.3). The examination of isolated bacteria (Cr-resistant, Cd-resistant and Pb-resistant) demonstrated the same morphological characteristics that depend on the bacterial growth conditions and imaging environment. Image of liquid cultured bacteria appeared to have smooth surfaces and apparently greater resolution of cell surface structure.

4.17.2. Determination of optimal growth conditions for isolated bacterial strain

The optimum incubation temperature and pH were determined for the isolated bacteria. Heavy metal ions resistant bacterial isolates are greatly influenced by incubation temperature. For determination of optimum temperature, culture medium was adjusted to pH 7.2 and incubation temperature was varied from 25°C, 30°C, 37°C and 40°C. The optical density (OD) of cultured bacteria was measured at 660 nm wave-lengths for 24 hrs incubation periods with 2 hrs intervals. The analyses results revealed that the most suitable temperature for individual metal ions, i.e., Cr, Cd and Pb, resistant bacterial isolates was found to be 37°C which are demonstrated in Figures.4.4, 4.5 and 4.6, respectively.

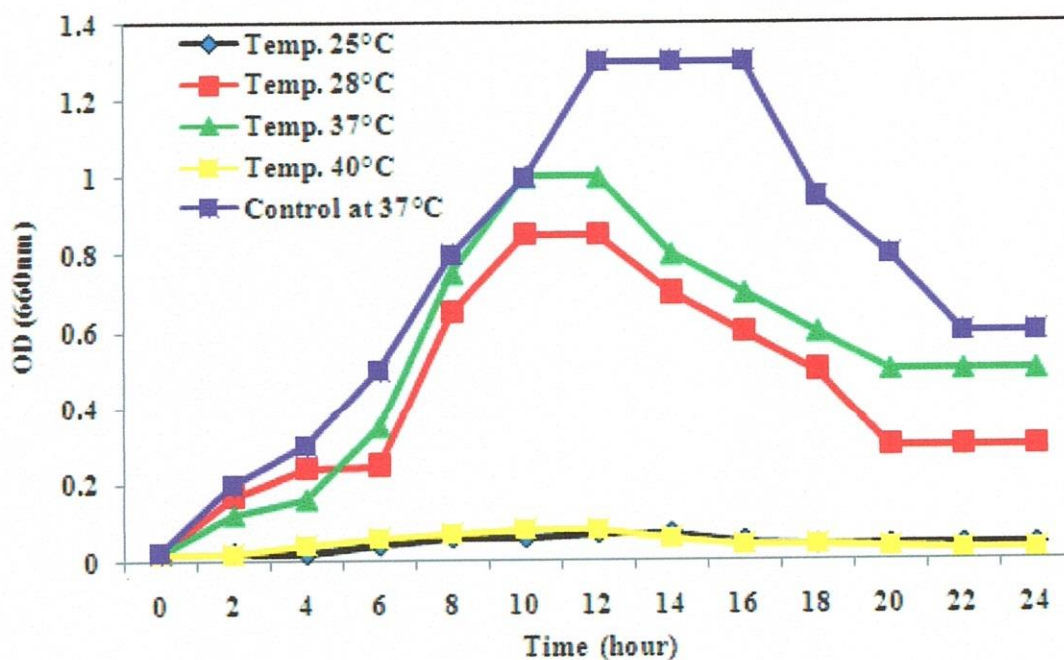


Figure 4.4: Effect of temperature on bacterial growth (nutrient: Cr)

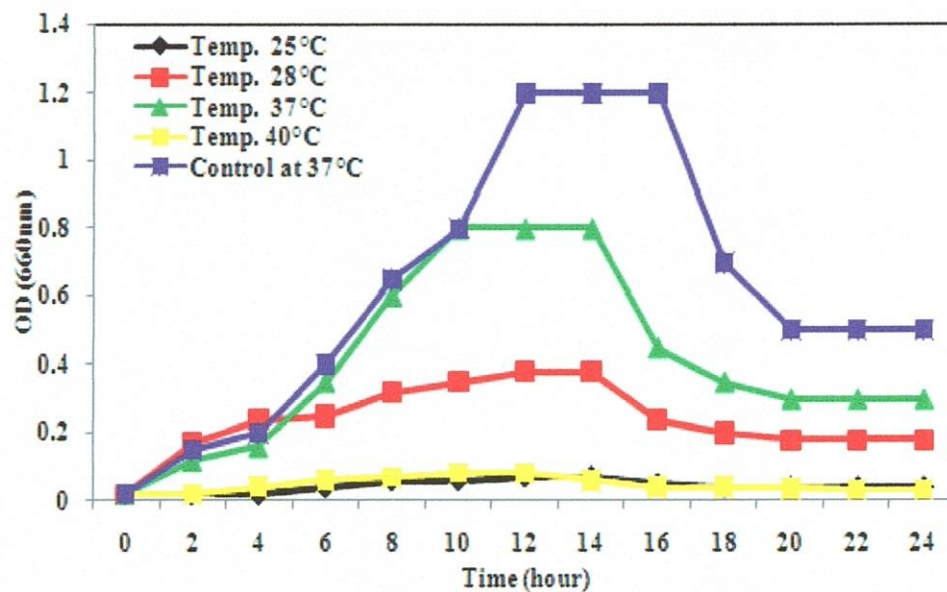


Figure 4.5: Effect of temperature on bacterial growth (nutrient: Cd)

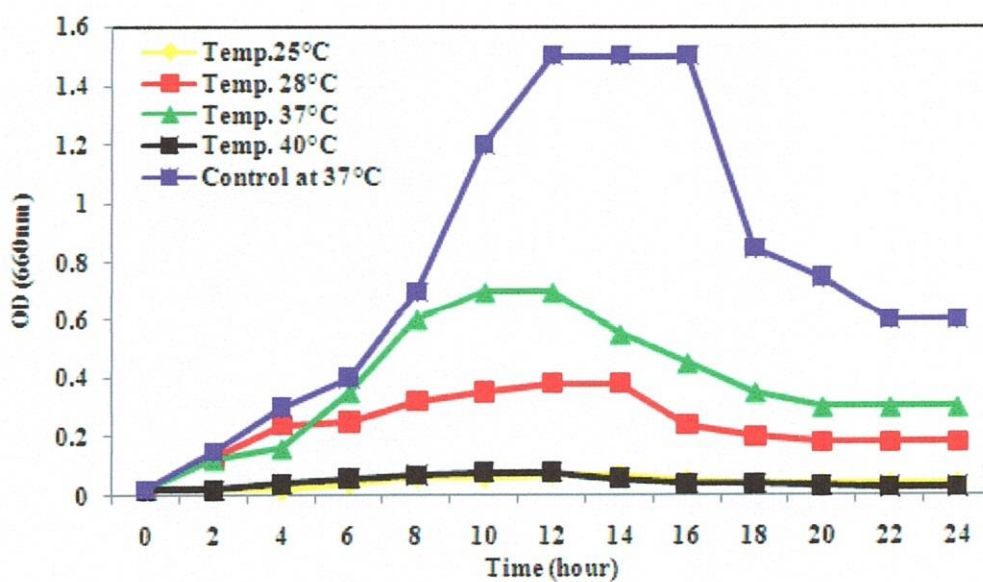


Figure 4.6: Effect of temperature on bacterial growth (nutrient: Pb)

The figures illustrate that metal ions resistant bacterial growth showed lower at low and high temperature (25 and 40°C) due to loss of viability or metabolic activity of cells on prolonged incubation. Sumeet *et al.* (2002) reported that temperature has an effect on bacterial growth and they found a critical temperature at 26°C and below the temperature, the rate of cell growth was inhibited.

The growth curve pattern was studied by growing the organism in the presence of each of the metal ions (20 µg/mL of Cr, Cd and Pb individually) and comparing with the control culture. The growth pattern of isolated bacteria (*Enterobacter species*) was significantly different from those of controls and the log phase was delayed up to 12 hours in the presence of each of the metal ions (Cr, Cd and Pb) individually, when compared with control. The study also reveals that the maximum growth was observed in 10 hrs and continued upto 12 hrs in the presence of 20 µg/mL Cr, or Pb and in the presence of 20 µg/mL Cd, the maximum growth was also observed in 12 hours and continued upto 14 hours. The present study also states that bacterial growth rate was lower in the presence of metal ions as compared to control culture where metal ions were not added into the culture medium at optimum temperature (37°C). A similar observation was made by Usman *et al.* (2011), studied a textile industrial effluents bacterium reported that the growth rate of bacteria was lower in presence of substrate than in control.

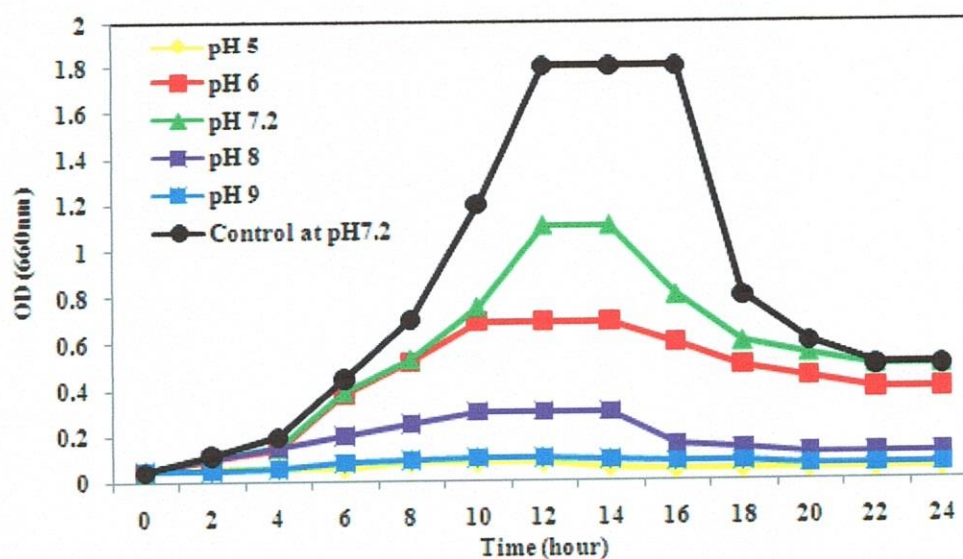


Figure 4.7: Effect of pH on bacterial growth (nutrient: Cr)

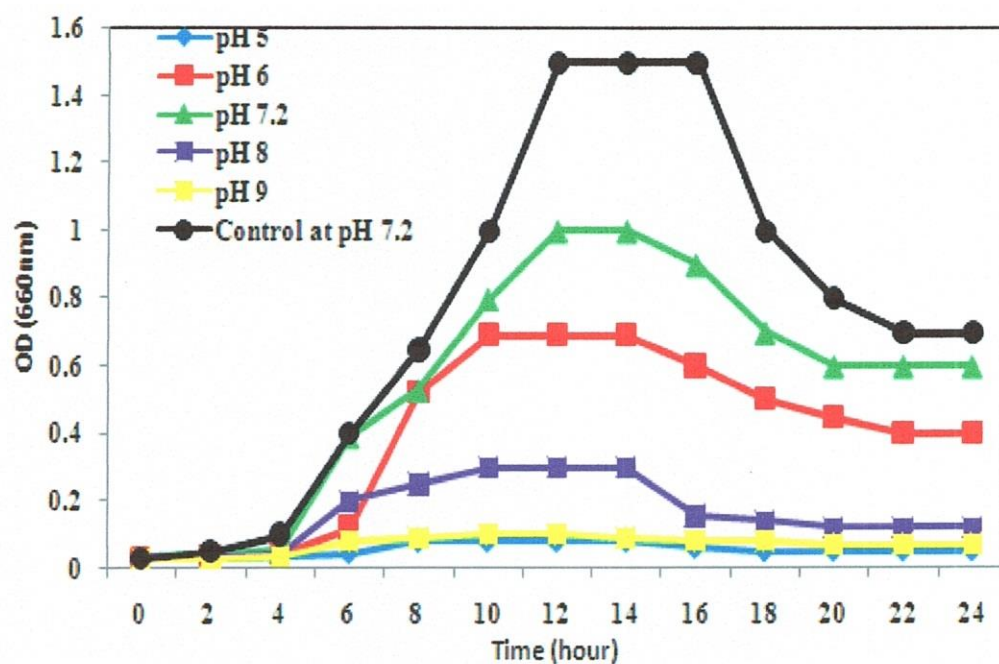


Figure 4.8: Effect of pH on bacterial growth (nutrient: Cd)

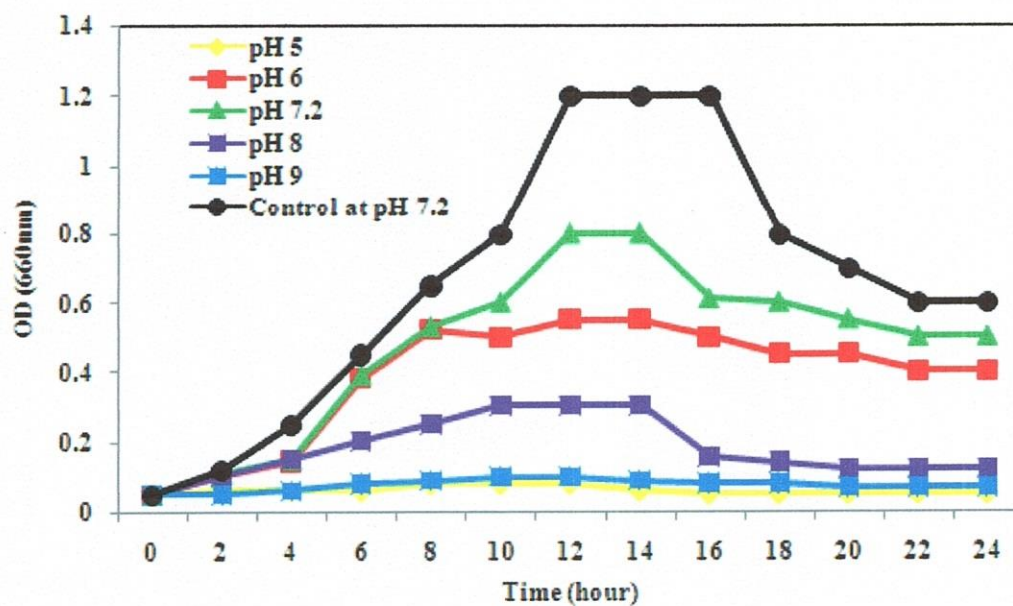


Figure 4.9: Effect of pH on bacterial growth (nutrient: Pb)

The pH of the culture medium was adjusted to 5, 6, 7.2, 8 and 9 then autoclaved and incubated at 37°C to determine the optimum pH. The incubation period and optical density were measured as above. The most effective pH for metal ions tolerance bacterial isolate was 7.2, which are illustrated in Figures 4.7, 4.8 and 4.9 respectively. The study results observe that all the metal ions resistant isolated bacteria showed maximum growth at neutral pH. The figures illustrate that the extreme PH were 5 and 9, where bacterial growth was strongly inhibited. The study also illustrates that all metal ion tolerance bacterial culture showed lag, log and exponential phases at pH 6, 7 and 8, but there was no distinct phase identified at pH 5 and 9. The highest free bacterial populations were observed in all cultural media at pH 7.2.

The growth pattern of isolated bacteria (*Enterobacter species*) was significantly different from those of controls at pH 7.2. Bacterial population were found highest in the control culture compare to each metal ion (Cr, Cd and Pb individually) tolerance bacteria. The study also reveal that the maximum growth was observed after 12hrs and then continued up to 14 hrs in the presence of each 20µg/ml of Cr, Cd and Pb ions individually (Figures 4.7-4.9). Cr-tolerance bacteria showed the maximum growth among the three metal ion tolerance bacteria (Figures 4.7 4.8 and 4.9). The death phase started after 14 hours of incubation in all three metal ion tolerances. In general the growth rate of the tannery effluents bacteria in the presence of metal ions was consistently slower than the control (Figures 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9) at an optimum condition. Edward Raja *et al.* (2009) reported that the growth rate of the sewage isolates in the presence of heavy metal ion (Cd, Ni, As and Pb) were consistently slower than that of the control media and the results are in good agreement with the present study.

4.17.3. Biochemical Tests

4.17.3.1. Utilization of sugars by isolating bacterial strain

Utilizing dextrose of isolated bacterial strain was observed in MS medium with respective carbohydrate, at 37°C and after incubation period 120 hours. The overall biochemical study results of the isolated bacterial strains are summarized in the Table-4.6 from the Plates (4.3, 4.4 and 4.5)

Table 4.6: Biochemical tests for the isolated bacterial strain

Carbon source	Pb resistant bacteria	Cd resistant bacteria	Cr resistant bacteria
Monosaccharide's			
Glucose	++	++	++
Gelatos	++	++	++
Mannose	++	++	++
Arabinose	++	++	++
Disaccharide's			
Maltose	++	++	++
Sucrose	++	++	++
Lactose	++	++	++
Citrate	++	++	++
Trisaccharide's			
Manitol			
Cellulose			
LIA (B/S)	y/p	y/p	y/p
TSI (B/S)	y/r	y/r	y/r
XLD	Well growth (media color)	Well growth (media color) some colony yellow	Well growth (media color)
BSA	+, ash color	+, ash color	+, ash color
BPA	+, poor growth Some blackish	+, poor growth Some blackish	+, poor growth Some blackish
Congo red agar	Clear, some black spot, blackish plate, positive for congo red binding and slime production	Clear, some black spot, blackish plate, positive for congo red binding and slime production	Clear, some black spot, blackish plate, positive for congo red binding and slime production
Skim milk agar	No clear zone, do	No clear zone, do	No clear zone, do

	not produce protease	not produce protease	not produce protease
MYP agar	No surrounding precipitation zone, phospholipase negative	No surrounding precipitation zone, phospholipase negative	No surrounding precipitation zone, phospholipase negative
Glucose	Yellow	yellow	Yellow
Sucrose	Yellow	yellow	Yellow
Lactose	Reddish	Reddish	Reddish
Fructose	Yellow	yellow	Yellow
Maltose	Yellow	yellow	Yellow
Trehalose	Yellow	yellow	Yellow
Rhamnose	Yellow	yellow	Yellow
Microscopy with only crystal violet	coccus	coccus	coccus
PEMBA	No blue color	No blue color	No blue color
Macconkey	Colorless (yellowish)	Colorless (yellowish)	Colorless (yellowish)
EMB	Slight pink	Slight pink	slight pink
Methyl red	negative	negative	negative
VP	negative	negative	negative
oxidase	positive	positive	positive
Catalase	positive	positive	positive
Control	--	--	--

The ‘++’ sign indicates the growth of the microorganisms, while ‘--’ sign indicates no growth species.

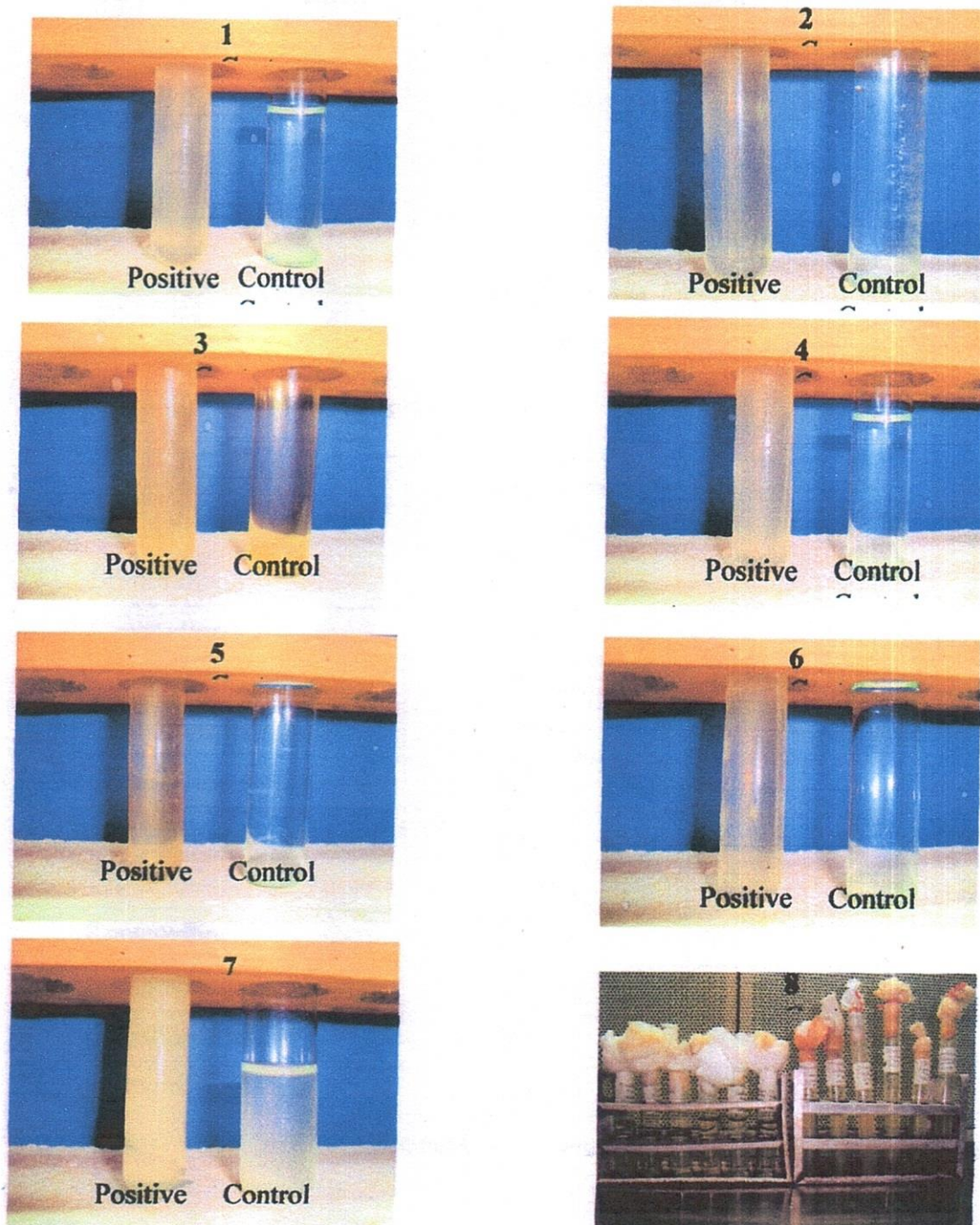
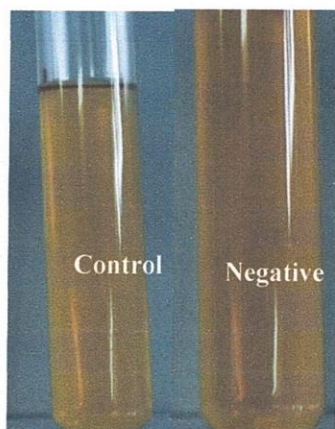
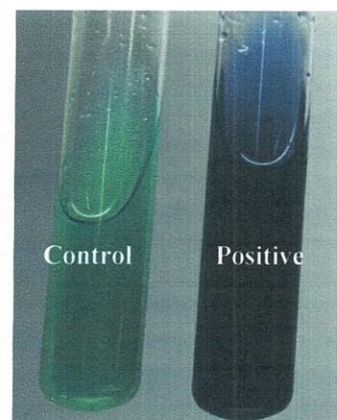


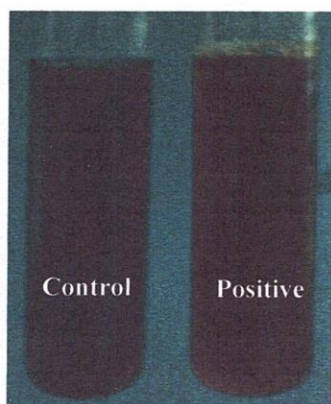
Plate-4.3: Sugar Utilization (1. Glucose, 2. Galactose, 3. Maltose, 4. Sucrose, 5. Lactose, 6. Mannitol, 7. Cellulose and 8. All).



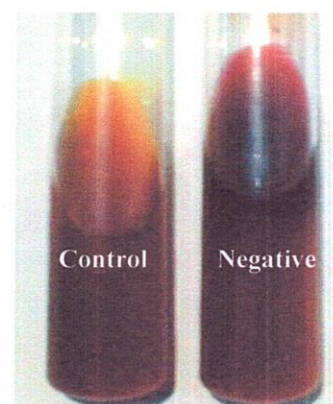
Indole, H₂S production test



Citrate utilization test

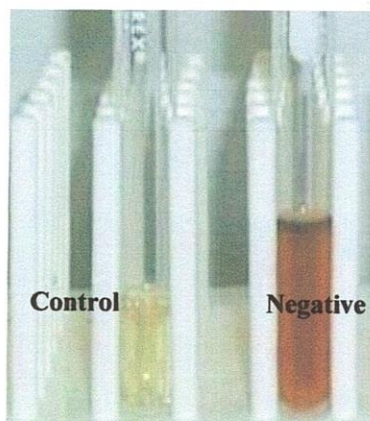


Triple Sugar Iron (TSI) test

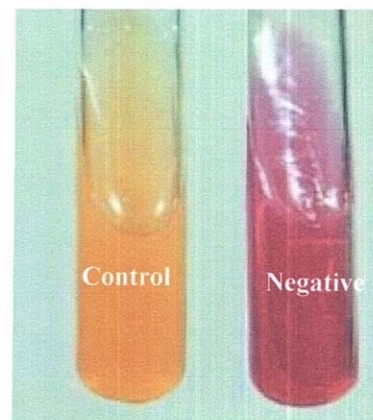


Methyl Red (MR) test

Plate-4.4: Biochemical tests



Voges Proskauer test



Urea hydrolysis test



Catalase test

Plate-4.5: Biochemical tests

4.17.3.2. Results of bio-chemical tests

Three isolated strains (supplemented with 20 μ g/mL of each Cr, Cd and Pb) were found to show the same phenomena for all the bio-chemicals studies. The analyses results of all bio-chemicals studies stated that three isolated bacterial strains were found to be the same species which was seems to be the *Enterobacter Speceies*.

4.17.4. Antibiotic sensitivity

Table 4.7 represents the anti-microbial zone of inhibition which was evaluated significance of zone diameters with disk potency. It is evident that the isolated

bacterium was resistant to 5 antibiotics out of ten antibiotics tested (Plate-4.6). The patterns of sensitivity and resistance of isolating bacterial cultures in 10 different antibiotics were tested by the disc method using on nutrient broth medium. Antibiotic disc containing LB agar plate (supplemented with each 20µg/ml of Cr, Cd, and Pb individually) was incubated at 37°C for overnight. If the diameter of the inhibition zone was grown, then inhibition zone was measured. Zone of inhibition surrounding the well represented the index of toxicity.

Table 4.7: Antibiotic sensitivity tests

Antimicrobial agent	Disk potency	Results
Cefuroxime sodium CXM(30µg)	15mm	I
Nitrofurantoin F(300µg)	10mm	R
Azithromycin AZM(15µg)	6mm	R
Penicillin P(10µg)	6mm	R
Ceftazidime CAZ(30µg)	18mm	S
Vancomycin VA(30µg)	6mm	R
Gentamycin GM(10µg)	6mm	R
Ciprofloxacin CIP(5µg)	24mm	S
Pefloxacin PEF(5µg)	11mm	I
Amoxycillin A(10µg)	15mm	I

(5-10 mm) = Resistance to antibiotic(R), (15-20 mm) = Sensitive to antibiotic(S), (10-15 mm) = intermediate resistance (I).

The analyses results observed that isolated strain was found different susceptibilities to drugs. The Cr, Cd and Pb resistant isolates were tested for their sensitivity to 10 common antibiotics. Isolated strain appeared to be most susceptible being inhibited by all considerable antibiotics. The strain was resistant to 5 antibiotics such as *Gentamycin*, *Vancomycin*, *Azithromycin*, *Penicillin* and *Nitrofurantoin* and it was sensitive to remaining 5 antibiotics and showed susceptible response to *ciprofloxacin* as described in Table 4.7. Maria De Souza *et al.*, (2006) reported that the bacterial isolates (*Pseudomonas*, *Flavobacterium*, *Micrococcus*, *Alcaligenes*, *Aeromonas*, *Vibrio* and *Acinetobacter*) isolated from Antarctic marine waters polluted with concentrate heavy metals showed varying degrees of resistance to antibiotics (*chloramphenicol*, *ampicillin*, *streptomycin*, *tetracycline* and *kanamycin*) and metals

and the results were in good agreement with the present study. Depending on the antibiotics the isolates showed different percentage of resistance. High incidence of resistance to both antibiotics and metals were common among the pigmented bacterial isolates. An increased in metal ion resistant decreased the ability of bacteria to express enzymes. Bezverbnaya *et al.* (2005) also reported that in plasmids these heavy metal resistance genes may be linked with genes of antibiotics resistance and genes responsible for pathogenic property. Thus individual bacterial strains have developed capacity to survive under toxicological stress which may hazardous to humans and animals. Hence it is essential to study the metal resistant bacteria which are being formed in response to selective anthropogenic pollution.

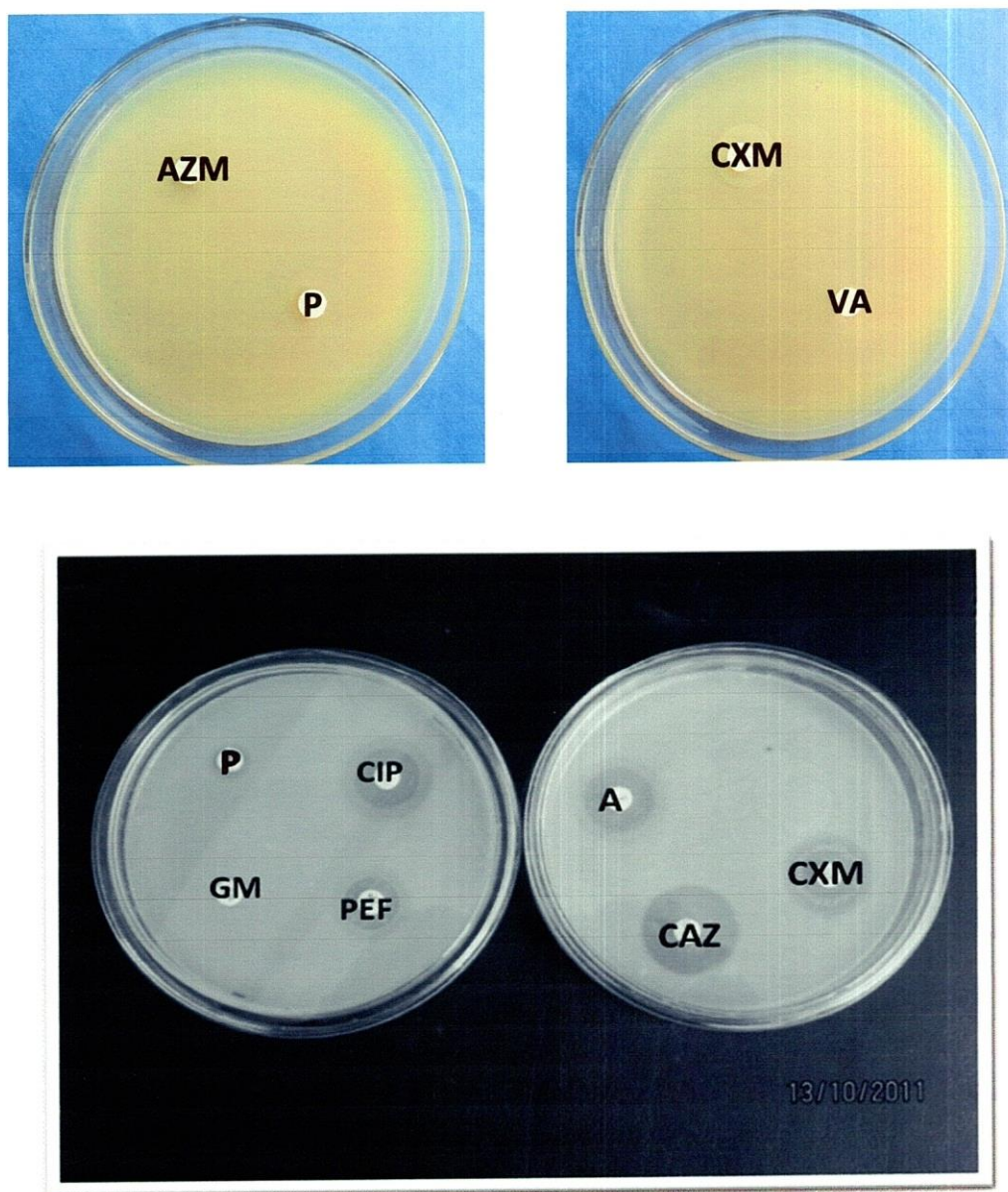


Plate-4.6: Antibiotic disc sensitivity tests

4.17.5. Extraction of Chromosomal DNA

The extraction of chromosomal DNA was done according to Sambrook *et al.* (1989). There was 1 band of Chromosomal DNA was found the agarose gel after illumination with the UV light. The 1 kb+ DNA marker was used for chromosomal size determination. Lane one indicates the DNA marker and lane 2 indicates the Bacterial Chromosomal DNA. The isolated chromosomal DNA was 1500 bp which compared with 1 kb+ DNA marker shown in Plate-4.7.

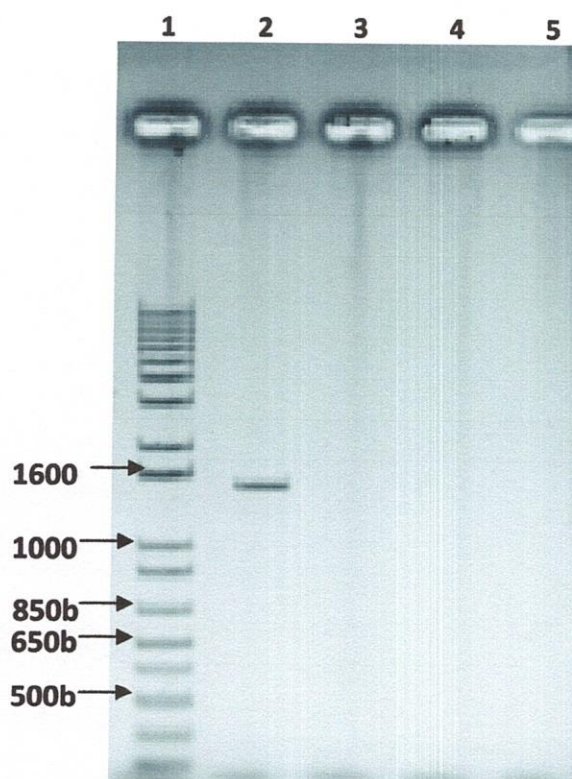


Plate-4.7: PCR Amplification of 16S rRNA gene yielded a 1500 bp PCR product, 1 kb+ DNA marker is used for size determination.

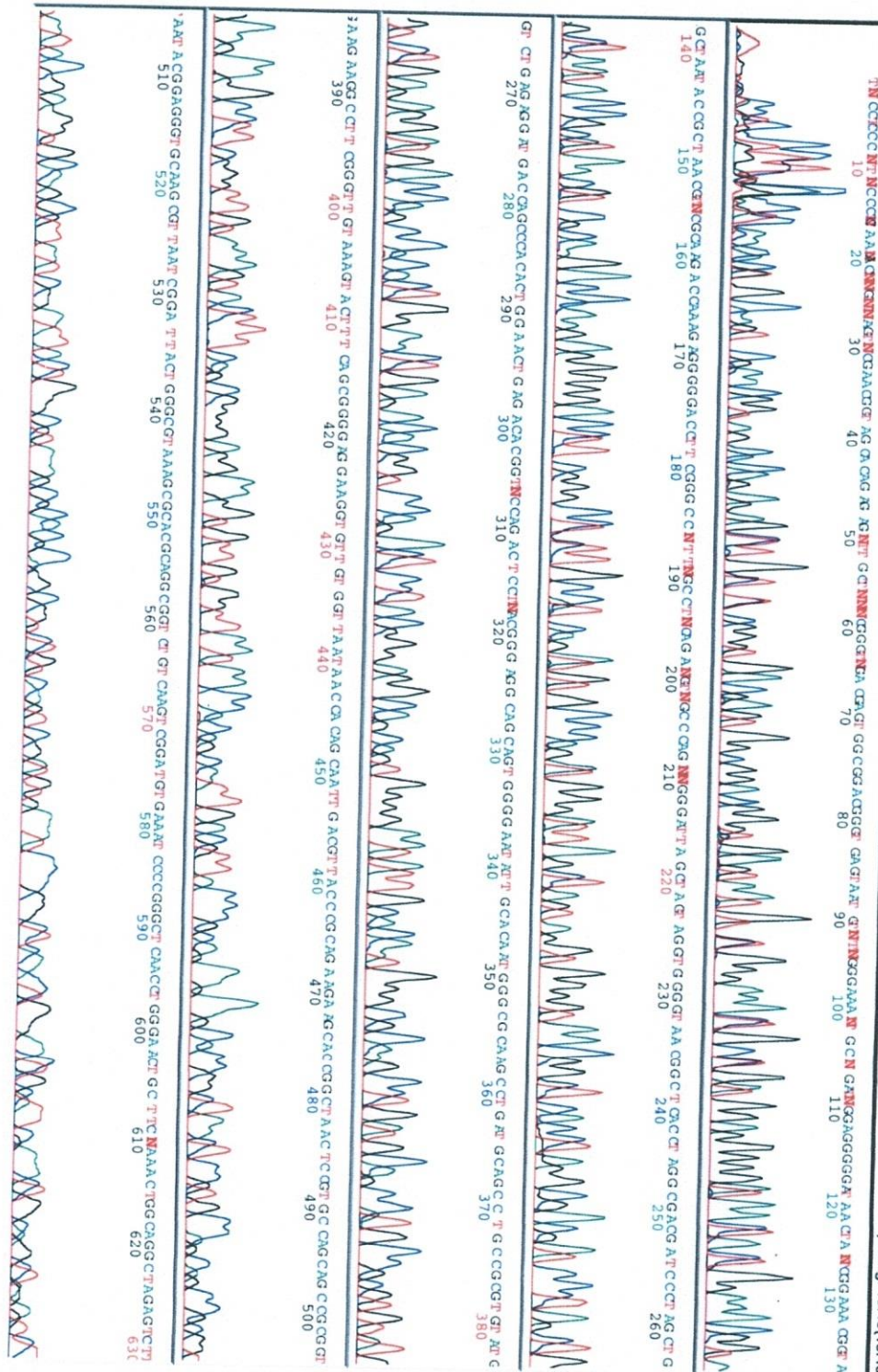
4.17.5.1. DNA sequencing analysis of 16S rRNA gene

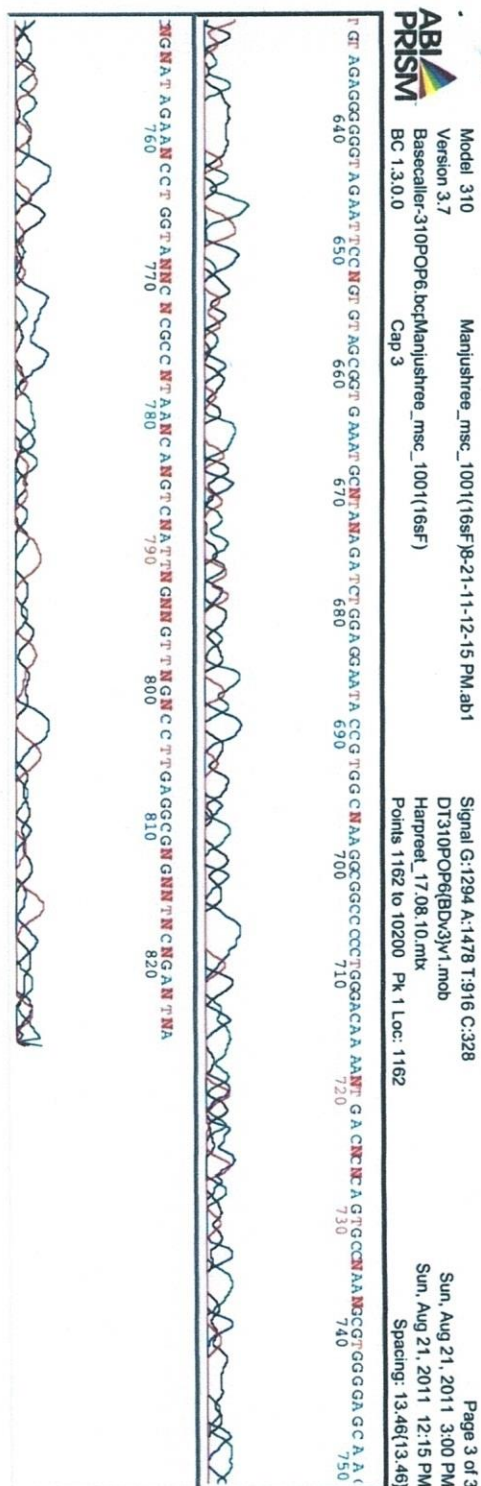
After the 16S rRNA gene sequencing and editing of sequences it was checked with the 16S rRNA gene sequences of other organisms that had already been submitted to Genbank database. From the genbank, several sequences are found with significant identity.

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
gil338858342 AB641896.1	<i>Enterobacter</i> sp. NCCP-287 gene for 16S rRNA, partial sequence	1368	1368	99%	0.0	100%
gil338858335 AB641889.1	<i>Enterobacter</i> sp. NCCP-280 gene for 16S rRNA, partial sequence	1368	1368	99%	0.0	100%
gil334852731 JF894166.1	<i>Enterobacter cloacae</i> strain FR 16S ribosomal RNA gene	1368	1368	99%	0.0	100%
gil329665845 JF489149.1	<i>Enterobacter cloacae</i> strain SF2 16S ribosomal RNA gene	1368	1368	99%	0.0	100%
gil326375384 JF281095.1	<i>Enterobacter</i> sp. EMB19 16S ribosomal RNA gene	1368	1368	99%	0.0	100%
gil319433526 HQ686272.1	<i>Salmonella</i> sp. enrichment culture clone CL107	1368	1368	99%	0.0	100%
gil209361530 FJ194527.1	<i>Enterobacter cloacae</i> strain EB89 16S ribosomal RNA gene	1368	1368	99%	0.0	100%
gil326885703 HQ336043.1	<i>Enterobacter dissolvens</i> strain AGYP1 16S ribosomal RNA gene	1364	1364	99%	0.0	99%
gil307713820 HM461143.1	<i>Enterobacter</i> sp. enrichment culture clone HSL19	1364	1364	99%	0.0	99%
gil307713810 HM461133.1	<i>Enterobacter</i> sp. enrichment culture clone HSL10	1364	1364	99%	0.0	99%
gil158514398 EU195905.1	<i>Bacterium</i> VNS3-1-2 16S ribosomal RNA gene	1364	1364	99%	0.0	99%
gil343206386 NR_044978.1	<i>Enterobacter cloacae</i> subsp. <i>dissolvens</i> strain LMG 2683	1364	1364	99%	0.0	99%
gil342209918 JN029950.1	<i>Pantoea</i> sp. T55 16S ribosomal RNA gene, partial sequence	1362	1362	99%	0.0	99%
gil340343756 JN089363.1	<i>Enterobacter</i> sp. IS.TYA 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339959025 JF513137.1	<i>Enterobacter cloacae</i> strain S125R 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339895672 HE572588.1	<i>Enterobacter</i> sp. SB2-GLA partial 16S rRNA gene, strain SB2-GLA	1362	1362	99%	0.0	99%
gil339283268 JF946808.1	<i>Enterobacter</i> sp. A732 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339283263 JF946803.1	<i>Enterobacter</i> sp. A715 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339282818 JN033080.1	Uncultured bacterium clone 4-31015 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339282770 JN033032.1	Uncultured bacterium clone 3-42048 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339282768 JN033030.1	Uncultured bacterium clone 3-42004 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil339282763 JN033025.1	Uncultured bacterium clone 3-41030 16S ribosomal RNA gene	1362	1362	99%	0.0	99%
gil338858347 AB641901.1	<i>Enterobacter</i> sp. NCCP-293 gene for 16S rRNA	1362	1362	99%	0.0	99%

From the biochemical test and 16S rDNA sequence analysis it can be indicated that the isolated bacterial strain is a member of the genera *Enterobacter*.





1 TNCTCCCT NCCNCANA CUNGNAGTN CGACGGTAG CACAGAGN TTCTNNNG GGTGACGAG 70
 71 TGGCGGACGG GTGAGTATG TTNNGGAAA NTGNGATNG GAGGGGATA ACTANTCGA AACGTAGCT 140
 141 AATACCCCTA ACCTNGGCA GACCAAGAG GGGACCTTC GGGCCNTNG CCTNCAGANG TNGCCAGNN 210
 211 GGGATTAGCT AGTAGGTGG GTAACGGCTC ACCTAGGCGA CGATCCCTAG CTGCTCTGAG AGGATGACCA 280
 281 GGGCACTG GACTGAGAC ACGGTNCCAG ACTCCTNACG GAGGGCAGCA GTGGGATAA TTGACAAATG 350
 351 GGGCGAAGCC TGATGCAGCC TGCCCGCTGT ATGAAGAAGG CCTTCGGGT TAAAGTACT TTCAGCGGG 420
 421 AGGAAGGTGT TGTGTTAT AACCAAGCA ATTGACCTTA CCGCAGAG GATTACTGG CGTAAGCGC ACGLAGCGG 490
 491 CCAGAGCCG CGTAATACG GAGGGTGCA GCGTTATCG GATTACTGG GATAAGCGC ACGLAGCGG 560
 561 TCTGTACAGT CGATGTGAA ATCCCGGGG TCAACCTGG AACTGCTTCN AACTGGCAG GCTAGAGTCT 630
 631 TGTAGAGGG GTAGAAATTC CNGTGTAGCG GTGAATGCG TANAGATCTG GAGGAATACC GTGGCNAAG 700
 701 CGGCCCTG GACCAAAANT GACNCCAGT GCCMAANGC TGCGAGCAA CNGATAGAA NCTGTGTANN 770
 771 CNGCCNTAA NCANGTCAAT TNGNNGTNG NCTTGAGGC GNGNNTNCG ANTNA 840

4.17.5.2. Analysis of sequencing results

The partially amplified 1500bp and sequenced 16SrRNA gene from local isolates (effluents isolates) was uploaded to the National Centre for Biotechnology Information (NCBI) website to search for similarity to known DNA sequences and to confirm the species of this local isolate. The BLAST query revealed that these are 100% homologous to *Enterobacter cloacae* strain FR species, 100% homologous to *Salmonella* species and 99% homologous to *Pantoea* species T55. The nucleotide sequences coding for the 16SrRNA gene of *Enterobacter* spp. EMB19, *Salmonella* spp. enrichment culture clone CL107 and *Pantoea* spp. PYPB15 have been submitted to the Genbank database using the BLASTN (<http://www.ncbi.nih.gov/BLAST>) algorithm. But its colonies characteristics on XLD and Mac-Conkey agar media and several biochemical reactions especially the methyl red test and its ability to ferment the amino acid lysine indicated that the strain must be a member of the genera *Enterobacter* spp. *Pantoea* species could not be fermented the amino acid lysine. On the other hand, the entire *Salmonella* sp (except *Salmonella pullorum*) are methyl red positive and it was non motile. So it would be concluded that the presented studied isolated strain was found to be *Enterobacter* spp.

4.17.6. Investigation of Plasmid DNA

After isolation, the bacterial strains were inoculated in the nutrient broth and incubated at 37°C in a shaker for overnight. The broth cultures were used for the isolation of plasmid DNA. The plasmid DNA was electrophoresis on the 0.8% and 1% high-melting agarose gel. Ethidium bromide was used to stain the gel. The DNA bands were resumed by ultraviolet illumination. Plasmid DNA band of bacteria that was found on the agarose gel after illumination with the UV light. These bands may be representing the plasmid DNA.

4.17.7. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was the lowest concentration of a drug at which inhibited the visible growth of bacteria. The results of MIC value of the *Gentamycin* against isolated bacteria are presented in the Table-4.8.

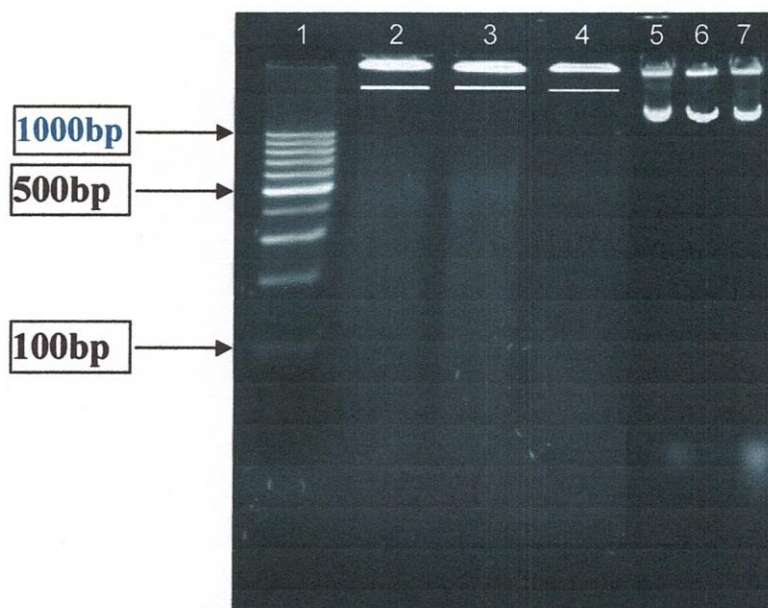


Plate-4.8: Agarose gel electrophoresis of the plasmid DNA isolated from bacterial sp. (lane 1, 2, 3 and 4). The DNA was resolved on 0.8 % agarose gel and stained with ethidium bromide (0.5 µg/mL).

Table-4.8: Minimum Inhibitory Concentration of *Gentamycin*

Test Organism	Growth response in different concentration					
<i>Enterobacter sp.</i>	<i>Gentamycin</i> (µg/ml)					
	50 ++	100 ++	200 ++	300++	400 ++	500 --

The '+' sign indicates the growth of the microorganisms while '-' sign indicates no growth.

The MIC of this antibiotic with *Enterobacter spp.* was 500 µg/mL, demonstrating that high concentration of this antibiotic was required to inhibit the growth of this microorganism.

4.17.8. Curing experiment

Nutrient broth cultures of bacterial strains were used for curing along with different curing agents of SDS, acriflavin and ethidium bromide were used as the curing agents (Table-4.9). LB broth media was inoculated with supplemented heavy metal ions in the presence of different curing agents.

Table 4.9: The elimination of plasmid from the bacterium using various curing agents

Curing agents	Growth response in different concentration of curing agents							
Ethidium bromide ($\mu\text{g/ml}$)	10 ++	15 ++	30 ++	70 ++	75 --	100 --		
Acriflavin ($\mu\text{g/ml}$)	10 ++	15 ++	30 ++	70 ++	75 ++	100 ++	150 --	
Sodium dodecyl sulphate (%)	1 ++	2 ++	3 ++	5 ++	7 ++	10 ++	25 --	30 --

However, ethidium bromide at high concentration was found to be successful to cure the plasmids (Table-4.9). The organisms were grown in the nutrient broth in presence of ethidium bromide of different concentration (10-200 $\mu\text{g/mL}$). The maximum concentration of ethidium bromide permitting growth (very little turbidity) was 100 $\mu\text{g/mL}$. Samples of this growth were diluted up to 10^{-7} and cultured on the nutrient agar plate containing antibiotic, *Gentamycin* (200 $\mu\text{g/ml}$) and another MS medium plates containing Cr, Cd and Pb (20 $\mu\text{g/mL}$) separately. The plates were incubated at 37°C for 24 hrs. After incubation, a very few colonies were found to grow in *Gentamycin* media MS+ Cr, Cd and Pb medium as most of the colonies had lost their ability to utilize Cr, Cd and Pb and *Gentamycin* in both LB and MS solid medium and

broth medium proved that the genes responsible for heavy metals metabolism might reside upon the plasmid DNA. These ethidium bromides treated bacteria were further sub cultured in nutrient broth and plasmid DNA was extracted from these bacteria. Agarose gel electrophoresis showed that the wild type strains had one band while the cured strains showed on plasmid band (Plate-4.9) the loss of bands in the cured strains proved that the bands found on the gel belongs to the DNA carrying the genes responsible for heavy metals degradation. Because, the chromosomal DNA could not be cured rather there might have been some mutation. Thus, the loss of bands with the treatment of ethidium bromide suggested a strong relation between the inability to metabolize heavy metals with the lost bands. This result strongly suggests that the genes responsible for the ability to metabolise Cr, Cd and pb might be plasmid DNA.

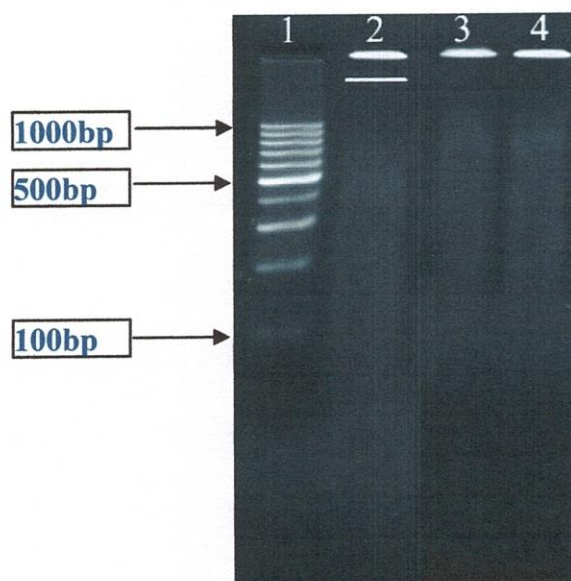


Plate 4.9: Cured strains of isolated *bacterial spp.* (lane 3 and 4). Other lane 1 and 2 denoted that 1Kb⁺ marker DNA, Plasmid DNA.

The wild type *Enterobacter species* was creamy white in color whereas the colony of the cured strain was white in color and also smaller in size compared to wild type. Moreover, colonies of the wild type *Enterobacter species* was raised, but the cured strain exhibited flat colonies. A similar finding was observed from a plasmid borne determinant of *Pseudomonas species* that was isolated from leaf of cotton (Saha *et al.*, 2001). Transformation and curing results of the study suggested that metal ions and Gentamycine resistance were conferred by plasmid DNA, while Cr, Cd and Pb resistance seems to be encoded by genes of the bacterial chromosome. The

development of the metal resistant population in a contaminated environment can result from vertical gene transfer (reproduction), horizontal gene transfer, including transposes and broad host range plasmids and selection pressures on spontaneous mutants due to the presence of heavy metal ions (Bogdanova, 1998). Transformation of Cr resistance gene suggests that horizontal gene transfer may be an important factor for the development of metal resistant populations in the environment. Indeed, further studies have to be performed to elucidate bioremediation of heavy metals and detoxification processes operating in this system.

4.17.9. Degradation rate of metal ions (Cr, Cd and Pb) by bacterial treatment

The isolated metal ions tolerance bacteria were applied to synthetic and row tannery effluents. The results of *Enterobacter species* treated synthetic effluents indicate that the concentration of Cr, Cd and Pb ions were found to be 0.01, 1.6 and 1.75 $\mu\text{g/mL}$, respectively. The results also illustrate that the bacterial spp. degraded metal ions (Cr, Cd and Pd individually) very efficiently (Figs. 4.10, 4.11 and 4.12).

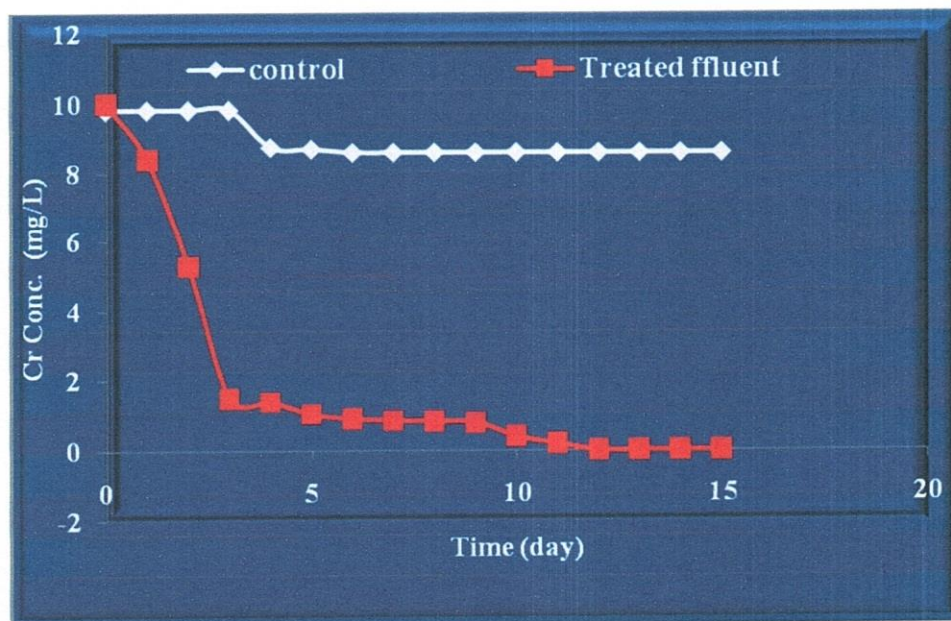


Figure 4.10: Effect of inoculation age on Cr reduction

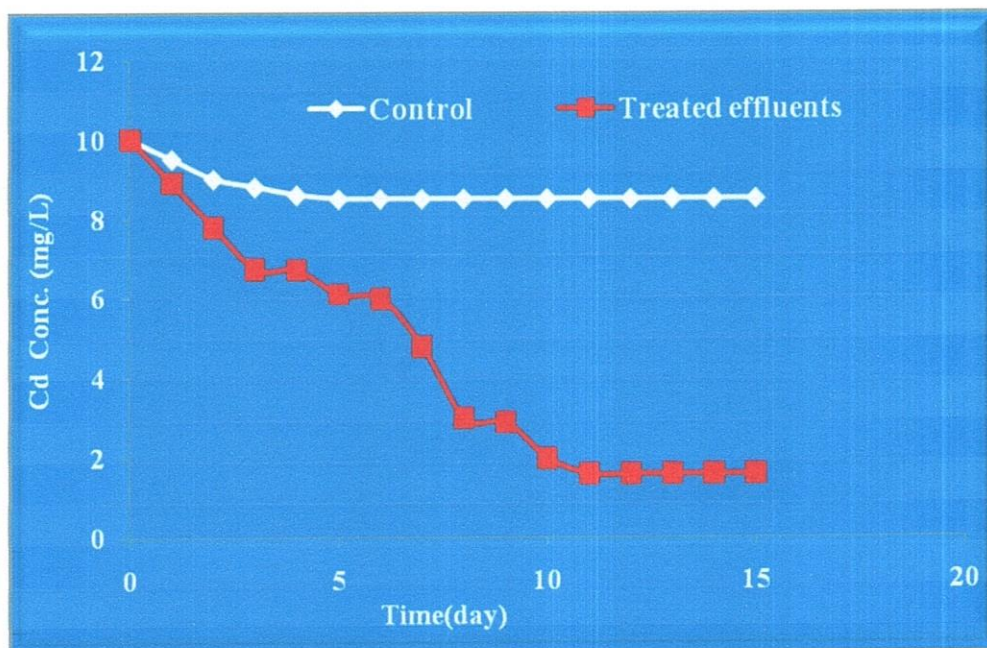


Figure 4.11: Effect of inoculation age of Cd reduction

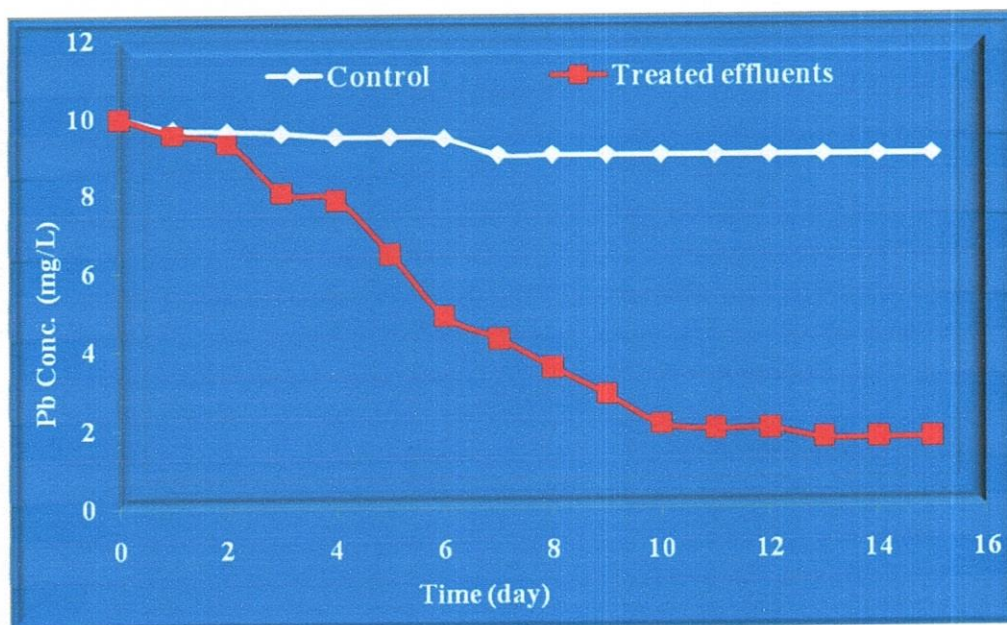


Figure 4.12: Effect of inoculation age of Pb reduction

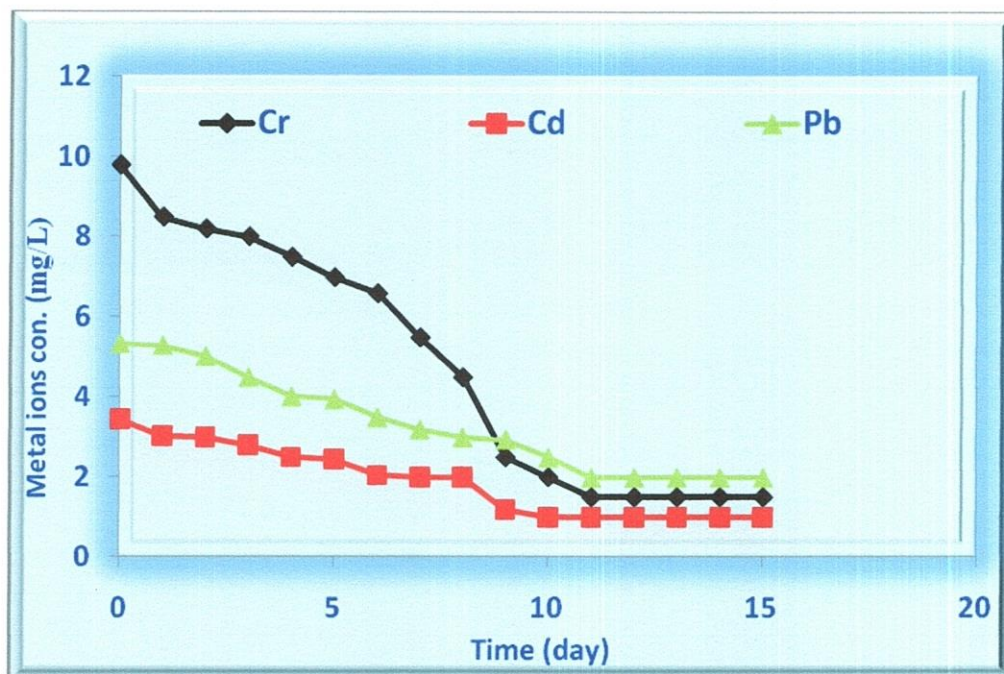
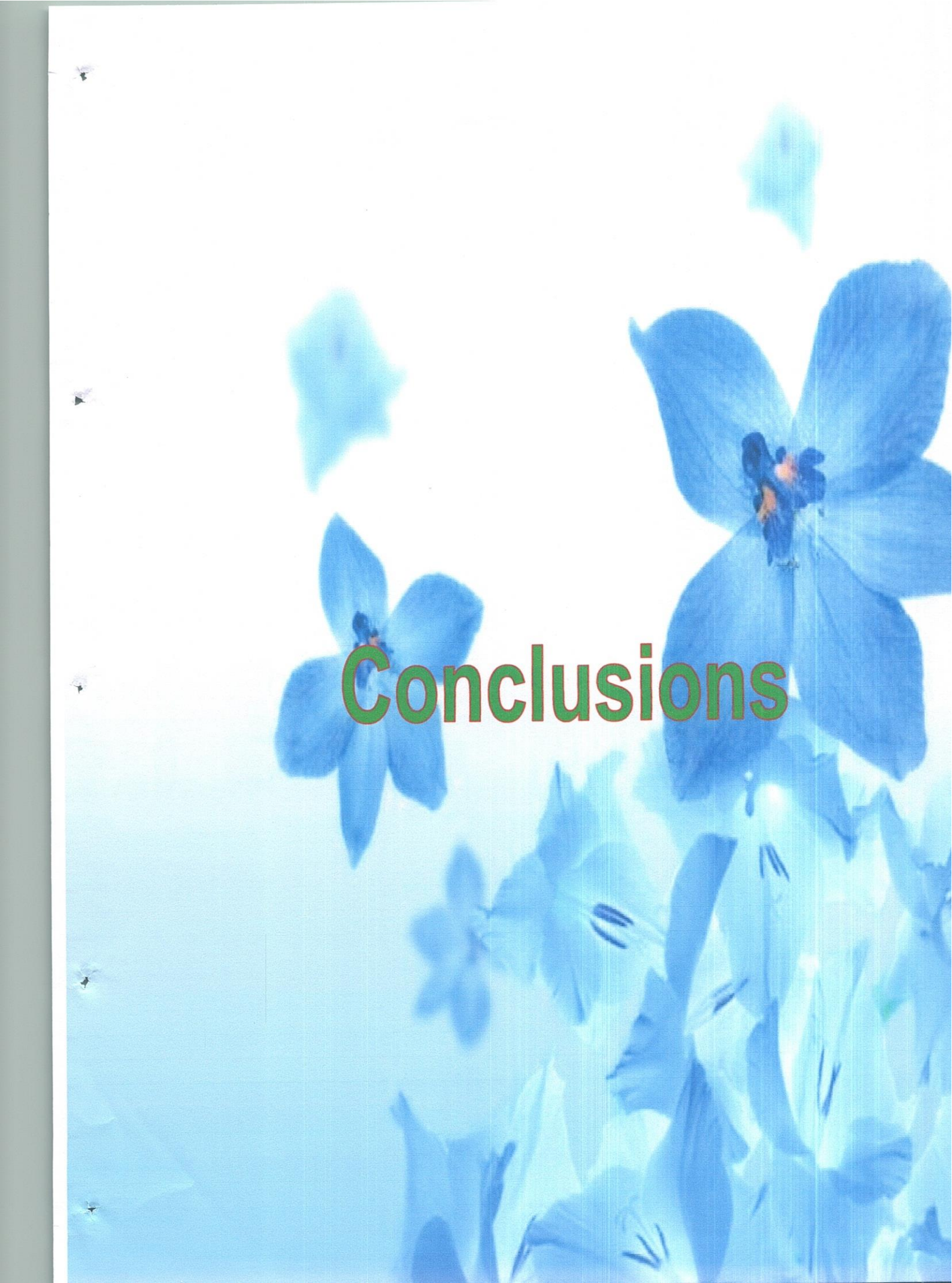


Figure 4.13 Effect of inoculation age on metal ions (Cr, Cd and Pb) reduction

The study results also reveal that Cr concentration was found almost zero (0) mg/L and removal efficiency was about 100% using indigenous strain *Enterobacter species* at a culture dose of 3ml (OD-1) at pH 7.2 and 37°C for incubation of 10-15 days. The analysis results also illustrate that the removal efficiency of Cd and Pb were 84 and 83%, respectively using the same conditions stated above. The analysis results for the raw tannery effluents show that the removal efficiency of Cr was 84% using *Enterobacter species* at a culture dose of 3ml (OD-1) at pH 7.2 and 37°C for inoculation period of 10-15 days but the strain showed comparatively lower efficiencies for degrading metal ions Cd and Pb. Rehman *et al.* (2008) stated that the ability of *Bacillus sp.ev3* to reduce hexavalent chromium into its trivalent form. *Bacillus sp.ev3* could tolerate Cr^{6+} (4800 $\mu\text{g/mL}$), Pb^{2+} (800 $\mu\text{g/mL}$), Cu^{2+} (200 $\mu\text{g/mL}$), Cd^{2+} (50 $\mu\text{g/mL}$), Zn^{2+} (400 $\mu\text{g/mL}$), Ni^{2+} (4000 $\mu\text{g/mL}$) and Hg^{2+} (50 $\mu\text{g/mL}$). They also reported that *Bacillus sp.ev3* could reduce 91% of chromium from the medium after 96 h and was also capable to reduce 84% Cr from the industrial effluents after 144 h. In comparison with above literature reports, this study results indicate comparatively better removal efficiencies in removing the parameters.

From the above result, it was also observed that the ability of microbial strains grown in the presence of heavy metal ions would be helpful in the waste water treatment where microorganisms were directly involved in the decomposition of organic matter in biological processes for waste water treatment. In the present study high degree of heavy metal ions resistance associated with multiple antibiotic resistances was detected in tannery effluents bacteria. In most of the studies, metal resistance has been reported to hold an association with antibiotic resistance (Verma *et al.*, 2001). Silver and Misra, (1988) stated that under conditions of metal stress, metal and antibiotic resistance in microorganisms possibly helps them to adopt faster by the spread of resistant factors than by mutation and natural selection. Ahmed *et al.* (2005) also demonstrated that bacteria exposed to high levels of heavy metal ions in their environment have adapted to this stress by developing various resistance mechanism. These mechanisms could be utilized for detoxification and removal of heavy metals from polluted environment. The study evaluates that the identified *Enterobacter spp.* could be used to reduce heavy metal ions from the discharged tannery effluents.

The background of the slide is a light blue gradient with a subtle pattern of blue flowers and butterflies. A large, detailed blue butterfly is prominent on the right side, with its wings spread. Several other blue flowers and butterflies are scattered throughout the background, some in sharp focus and others blurred. The word "Conclusions" is centered in a green, serif font.

Conclusions

Conclusions

Leather industrial effluents pollution is a major environmental and social concern in Bangladesh. The effluents contained pollutants can cause undesirable effect on the ecosystems and environment. To minimize the effects, this study considered treatment processes including physico-chemical and biological approaches to minimize the toxic levels before discharging the tannery effluents into the environment.

The study results show that the collected tannery effluents from different manufacturing units (MU) in three selected leather industries contained extremely high values of TSS, TDS, TS, BOD₅, COD, SO₄²⁻, Na, Ca, Cr, As, Cd and Pb. The analysis results illustrate that the highest value of SO₄²⁻ and As were found in the liming and un-hairing effluents, the highest values of TDS, Cl⁻, Cr, Cd and Pb were found in the pickling and chrome tanning effluents and the highest value of COD and BOD₅ were found in the re-tanning and dyeing effluents. These values were far above the standard permissible limits for ISW in BDS, ISI-2000 and USEPA-2000. The mean concentration of TSS, TDS, SO₄²⁻, Cl⁻, BOD₅, COD, Na, Cr, As, Cd and Pb in the collected composite tannery effluents in all monitoring points were found in the range 980-6880, 7050-14080, 1100-4000, 1800-5005, 820-920, 2740-3980, 1200-3840, 6.4-9.81, 0.67-2.15, 0.96-3.45 and 1.5-5.35 mg/L, respectively. The analyses results indicate that the major physico-chemical parameters of the collected composite samples (at all monitoring points) were also exceeded the standard disposal limits. The concentration of metal ions in the composite tannery effluents were in the following order: Na > Mg > Ca > Cu > Zn > Cr > Pb > Ni > Cd > Mn > As. Depending on the concentration of all parameters, the locations were followed in the order: P₁ > P₂ > P₃. The value of all parameters at monitoring point P₁ were comparatively higher than the other monitoring points P₂ and P₃ as the samples at the point P₁ was the composite mixture of effluents collected from different manufacturing units inside the tannery industry. The values at points P₂ and P₃ were decreased due to sediment in some way and dilute through sewerage system. The results also observe that there was no significant effect of seasonal variation on the concentration of most of the parameters in the collected composite samples at monitoring points P₁, P₂ and P₃. However DO value - was found to be 2.7 mg/L in monsoon and it was almost "zero" (0 mg/L) in dry season indicating acute toxicity to aquatic life. The study also

reveals that the concentration of various metal ions in the sludge contained very high values, Cr (10000-9600 mg/Kg) in particular. The concentration of ions in the sludge was in the following order: Cr > Na > Ca > S > Mg > P > Cu > TN > Zn > Organic compound > Pb > As > Cd. The study suggests that the untreated leather industrial effluents were not suitable for discharging into surface water and land, which poses potential threats to human health, aquatic life and the environment.

The study carried out various physical, chemical and biological treatment processes to reduce toxic pollutants from the discharged tannery effluents. The results illustrate that the sand-stone filtration process could reduce certain pollution levels but not sufficient enough to consider the process alone using as an effluent treatment process. On the other hand, sawdust and combined filter (saw dust combine with sand) showed lower efficiencies in removing most of the parameters compare to the sand-stone filtration process. However, the saw dust filtered showed comparatively better removal efficiencies for Cl^- , Cu and Cr ions and the efficiencies were 72, 35 and 31%, respectively. The study also carried out chemical treatment process using individually three coagulants namely, FeCl_3 , Fe_2O_3 and FeSO_4 . The study optimized the coagulant dose and pH for each coagulant. The optimum dose for the coagulant, FeCl_3 and FeSO_4 was 150mg/L and for Fe_2O_3 was 100 mg/L. The optimum pH for the coagulants FeCl_3 and FeSO_4 was near neutral pH and for the coagulant Fe_2O_3 was 9. The analyses results reveal that removal efficiency of color and TSS achieved were about 100% for all coagulants. The coagulant Fe_2O_3 removed Cu and Cr almost 100%. The mean removal efficiency for all parameters were 91%, 89% and 85%, for the coagulants Fe_2O_3 , FeCl_3 and FeSO_4 , respectively, indicating that Fe_2O_3 showed the best removal performance among the coagulants. It may be mentioned that Fe_2O_3 showed lower removal efficiency only for $\text{NH}_4\text{-N}$ and TN compared to coagulant FeCl_3 . According to the removal performance of different parameters, the coagulants can follow the order: $\text{Fe}_2\text{O}_3 > \text{FeCl}_3 > \text{FeSO}_4$. The coagulants used in the study have showed their potentiality in removing pollutants from the tannery effluents. The concentrations of the toxic pollutants such as TSS, BOD_5 , COD, SO_4^{2-} and metal ions; Cr, Cd, As and Pb were decreased significantly in treated effluents and the values were found to be within the permissible standards limit for ISW-BDS. Various chemicals and other treatment processes have been practicing for removing different inorganic and organic pollutants from tannery effluents. Most commonly used chemicals are FeCl_3 , FeSO_4 , $\text{Fe}_2(\text{SO}_4)_3$, alums, and TiO_2 as coagulants, MgSO_4 and

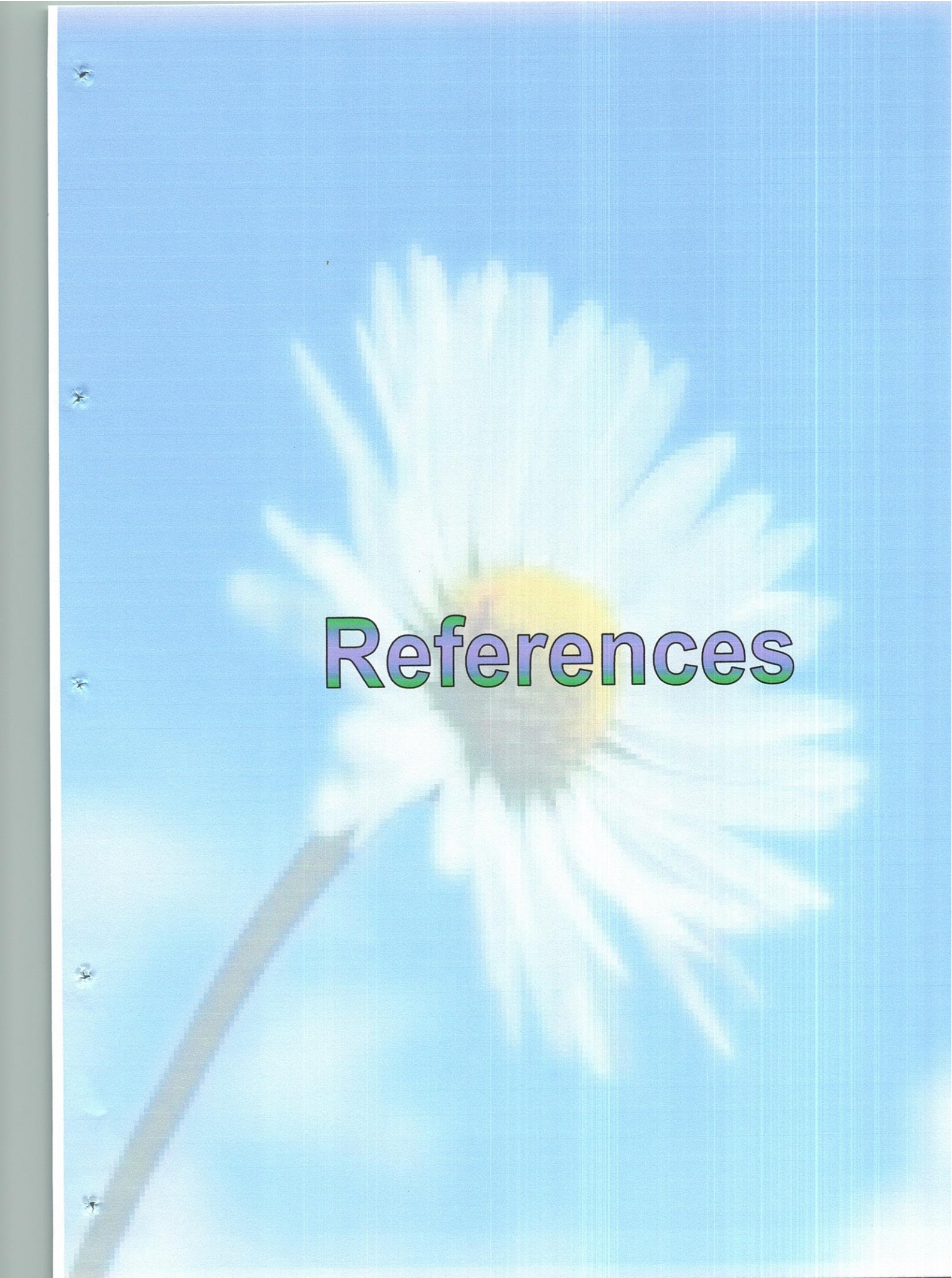
NiSO_4 as oxidizing agent, FeSO_4 , and H_2O_2 in Fenton-photo process for COD, Cr and other cations removal. The removal efficiencies of some major parameters of the study were compared to other treatment processes reported in the literatures. The comparison results illustrate that the coagulants used in the study showed the best performance in terms of removal efficiencies and the coagulant doses. Moreover, FeCl_3 , Fe_2O_3 and FeSO_4 salts are inexpensive compare to TiO_2 , alum, copper salts and other commonly used coagulants. The coagulant costs become much evident for the suitability of the process. The study also reveals that the other processes mentioned in the literature have used huge amount of chemicals compare to the present study that which might decrease the sludge production and thus reducing the environmental burden. The coagulants FeCl_3 , Fe_2O_3 and FeSO_4 used in this study were potentially suitable for removing major chemical parameters from the tannery effluents and hence it suggests that the treatment process could be applied to tannery industry effluents and the treated effluents could be safe for discharging into the environment.

The present research also carried out microbial treatment with bacterial species for the composite raw and synthetic tannery effluents to remove heavy metal ions. The study isolated Cr, Cd and Pb tolerance bacteria to check their degradation ability of heavy metal ions to clean up the environment. All the isolates showed effective tolerance against the selected heavy metal ions without producing any byproduct. Hence, the species could be used as a bio-sorption or bioaccumulation tool for the treatment of leather industrial effluents. The bioaccumulation properties of each Cr, Cd and Pb tolerant isolates were investigated. Bacterial strains were capabilities of growing at 1500, 1300 and 1200 mg/L of Cr, Cd and Pb, respectively in LB media indicating resistivity of the above metal ions. The strains were also capable of resisting Cr, Cd and Pb up to 60, 55 and 50 mg/L, respectively in MS media. The study results observed that the order of metal ions resistance bacteria was found to be $\text{Cr} > \text{Cd} > \text{Pb}$ on the MS and LB agar plates. The effects of different operating conditions such as initial pH, temperature and initial metal ions concentration on bioaccumulation of Cr, Cd and Pb ions tolerance cultures were studied in a batch system. The experimental investigation showed the optimization of Cr, Cd and Pb ions uptake by bacterial isolates for 10 days incubation period at pH 7.2 and temperature at 37°C . As the initial concentration of Cr increased from 5 to 50 mg/l, the uptake of Cr also increased. At about 20 mg/L initial Cr concentrations, uptake of the selected potential strain exceeded 84% within 72 hours of incubation. The study results also illustrated that the bacterial strain was capable of removing 100, 84 and 83% of Cr, Cd and Pb,

respectively within 10 days from the synthetic effluents. The study results also described that the bacterial strain was also capable of removing Cr 84%, Cd 65% and Pb 62% within 10 days from raw tannery effluents indicating the efficiency the bacterial strain.

The bacterial isolates were identified by 16S rRNA gene sequencing and biochemical results showed that isolated strains was found to be *Enterobacter species*. Moreover the protein plasmid DNA genes code for a wide range of metabolic activities and enable bacteria which would be accumulated the metal ions pollutants on the bacterial cell surface. Plasmid DNA was extracted from the isolated bacterial species to evaluate its role on degrading metal ions. Each of the three isolated bacterial colonies contained individual endogenous plasmid. The study results showed the presence of plasmid DNA in the bacterial isolates, which contained extra molecular weight having degrading capability of metal ions. All of the plasmids were lost when the organisms were treated with ethidium bromide, acriflavin and sodium dodecyl sulphate (100,150 µg/ml and 25%, respectively). The organisms without plasmid DNA were unable to grow on the LB, MS agar and broth media containing Cr, Cd and Pb. It may be concluded that the endogenous plasmids were necessary to allow the bacterial growth in presence of substrate. Phenotypic testing of wild type, cured strains revealed that the gene(s) responsible for Cr, Cd and Pb degradation may be borne on the plasmids. The roles of plasmids in degradation of toxic metal ions have provided exciting performance in terms of pollution control. The study results indicate that the isolated strain could be potentially applied for biological treatment of effluents containing higher levels of Cr, Cd and Pb.

This study has shown that the tannery effluents contained a number of toxic pollutants, when discharged into surface water bodies caused severe threat to environment. Therefore, it is imperative to routinely monitor water quality, integrating both surface water and sediments. The study recommends a combined treatment process consists of filtration, coagulation, sedimentation and microbial approach would be the best possible option in order to preserve water quality and the environment.

A large, close-up photograph of a white daisy flower with a bright yellow center. The flower is positioned slightly to the right of the center, with its stem extending towards the bottom left. The background is a clear, bright blue sky. The word "References" is superimposed over the center of the flower in a stylized, multi-colored font.

References

- Aboulhassan M. A., Souabi, S., Yaacoubi, A., Baudu, M., (2006). Removal of surfactant from industrial wastewaters by coagulation flocculation process, *International Journal of Environmental Science and Technology*, **3(4)**: 327-332.
- Ademoroti C.M.A. (1996). Standard method for water and Effluents Analysis. Foludex press Ltd Ibadan pp. 22-23, 44-54, 111-112 and 182.
- Adil Al-Hemiri., Hameed Al-Anbari and Ibtiha K. Shakir,(2007). Dye removal from wastewater using iron salts. *Iraqi Journal of chemical and petroleum engineering*. **9(3)**: 17-24.
- Ahemad M, Khan MS, Zaidi A, Wani PA. (2009). Remediation of herbicides contaminated soil using microbes. In: *Microbes in sustainable agriculture*, Khan MS, Zaidi A, Musarrat J, (eds.) *Nova Science Publishers* New York, p. 261–284.
- Ahemad M, Khan MS. (2010). Ameliorative effects of *Mesorhizobium* sp. MRC4 on chickpea yield and yield components under different doses of herbicide stress. *Pestic Biochem Physiol* **98**: 183–190.
- Ahemad M, Khan MS. (2011). Pesticide interactions with soil microflora: importance in bioremediation. In: *Microbes and microbial technology: agricultural and environmental applications*, Ahmad I, Ahmad F, Pichtel J., (eds.) Springer, New York, p. 393–413.
- Ahmed A. U. and Reazuddin. (2000). Industrial Pollution of water systems in Bangladesh. (University Press Limited, Dhaka, Bangladesh), 175-178.
- Ahmed N. Nawaz, A. and Badar, U. (2005). Screening of copper tolerant bacterial species and their potential to remove copper from the environment. *Bulletin of Environmental and Contamination Toxicology* **74**: 219- 226.
- Ahmedna M., Marshall, W.E., Rao, R.M., (2000). Production of granular activated carbons from selected agricultural by-products and evaluation of their physical, chemical and adsorption properties, *Bioresour., Tech.*, **71**: 113-123.
- Ajayi S.O. and Osibanji O. (1981). Pollution studies on Nigeria Rivers 11; Water quality of some Nigerian Rivers. *Environ. Pollut. Series 2*: 87-95.

- Ajmal M, Khan AH, Ahmad S, Ahmad A. (1998). Role of sawdust in the removal of copper(II) from industrial wastes. *Water Res.* **32**:3085–3091.
- Ajmal M, Rao R, Ahmad R, Ahmad J and Rao L (2001). Removal and recovery of heavy metals from electroplating wastewater by using Kyanite as an adsorbent. *J. Hazard. Mater. B* **87**:127-137.
- Ajmal M., Afsar M. Sulaiman, Akhtar Hussian Khan, (1993). Surface entrapment of toxic metals from electroplating waste and their possible recovery. *Water, Air and Soil pollution.* **68(3-4)**: 485-492.
- Ajmal M., Rao RAK, Siddiqui BA. (1996). Studies on removal and recovery of Cr(VI) from electroplating wastes. *Water Res.* **30**:1478–1482.
- Ajmal M.; Hussain Khan, A.; Ahmad, S.; Ahmad, A., (1998a). Role of sawdust in the removal of copper(II) from industrial wastes, *Wat. Res.*, **32 (10)**: 3085-3091.
- Akan J. C., Abdulrahman FI, Dimari GA, Ogugbuaja VO, (2008). Physicochemical determination of pollutants in wastewater and vegetable samples along the Jakara wastewater channel in Kano metropolis, Kano state, Nigeria. *European Journal of Scientific Research*, **23 (1)**: 122-133.
- Akan J. C., F.I. Abdulrahman, J.T. Ayodele and V.O. Ogugbuaja (2009). Impact of Tannery and Textile Effluent on the Chemical Characteristics of Challawa River, Kano State, Nigeria. *Australian Journal of Basic and Applied Sciences*, **3(3)**: 1933-1947.
- Akan J.C., E. A Moses, and Ogugbuaja, (2007). "Assessment of tannery industrial effluent from Kano metropolis, Nigeria," *Asian Network for Scientific Information, Journal of Applied Science*, **7(19)**: 2788—2893.
- Aksu Z. and Isoglu IA, (2005). Removal of copper (II) ions from aqueous solution by biosorption onto agricultural waste sugar beet pulp process *Biochemistry*, **40**: 3031-3044.
- Alam Z. M, Malik A. (2008). Chromate resistance, transport and bioreduction by *Exiguobacterium* sp. ZM-2 isolated from agricultural soil irrigated with tannery effluent. *J Basic Microbiol* **48**: 1–5.

- Ali M. S. and N. Deo, (1992). Effect of pH on adsorption process of chromium (VI) with a new low cost adsorbent. *J. Environ, Prot* , **12** : 202-209.
- Ali M. S., M. R. Khan and Afroz T., (2008). Potable water from industrial wastewater. *Chemical Engineering Research Bulletin*, **12**:20-23.
- Altschul S.F., Madden T.L., Schaffer A., Zhang J., Zhang Z., Miller W. & Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**: 3389-3402.
- Amuda O.S, Amoo I. A. Ajayi O.O., (2006). Performance optimization of some coagulants/ flocculant in the treatment of a beverage industrial wastewater J. *Hazard. Mater. B* **129**: 69-72.
- Andualem Mekonnen Hiruy., (2000). Developing a laboratory scale sequencing batch reactor and evaluating its performance for the treatment of tannery wastewater. Addis Ababa.
- Anglada A., Urtiaga, A. and Ortiz, I. (2009). Contributions of electrochemical oxidation to waste-water treatment: fundamentals and review of applications, *J. Chem. Technol. Biot.*, **84 (12)**: 1747-1755.
- APHA (American Public Health Association). (1998). *Standard Methods for the Examination of Water and Wastewater*. 20th ed. APHA, Washington, DC, USA.
- APHA-AWWA-WPCF (2005). In: *Standard methods for the examination of water and waste water*. American Public Health Association, Washington, D. C 20th ed., New York.
- Apte A. D., S. Verma, V. Tare, and P. Bose (2005). "Oxidation of Cr(III) in tannery sludge to Cr(VI): field observations and theoretical assessment," *Journal of Hazardous Materials*, **121(1-3)**: 215-222.
- Aravindhan R., Madhan, B., Rao, R., Nair, B. and Ramasami, T. (2004). Bioaccumulation of chromium from tannery wastewater an approach for chrome recovery and reuse, *Env. Sci. Technol.*, **38**: 300-306.

- Armienta M.A., O. Morton, R. Rodriguez, O. Cruz, A. Aguayo and N. Ceneceros, (2001). Chromium in a tannery waste water irrigated area, Leon Valley, Mexico. *Bull. Env. Conta. Toxicol.*, **66**: 189-195.
- Ates E, Orhon D, Tunay O. (1997) Characterization of Tannery wastewater for pre treatment- selected case studies. *Water Sci. Tech.*, **36**: 2-17.
- ATSDR (1994) Toxicological profile for zinc. US Department of Health and Human Service, Public Health Service., 205-88-0608.
- Awan A. M. (2004). Reduction of chemical oxygen demand from tannery wastewater by Oxidation. *EJEAF Che.*, **3(1)**: 625-628.
- Aygan A T. Yilmaz, (2010). Improvement of coagulation-flocculation process for treatment of detergent wastewaters using coagulant aids. *International Journal of Chemical and Environmental Engineering*.
- Azom M. R., K. Mahmud, S. M. Yahya, A. Sontu, and S. B. Himon (2012). Environmental Impact Assessment of Tanneries: A Case Study of Hazaribag in Bangladesh. *International Journal of Environmental Science and Development*, **3(2)**:152-156.
- Azumi S.Dan and Bichi, M.H. (2010). Industrial pollution and heavy metals profile challawa river in Kano Nigera. *Journal of Applied Sciences in Environmental Sanitation* **5 (1)**: 23-29.
- Baisali Sarkar, P. P. Chakrabarti, A. Vijakumar, Vijay Kale, (2005). "Wastewater treatment in dairy industries- possibility of reuse", *Lipid Science and Technology Division, Indian Institute of chemical Technology, Hyderabad 500007, India*.
- Bajza Z. and Vrcek, I.V., (2001). Water quality analysis of mixtures obtained from tannery waste effluent. *Eco toxicol. and Environ. safe*, **50** : 15 - 18.
- Balasubramian S, Pugalenth V, Anuradha K, Chakradhar SJ. (1999). Characterization of tannery effluents and the correlation between TDS, BOD and COD. *Environ. Sci. Health*, **34** : 4-16.

- Baldwin BR, Peacock AD, Park M, Ogles DM, Istok JD, McKinley JP, Resch CT, White DC. (2008). Multilevel samplers as microcosms to assess microbial response to biostimulation. *Ground Water* **46**: 295–304.
- Balusubramanian S, Pugalenth V (2000). A Comparative study of the determination of sulphide in tannery wastewater by ion selective electrode (ISE) and Iodimetry. *Water Res* **34**: 4201–4206.
- Bansoda R.R, Losso, J.N, Marshall, W.E, Rao, R.M, Portier, R.J, (2004). Pecan shell-based granular activated carbon for treatment of chemical oxygen demand (COD) in municipal wastewater, *Bioresour, Tech.*, **94**: 129-135.
- Banu R and Kaliappan S. (2007). Treatment of tannery wastewater using hybrid upflow anaerobic sludge blanket reactor, *J. Environ. Eng. Sci.*, **6**: 415-421.
- Baquero F, Negri MC, Morosini MI, Blazquez J. (1998). Antibiotic-selective environments. *Clinical Infectious Diseases*, **27**: 5–11.
- Barceloux D (1999). Chromium. *Journal of Toxicology and Clinical Toxicology* **37**: 173– 194.
- Barnhart, M.C. and F. A. Riusech. (1997). Re-infection of host fishes with glochidia of the fat pocketbook, *Potamilus capax*. Triannual Unionid Report **13**:36. Abstract.
- Bartkiewicz B (2000). Industrial wastewater purification. Warszawa, 271-279.
- Base CF (Jr.) and Mesmer RE (1976). The hydrolysis of cat ions. John Wiley and Sons, New York.
- Bashaar Y. A. (2004). Nutrients requirements in biological industrial wastewater treatment. *African Journal of Biotech*, **3 (4)**: 236-238.
- Basudev K Das and Rafiqul Islam (1999). Removal of Arsenic from Ground Water A Method Useable by Rural Folk. *Journal of Science* Vol.**27**: 55-60.
- Batzias FA, Sidiras DK (2004). Dye adsorption by calcium chloride treated beech sawdust in batch and fixed-bed systems. *J Hazard Mater* **114**:167–174.

- Bauer A.W.; Kirby, M.D.K.; Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by standard single disc diffusion method. *Amer J Clin Path.* **45**:493-486.
- BBS (2011). (Bangladesh Bureau of Statistics), Statistics Division, Ministry of Planning, The Peoples Republic of Bangladesh, Official website, Date accessed: 27 November .
- Bergey's Manual of Determinative Bacteriology ninth edition, David Hendricks Bergey, John G.Holt.
- Bernal D.A., Contreras, R. A., Trujillo, T. , Olalde, P. V. and Dendooven, L.(2006). Effects of tanneries wastewater on chemical and biological soil characteristics. *App. Soil Ecol*, **33**: 269–277.
- Bezverbnaya I.P., L.S. Buzoleva and S. Khristoforova, (2005). Metal-resistant heterotrophic bacteria in coastal waters of primorye. *Russ. J. Mar. Biol.*, **31**: 73-77.
- Bhadra B. Nanda, A.K. and Chakraborty, R. (2006). Inducible nickel resistance in a river isolate of India phylogenetically ascertained as a novel strain of *Acinetobacter junii*. *World Journal of Microbiology and Biotechnology* **22** : 225-232.
- Bhalli J.A.; Khan, Q. M., Hag, M.A.; Khalid. & Nasim, A. (2006). Cryptogenic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticides producing industry *Mutag*. **21**: 143-148.
- Bhatia S. C., (2005). Environmental pollution and Control in chemical process industries. Publish by Romesh Chander Khanna for Khanna publishers 2-B Nath Market, Nai Sarak Delhi- 110006. pp 772-831.
- Bhinde J. V., P.K. Dhakephalkar and K.M. Paknikar,(1996). Microbiological process for the removal of Cr(VI) from chromate bearing cooling tower effluents. *Biotechn. Lett.*, **18**: 667-672.
- Bi S., Wang, C., Cao, Q. and Zhang, C.(2004). Studies on the mechanism of hydrolysis and polymerization of aluminium salts in aqueous solution: correlations between the Core-links model and Cage-like keggins-Al13 model, *Coordination Chemistry Reviews*, **248**: 441-455.

- Bogdanova E. S., Bass, I. A., Minakhin, L. S., Petrova, M. A., Mindin, S. Z., Volodin, A. A. Kalyaeva, E. S. Tiedje, G. M. Hobman, J. L. Brown, N. L. Nikiforov, V. G., (1998). Transfer of monomer operons amongst environmental isolates of gram-positive bacteria. *Microbiology*, **144** (3): 609-620.
- Bois L., Bonhomme, A., Ribes, A., Pais, B., Raffin, G., Tessier, F., (2003). *Functionalized silica for heavy metal ions adsorption*. *Colloids and Surfaces A: Physicochemical Engineering Aspects*, **221**: 221-230.
- Boshoff G., Duncan, J. and Rose, P. D. (2004). Tannery effluent as a carbon source for biological sulphate reduction. *Wat. Res.* **38**(11): 2651-2658.
- Bosnic M., Buljan, J., Daniels, R. P., (2000). Regional program for pollution control in the Tanning industry US/RAS/92/120 in South East Asia Pp 1-14.
- Brayant P.S., Petersen, J.N., Lee, J.M., and Brouns, T.M., (1992). Sorption of heavy metals by untreated red fir sawdust, *Applied Biochemistry Biotechnology*, **34-35**: 777-788.
- Brierley J. L. Brierley, C. L. Goyak, G. M. AMT-BIOCLAIM (1986). A new wastewater treatment and metal recovery technology. In *Fundamental and Applied Biohydrometallurgy*; Lawrence, R. W., Branion, R. M. R.; Ebner, H. G., Eds.; Elsevier: Amsterdam., pp 291-304.
- Brierly C.L., and Brierley J. A. (1993). Immobilisation of biomass for industrial application of biosorption in Biohydrometallurgical Technologies. *Proceedings of the international biohydrometallurgical symposium*, Torma A. E., Apel, M. I. and Brierley, C. L., (eds), The Minerals, Metals and Materials Society, warrendale, PP, 35—44.
- Brinboim HC, Doly JA (1979). Rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl. Acid Res.* **1**: 1513-1523.
- Broda P. (1979). *Plasmids*. W.H. Freeman and Company. P. 197.
- Buljan J. (1994). Environmental aspects of processing and trade in hides, skins and leather. UNIDO, Geneva.

- Calheiros C.S.C, Rengel A.O.S.S, Castro, P.M.L. (2008). Evaluation of Different Substrates on the growth of *Typhalatifolia* in Constructed Wetlands treating Tannery wastewater over longterm operation. *Bio resource Technology*.
- Camargo F.A.O., Bento, F.M., Okeke, B.C. and Frankenberger, W.T. (2003). Chromate reduction by chromium-resistant *J Environ qual* **32**: 1228–1233.
- Camargo J. A., A. Alonso, and A. Salamanca. (2005). Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* **58**:1255–1267.
- Caravelli Alejandro H., Leda Giannuzzi and Noemi E. Zaritzky (2008). Reduction of hexa- valent chromium by *Sphaerotilus natans* a filamentous micro-organism present in activated sludges. *Journal of Hazardous materials*. **156(1-3)**: 214-222.
- Carlos K., Katarzyna, W., Kazimierg, W., Juan, M.M., Laura, E., Nava L., and Gomez, R. G. (2002). Possible Adverse Effects of Chromium in Occupational Exposure of Tannery Workers. *Industrial Health*, **40**: 207-213.
- Carniglia C.E. (1981). Aromatic hydrocarbons: Metabolism by bacteria, fungi and algae. *Reviews of biochemical toxicology*. **3**: 321-361.
- Cassano A., Criscuoli, A., Drioli, E. and Molinari, R. (1999). Clean operations in the tanning industry: aqueous degreasing coupled to ultra filtration, *Clean Products and Processes*, **1**: 257–263.
- Cassano A., Molinali, R., Romomo, M. and Drioli, E. (2001). Treatment of aqueous effluents of the leather industry by membrane processes a review, *J. Membrane Sci.*, **181** : 111- 126.
- Cervantes C., Campos-Garcia, J., Debars, S., Gutierrez-Corona, F.; Loza-Tavera, H., Carlos-Tarres-Guzman, M. and Moreno-Sanchez, R. (2001). Interaction of chromium with Microgenesis and plants. *FEMS Microbiol. Rev.* **25**: 335-347.
- Chadderton W.L., Grainger, N. and Dean, T. (2003) . Appendix 1 – Prioritising control of invasive freshwater fish. Pages 171 – 174 in Department of Conservation, Managing invasive freshwater fish in New Zealand.

- Chain K.K., Ng, W.J., Ma, A.N., and Wong, K.K. (1987). Treatability studies of palm oil refinery wastewaters. *Wat. Sci. Tech.* **19**: 23-29.
- Chakrapani G.J., (2005). Major trace element geochemistry in upper Ganga River in the Himalayas. *Indian Environ. Geol.* **48**: 189-201.
- Chaudhry G.R.; Huang, G.H. (1988). Isolation and characterization of a new plasmid from a *Flavobacterium* spp. which carries the genes for degradation of 2,4-dichlorophenoxyacetate. *J. Bacteriol.*, **170**: 3897-3902.
- Chaudry M. A., Ahmad, S. and Malik M. T. (1997). Supported liquid membrane technique applicability for removal of chromium from tannery wastes. *Waste Management.* **17** (4):211-218.
- Chen S. S., Tzeng, J. H. Tien, Y. and Wu, L. F. (2001). Rapid determination of Chemical Oxygen Demand (COD), using focused microwave digestion followed by a Titrimetric method. *Analytical, Science.* **17**: 551-553.
- Chhatwal G.R., Mehra M. C., Satake M., Katyal T., Katyal M. and Nagahiro T. (1989). *Environmental Water Pollution and Its Control*. Vol.4 PP 7, 341 Anmol publications private limited.
- Chubar N., Calvalho, J.R., Correia, M.J.N., (2004). Heavy metals biosorption on cork biomass: effect of the pre-treatment. *Colloids Surf. A* **238**: 51-58.
- Claudia V Genovese and J Froilan Gonzalez (1998). Solids removal by coagulation from fisheries waste waters. *Water SA* **24** (4): 371-372.
- Claus D. and Berkeley, R.C.W. (1986): *Genus Pseudomonas*. In: Bergey's Manual of Systematic Bacteriology, Vol 1, eds. Sneath PHA, Mair NS, Sharpe ME . pp. 140-219, Baltimore: Williams and Wilkins. ISBN 0-683-04108-8.
- Colak S., Zengin, S., Ozgunayh, S., Sarikahyah, Sari O., Yuceer L (2005). Utilisation of leather Industry Pre fleshings in Biodiesel Production. *JalCa* **100** (3) : 4, 137.
- Cooman K., Gajardo, M., Nieto, J., Bornhardt, C. and Vidal G. (2002). Tannery wastewater characterization and toxicity effects on *Daphnia* spp. *Environ. Toxic.* **18** (1): 45-51.

- Cooman K. Gajardo, M., Nieto, J., Bornhardt, C., & Vidal, G. (2003). Tannery Waste Water Characterization and Toxicity Effects on *Daphnia Spp*. Environmental Toxicology, **18**: 45-51.
- Cooper P. (1995). 'Colour in dye house effluent'. Society of dyers and colourists, Bradford.
- Costa C. R. and Olivi, P. (2009). Effect of chloride concentration on the electrochemical treatment of a synthetic tannery waste water, Electrochim. Acta, **54 (7)**: 2046-2052.
- Costa M. and Klein, C.B. (2006). Toxicity and carcinogenicity of chromium compounds in humans. Critical Reviews in Toxicology. **36 (2)**: 155-63.
- Covarrubias C., Garcia, R., Yanez, J. and Arriagada, R. (2008). Preparation of CPB-modified FAU zeolite for the removal of tannery wastewater contaminants, J. Porous Mat. **15 (4)**.
- Dantas T. L. P., Jose, H. J. and Moreira, R. F. P.M. (2003). Fenton and Photo – Fenton oxidation of tannery waste water, Acta Scientiarum. Tech., **25 (1)**: 91-95.
- Das P., Das B. and Khan YSA. (2006). Environmental assessment of tannery wastes from Chittagong, Bangladesh. Asian Journal of water Environmental pollution. **3 (1)**: 83-90.
- Dhungana TP, Yadav PN (2009). Determination of Chromium in Tannery Effluent and Study of Adsorption of Cr (VI) on Sawdust and Charcoal from Sugarcane Bagasses. J. Nepal Chem. Soc., **23**: 93- 101.
- DHV (1998). Megna estuary study, draft master plan, Volume **1**: Main report for Bangladesh ground water, Dhaka, Bangladesh.
- DoE (1997). Water quality data of rivers Buriganga, Meghna, Balu, Shitalakhya Jamuna(1991-2000). Department of Environment, Dhaka, Bangladesh.
- DoE (2001). The general over view of pollution status of rivers of Bangladesh, Department of Environment, Dhaka, Bangladesh.
- Dorman CD, Breneman KA, Melanie FS, Miller KL, James AR, Marshall MW, Foster PMD (2000). Fertility and developmental neurotoxicity effects of inhaled hydrogen sulphide in Sprague-Dawley rats. Neurotoxicol Teratol **22**: 71–84.

- DPCD (Diffuse pollution Conference Dublin), (2003). Aquatic ecology and dangerous substances: Bangladesh perspective. Institute for Environment and development studies. Ecology, **8 (C)**: 8-65.
- Du M., (1990). Treatment of dying and printing wastes by the combined processes of polyferric sulphate coagulation and chlorination oxidation. Environ. Eng. **9**: 1- 4.
- Dua M, Sethunathan N, Johri AK. (2002). Biotechnology bioremediation success and limitations. *Appl Microbiol Biotechnol* **59**: 143–152.
- DWAF (1996). South African Water Quality Guidelines (2nd edition). Volume 7: Aquatic Ecosystems. Department of Water Affairs and Forestry, Pretoria.
- ECR (1997). Ministry of Environment and Forest, Gazette notification 27, Aug.
- Edward Raja C., *G. S. Selvam, (2009). Plasmid profile and curing analysis of *Pseudomonas aeruginosa* as metal resistant. Int. J. Environ. Sci. Tech., **6 (2)**: 259-266.
- Edward Raja C., G. S. Selvam* and Kiyoshi Omine, (2009). Isolation, identification and characterization of heavy metal resistant bacteria from sewage. International Joint symposium on geo-disaster prevention and geo-environment in Asia.
- Edward Raja, C. Anbazhagan, K. and Selvam, G.S. (2006). Isolation and characterization of a metal resistant *Pseudomonas aeruginosa* strain. World Journal of Microbiology and Biotechnology **22**: 577- 586.
- Elke G., Werner, H. and Christian, M. (1996). Biological sulfate removal from tannery wastewater in a two-stage anaerobic treatment. War. Res., **30(9)**: 2072-2078.
- Elmaleh S. & Ghaffor N. (1996). Upgrading oil refinery effluents by cross-flow ultrafiltration. *Water Science and Technology*, **34 (9)**: 231–238.
- EPA (1999). Sediment Quality Guidelines developed for the national status and trends program. Report No. 6/12/99.
<http://www.epa.gov/waterscience/cs/pubs.htm> (Accessed in May 2004).

- EPA (2003). Effects of Acid Rain: Lakes and Streams. United States Environmental Protection Agency, Washington, DC. (<http://www.epa.gov/acidrain/effects/surfacewater.html>. Last accessed on 29 November, (2010).
- EPA (1990). Environmental Standards for Tannery effluents, EPA notification (G.S.R 742(E) dt.30 th August.
- EPB (2010). (Export Promotion Bureau), Ministry of Commerce, the Peoples Republic of Bangladesh, Annual Report, Chapter 9.
- Errais E., Duplay, J., Darragi, F., (2010). Textile Dye Removal by Natural Clay – Case Study of Fouchana Tunisian Clay, *Environmental Technology*, **31(4)**: 373-38.
- ESCAP (1982). Report on Industrial Pollution Control Guidelines Part VII." Tanning Industry. United Nations, Bangkok.
- Esmaeili A., Mesdaghi nia, A. and Vazirinejad, R. (2005). Chromium (III) Removal and Recovery from Tannery Wastewater by Precipitation Process, *American J. Appl. Sci.*, **2 (10)**: 1471-1473.
- Espinoza-Quinones, F., Fornari, M., Modenes, A. N., Palacio, S. M., Silva, F. G., Szymanski, N., Kroumov, A. D. and Trigueros, D. (2009). Pollutant removal from tannery effluent by electrocoagulation, *Chem. Eng. J.*, **151**: 59-65.
- ETPI (1998). Environmental Report of the Leather Sector, Draft Final Report, Environmental Technology Program for Industry, Karachi.
- Ewers U., Schlipköter, H-W.(1991). Lead. In: Merian, E. (Ed.) *Metals and their compounds in the environment*. VCH, Weinheim.
- FAO (1996). Management of Waste from Animal Product Processing. Wageningen, Netherlands.
- Farabegoli G., Carucci A., Majone M. and Rolle E. (2004). Biological treatment of tannery wastewater in the presence of chromium, *J. Environ. Manage.*, **71**: 345-349.

- Fathima N. N., B. Madhan; J. R. Rao, B.U. Nair (2003). Mixed Metal Tanning Using Chrome-Zinc-Silica: A new Chrome-Saver Approach JALCA, , **98 (4)**: 139-146
- Filali B.K. Taoufik, J. Zeroual, Y. Dzairi, F.Z. Talbi, M. and Blaghen, M. (2000). Waste water bacteria resistant to heavy metals and antibiotics. Current Microbiology **41**: 151-156.
- Flynn CM (1984). Hydrolysis of inorganic iron (III) salts. Chemical Reviews, **84**: 31-41.
- Fox P., Suidan, M. T., and Bandy, J. T. (1990). A comparison of media types in acetate fed expanded- bed anaerobic Reactors. Water research (G. B.), **24**: 827.
- Gadd GM. (1992). Metals and microorganisms: a problem of definition. *FEMS Microbiol Lett* **100**: 197-204.
- Ganesh R. and Ramanujam R. A. (2009). Biological waste management of Leather tannery effluent in India: Current options and Future research needs, Int. J. Env't. Eng., **1**: 165-186.
- Ganguly C., Matsumoto M.R., Rabideau A.J., Van Benschoten J.E.,(1998). Metal ion leaching from contaminated soils: model development. J. Environ. Eng., **124 (3)**: pp.278-287.
- Garcia-Calderon, D., Buffiere, P., Moletta, R. and Elmaleh, S. (1998). Anaerobic Digestion of wine distillery wastewater in down-flow fluidized bed. Water Res., **32**: 3593-3600.
- Garcia-sanchez, A. and E. Alvarez-ayuso, (2003). Arsenic in soils and water and its relation to geology and mining activities (Salamanca Province, Spain). J. Geol. Explor., **80**: 69-79.
- GATT G.M., (1994). Trade and the Environment, News and Views from GATT, Symposium on Trade, Environment and Sustainable Development, TE 008.
- Genovese CV., Gonzalez JF. (1998). Solids removal by coagulation from fisheries wastewaters. Water Science. **24 (4)**: 371-372.
- Gibson D.T. (1984). Microbial degradation of organic compounds Marcel Dekker New York.

- Giller KE, Witter E, McGrath SP. (1998) Toxicity of heavy metals to microorganisms and microbial process in agricultural soils: a review. *Soil Biol Biochem* **30**: 1389–1414.
- Gogate P. R. and Pandit, A. B. (2004). 'A review of imperative technologies for wastewater treatment II: hybrid method'. *Advan. Environ. Res.* **8**: 553 – 597.
- Gomez AJL, Giráldez I, Sánchez-rodas D, Morales E (2000). Comparison of the feasibility of three extraction procedures for trace metal partitioning in sediments from south west Spain. *Sci. Total. Environ.* **246**: 271-283.
- Gouhua C., (2004). Electrochemical technologies in wastewater treatment, *Sep. Purif. Tech.* **38**: (1): 11-41.
- Gourdon R, Bhende S, Rus E and Sofer S S, (1990). Comparison of cadmium biosorption by Gram- positive and Gram- negative bacteria from activated sludge, *Biotechnol Lett*, **12**: 839-842.
- Gray EJ, Smith DL. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol Biochem* **37**: 395–412.
- Grosell M.H., C. Hogstrand and C.M. Wood, (1997). Copper uptake and turnover in both copper acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **38**: 257-276.
- Gupta P. (1998). Cadmium toxicity and thyroid function with special reference to 5' monodeiodinase enzyme activity a comparative study in birds and mammal. Ph.D.Thesis.
- Gupta V.K., Ali, I., (2000). Utilization of bagasse fly ash (a sugar industry waste) for the removal of copper and zinc from wastewater. *Separation and Purification Technology*, **18**: 131-140.
- Gupta P.K (2003). Achieving production effectiveness and increasing business competitiveness through cleaner production. Leather sector profile National production center, New Delhi, India.

- Gürses S. Karaca, C. Dogar, R. Bayrak, M. Acikyildiz and M. Yalcin,(2004). Determination of adsorptive properties of clay/water system: methylene blue sorption, *J. Colloid Int. Sci.* **269**: 310–314.
- Habi S, Daba H. (2009). Plasmid incidence, antibiotic and metal resistance among enterobacteriaceae isolated from Algerian streams. *Pak J Biol Sci* **12**: 1474–1482.
- HACH (1997). *Water Analysis Handbook*, 3rd edition, HACH Company, Loveland, Colorado, USA.
- Harriman R., Watt, A.W., Christie, A.E.G., Collen, P., Moore, D.W., McCartney, A.G., Taylor, E.M. and Watson, J. (2001). Interpretation of trends in acidic deposition and surface water chemistry in Scotland during the past three decades. *Hydrology and Earth System Sciences*, **5**: 407–420.
- Hascakir B., Dolgen, D.,(2008). Utilization of Clay Minerals in Wastewater Treatment: Organic Matter Removal with Kaolinite, *Ecology*, **66**: 47-54.
- Haydar S, Aziz JA, Ahmad MS (2007). Biological Treatment of Tannery Wastewater Using Activated Sludge Process. *Pak. J. Eng. Appl. Sci.*,**1**: 61-66.
- Haydar S. and Aziz, J. A. (2009). Coagulation–flocculation studies of tannery wastewater using cationic polymers as a replacement of metal salts, *Water Sci. Technol.*, **59** : 381- 390.
- He ZJ, Li S and Zhang L (1987). Treatment of industrial wastewater and sludge dehydration with inorganic high molecular weight coagulants. *Shanghai Environ. Sci.* **6**: 32 – 34.
- Hellawell J.M. (ed.) (1986). *Biological Indicators of freshwater pollution and Environmental Management*, Elsevier Applied Science Publishers Ltd., London and New York, pp.1-546 .
- Hesham M. Abdulla 1, Engy M. Kamal 1*, Amr H. Mohamed 2, Ahmed D. El-Bassuony (2010). Chromium removal from tannery wastewater using chemical and biological techniques aiming zero discharge of pollution. *Proceduring of fifth scientific environmental conference, Zagazig Uni.*, 171 – 183.

- Holt J.G., Krieg R.N, Sneath A.H.P, Staley T.J, Williams T.S (1994). Bergey's Manual of Determinative Bacteriology, 9th Edition (International Edition).
- Hung C.M., Lou, J.C., Lin, C.H., (2003). Removal of ammonia solutions used in catalytic wet oxidation processes. *Chemosphere*. **52**: 989-995.
- IFC (2007). Environmental, Health, and Safety Guidelines Tanning and Leather finishing. Washington, D.C.
- Ignacio G.J., Manuel B., Pablo, C. and Manuel J. B. (1995). Treatment of tannery effluents by a two step Coagulation/Flocculation process. *Wat. Res.*, **29 (6)**: 2605- 2608.
- Ilhan S, Noubakhsh M. N, Kilicarslan S and Ozdag H, (2004). Removal of chromium, lead and copper ions from industrial wastewater by *Staphylococcus saprophyticus* Turk, *Electronic J Biotechnol*, **2**: 50-57.
- Imamul Huq, S. M., (1998). Critical Environmental Issues Relating to Tanning Industries in Bangladesh. In: Naidu. (eds), Towards Better Management of Soils Contaminated with Tannery waste, Proceedings of a workshop held at the Tamil Nadu Agricultural University, Coimbatore, India, 31 January to 4 February, 1998.
- ISI, Indian Standards Institution, (2000). Guide for Treatment and Disposal of Effluents of Tanning Industry. New Delhi, India.
- Islam K. M. N., Khaled Misbahuzzaman, Ahmed Kamruzzaman Majumder and Milan Chakrabarty (2011). Efficiency of different coagulants combination for the treatment of tannery effluents: A case study of Bangladesh. *African Journal Environmental Science and Technology* **5(6)**: 409-419.
- ISW-BDS (inland surface water in Bangladesh), ECR (1997). Ministry of Environment and Forest, Gazette notification 27, Aug.
- Javed M. (2005). Heavy metal contamination of fresh water fish and bed sediments in the River Ravi stretch and related tributaries. *Pak. J. Biol. Sci.* **8**: 1337-1341.

- Jeanmougin F. Thompson, J.D. Gouy, M. Higgins, D.G. and Gibson, T.J. (1998): Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences* **23**: 403-405.
- Jeyasingh J, Ligy Philip, (2005). Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions, *Journal of Hazardous Materials, B* **118**: 113–120.
- Jiang J.Q., (2001). Development of coagulation theory and pre-polymerized coagulants for water treatment., *Sep. Purif. Methods*, **30 (1)**: 127–141.
- Jia-qian Jiang and Nigel JD Graham (1998). Pre-polymerised inorganic coagulants and phosphorus removal by coagulation- A review. *Water SA* **24 (3)**: 237-244.
- Jing-wei F., Ya-bing, S., Zheng, Z., Ji-biao, Z., Shu, L. and Yuan-chun, T. (2007). Treatment of tannery wastewater by electrocoagulation, *J. Environ. Sci.*, **19**: 1409–1415.
- Junior H., Silva J., Arenzon A. Portela C., (2006). Evaluation of genotoxicity and toxicity of water and sediment samples a Brazilian stream influenced by tannery industries. *Chemosphere*: 1-7.
- Junior H.M., Silva, J.D., Arenzon, A., Portela, C.S. and Henriques, J.A. (2007). Evaluation of genotoxicity and toxicity of water and sediment samples from a Brazilian stream influenced by tanneries. *Chemospher*, **67**: 1211-1217.
- Justina C., Elsa, M., Ana, P., Ana, L., Luis, S. and Maria, N. (2009). Membrane-based treatment for tanning wastewaters, *Can. J. Civil Eng.*, **36 (2)**: 356-362.
- Kabdasli I., Olmez, T. and Tunay O. (2003). Nitrogen removal from tannery wastewater by protein recovery, *Water Sci. Technol.* **48 (1)**: 215–223.
- Kabdasli I., Tunay,O., and Orhon, D. (1999). Wastewater control and management in a leather tanning district. *Wat. Sci. Tech.*, **40 (1)**: 261-267.
- Kabir G. and Ogbeide, S. E. (2008). Removal of Chromate in Trace Concentration Using Ion Exchange From Tannery Wastewater. *Int. J. Environ. Res.*, **2 (4)**: 377-384.

- Kamaludeen SPBK, Arunkumar KR, Avudainayagam S, Ramasamy K. (2003). Bioremediation of chromium contaminated environments. *Indian J Exp Biol* **41**: 972–985.
- Kannan A.R. and R.K.Upreti, (2008). Influence of distillery effluent on germination and growth of mungbean (*Vigna radiate*) seeds. *J. Hazard.Mater.*, **153**: 609-615.
- Kannan V., R. Ramesh, and C. Sasikumar, (2005). Study on ground water characteristics and the effects of discharged effluents from textile units at Karur District. *Journal of Environmental Biology* **26 (2)**: 269-272.
- Kao PH, Huang CC, Hseu ZY. (2006). Response of microbial activities to heavy metals in a neutral loamy soil treated with biosolid. *Chemosphere* **64**: 63–70.
- Khan MS, Zaidi A, Wani PA, Oves M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environ Chem Lett*, **7**: 1–19.
- Khan MS, Zaidi A, Wani PA, Oves M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environ Chem Lett*, **7**: 1–19.
- Khan S. and Noor M. (2002). Investigation of pollutants in wastewater of Hayatabad Industrial Estate Peshawar. *Pakistan Journal of Agriculture Sciences* **2**: 457-461.
- Khan S. R., Khwaja, M. A., Khan, S., Kazmi and Ghani, H. (1999). "Environmental Impacts and Mitigation Costs of Cloth and Leather Exports from Pakistan," SDPI Monograph Series M. 12, Islamabad, Pakistan.
- Kim J, Nason JA & Lawler DF (2008). Influence of surface charge distributions and particle size distributions on particle attachment in granular media filtration. *Environmental Science and Technology* **42**: 2557- 2562.
- Kim S-H, Moon B-H & Lee H-I (2001). Effects of pH and dosage on pollutant removal and floc structure during coagulation. *Micro-chemical Journal* **68**: 197–203.

- Kokila A., Parmar, Sarju Prajapati, Rinku Patel and Yogesh Dabhi (2011). Effective use of ferrous sulphate and alum as a coagulant in treatment of dairy industry wastewater. *ARPN journal of engineering and applied Sciences*. **6 (9)**: 42-45.
- Kornhauser Carlos, Katarzyna Wrobel, Kazimierz Wrobel, Juan Manuel Malacara, Laura Eugenia Nava Leobardo GÓMEZ and Rita Gonzalez.,(2002). Possible Adverse Effect of Chromium in Occupational Exposure of tannery Workers.*Industrial Health*, **40**: 207–213.
- Krishnamoorthi S, Sivakumar V, Saravanan K, Prabhu S (2009). Treatment and Reuse of Tannery Waste Water by Embedded System. *Modern Appl. Sci.*, **3 (1)**: 129-134.
- Kudo C. I. and Liu, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmid. *J. Bacterol.* **145**: 1365-1373.
- Kumar A, Bisht BS, Joshi VD, Dhewa T. (2011). Review on bioremediation of polluted environment: a management tool. *Int J Environ Sci*, **1**: 1079–1093.
- Kyung-Soak M. JaeJeong, Y. and Zuwhan, Y. (2004). Removal of ammonium from tannery wastewater by electrochemical treatment. *Environ. Sci. Health*, **39 (7)**: 1867 – 1879.
- Lakshmi Jothinathan, Subramanyan, Vasudevan, Murthy packiyan (2010). Electro coagulation studies on removal of Cd using magnesium electrode. *Journal Applied Electrochem.***40**: 2023-2032.
- Lederberg J. (1952). Cell genetics and hereditary symbiosis. *Physiological Reviews*. **32**: 403.
- Lee W. and L. Westerhoff. (2006). Dissolved organic nitrogen removal during water treatment by aluminium sulphate and cationic polymer coagulation. *Journal of Water Research*. **40 (20)**: 3767-3774.
- Lefebvre O., Vasudevan N., Torrijos M., Thanasekaran K. and Moletta R. (2006). Anaerobic digestions of tannery soak liquor with an aerobic post-treatment, *Water Res.* **40**: 1492 – 1500.

- Leghouichi E., Laib E. and Guerbet, M. (2009). Evaluation of chromium contamination in water, sediment and vegetation caused by the tannery of Jijel (Algeria): A case study. *Environmental monitoring and assessment*, **153**: 111-117.
- Li W., Zhang. S., and Shan. X. Q, (2007). Surface modification of goethite by phosphate for enhancement of cu and cd adsorption, *Colloids and Surfaces A:Physiochem.eng.Aspects*, **293**: 13-19.
- Linda A. L. and Peter K. J. (1999). Physico-chemical treatment methods for the removal of microcystins (cyanobacterial hepatotoxins) from potable waters. *Chem. Soc. Rev.* **28**: 217-224.
- Liu WH, Zhao JZ, Ouyang ZY, Soderlund L, Liu GH (2005): Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environment international* **31**: 805-812.
- Loffler J., Hebart, H., Brauchle, U., Schumacher, U. and Einsele, H. (2000). Comparison between plasma and whole blood specimens for detection of *Aspergillus* DNA by PCR. *J Clin Microbiol* **38**: 3830-383.
- Lofrano G. Belgiorno, V. and Gallo, M. (2006). Toxicity reduction in leather tanning wastewater by improved coagulation flocculation process. *G. NEST J.*, **8 (2)**: 151-158.
- Lokhande R.S., Singare, P.U., and Pimple, D.S., (2011). Quantification Study of Toxic Heavy Metals Pollutants in Sediment Samples Collected from Kasardi River Flowing along the Taloja Industrial Area of Mumbai, India., *The New York Science Journal* **4 (9)**: 66-71.
- Ma X, Novak PJ, Ferguson J, Sadowsky M, LaPara TM, Semmens MJ, Hozalski RM. (2007). The impact of H₂ addition on dechlorinating microbial communities. *Journal of Bioremediation* **11**: 45-55.
- Mahesh A., James, R., Peterson, N. Johnstone, L., Johnstone, C., Turick, E., David, Ry., and William A. A. (1997). Bacterial reduction of hexavalent chromium by *Enterobacter cloacae* strain HO1 grown on sucrose. *Biotechnol, Lett.* **19**: 691-694.

- Mahvi A.H., Maleki, A., Roshani, B., (2004). Removal of Anionic Surfactants in Detergent Wastewater by Chemical Coagulation, *Pakistan Journal of Biological Sciences*. **7 (12)**: 2222- 2226.
- Mahvi A.H., Nabizadh, R., Pishrafti, MH., Zarai, Th., (2008). Evaluation of single stage USBF in removal of nitrogen and Phosphorus from wastewater, *EUR. J. Sci. Res.* **23 (2)**: 204-211.
- Maiti S. K. (2002). Chemical analysis of waste – water and effluent. In *Handbook of Methods in Environmental studies*, pp. 22 -24, 174- 176. India: ABD Publishers.
- Mandal T., Dalia Dasgupta, Subhasis Mandal, Siddhartha Datta. (2010). Treatment of leather industry wastewater by aerobic biological and Fenton oxidation process. *Journal of Hazardous Materials* **180**: 204-211.
- Manu K. J., Mohana V. S. and Ganeshaiah K. N (2011). Effluent Generation by the Dairy Units: characterization and Amelioration for Irrigation International *Journal of Research in Chemistry and Environment*. **1 (2)**: (173-182).
- Maria-Judith De Souza, Shanta Nair, P A Loka Bharathi, D Chandramohan, (2006). Metal and antibiotic-resistance in psychrotrophic bacteria from Antarctic Marine waters. *Microbiology Laboratory, Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa 403 004, India*. **15 (4)**:379-384.
- Martinez J.M., Goltara, A. and Mendez R. (2003). Tannery wastewater treatment: comparison between SBR and MSBR, *Water Sci. Technol.: Water Supply*, **3 (5-6)**: 275-282.
- Martinez N.S., Fernandez, J.F., Segura, X.F., Ferrer, A.S., (2003). Pre-oxidation of an extremely polluted industrial wastewater by the Fenton's reagent, *J.Hazard. Mater.* **B101**: 315-322.
- Matsumoto ST, Mantovani MS, Mallaguti MI and Marin-Morales MA (2003). Investigation of the genotoxic potential of the waters of a river receiving tannery effluents by means of the in vitro comet assay. *Cytologia* **68**: 395-401.

- Megharaj M, Avudainayagam S, Naidu R.,(2003). Toxicity of hexavalent chromium and its reduction by bacterium isolated from soil contaminated with tannery waste. *Carr Microbiol.* **47**: 51-54.
- Mengoni A. Barzanti, R. Gonnelli, C. Gabbrielli, R. and Bazzicalupo, M. (2001). Characterization of nickel resistant bacteria isolated from serpentine soil. *Environmental Microbiology* **3**: 691-708.
- Mesdaghinia A. R. and Yousefi, Z. (1991). The use of oxygen in catalytic oxidation of sulphides in tannery wastes, *Iranian J. Publ. Health.* **20**: 1-4.
- Metcalf and Eddy, Inc. (1979). *Wastewater Engineering: Treatment, Disposal, and Reuse.* 2nd ed. McGraw-Hill.
- Metes A., Kovacevic, D., Vujevic,D. and Papic,S. (2004). The role of zeolites in wastewater treatment of printing inks. *Wat. Res.* **38 (14)**: 3373–3381.
- Mikami Y, Slatar Y and Yiasa M (1984). Treatment of dying waste water by coagulation. *PPM* **15** : 14 - 19.
- Mohanta M. K., M. A. Salam, A. K. Saha, A. Hasan, A. K. Roy, (2010). Effects of Tannery Effluents on Survival and Histopathological Changes in Different Organs of *Channa punctatus*, *Asian J. Exp. Biol. Sci.* **1(2)**: 294 – 302.
- Ms. Khan, M. S. Ali, and T.Afroz, et al (2009). Potable water from industrial wastewater. *Chemical Engineering Research Bulletin*, **12**: 20-23.
- Mullen MD, Wolf DC, Ferris FC, Beveridge TJ, Flemming CA, Bailey FW. (1989). Bacterial sorption of heavy metals. *Appl Environ Microbiol.* **55**: 3143–3149.
- Munees Ahemad, (2012). Implicaions of bacterial resistance against heavy metals in bioremediation: A review *IIOABJ*; **3 (3)**: 39–46.
- Munz G., Gori, R., Cammilli, L. and Lubello, C. (2008). Characterization of tannery wastewater and biomass in a membrane bioreactor using respirometric analysis. *Bioresource Technol.*, **99**: 8612-8618.
- Murat S., Insel G., Artan N. and Orhon D. (2006). Performance evaluation of SBR treatment for nitrogen removal from tannery wastewater. *Water Sci. Technol.*, **53 (12)**: 275-284.

- Murugananthan M., Raju, B. G. and Prabhakar, S. (2004). Separation of pollutants from tannery effluents by electro flotation. *Sep. Purif. Technol.* **40**: 69-75
- Mythili K. and Karthikeyan, B., (2011). Bioremediation of tannery effluent and its impact on seed germination (blackgram and sunflower), *Current Botany*, **2** (8): 40-45.
- Nabi G. R. Bidhendi, A. Torabian, H. Ehsani, N. Razmkhah (2007). Evaluation of industrial dyeing wastewater treatment with coagulants and polyelectrolyte as a coagulant aid. *Iran. J. Environ. Health. Sci. Eng.* **4** (1): 29-36.
- Nandy T., and Kaul, S.N. (2001). Anaerobic pre-treatment of herbal- based pharmaceutical wastewater using fixed -film reactor with recourse to energy recovery. *Water res.* **35** (2): 351-362.
- Narashimha G., Babu, G. V. A. K., and B.R., (1999). Cellulolytic activity of cultures isolates from soil contaminated with effluents of cotton ginning industry. *Journal of Environ. Biol.* **20** (3): 235-239.
- Nariagu J. O. Pacyna, J.M (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* **333**: 134-139.
- Naumczyk J. Szpyrkowicz, L., De Faveri D. M. and Zillio G. F. (1996). Electrochemical treatment of tannery wastewater containing high strength pollutants. *Trans I ChemE*, **74** (Part B): 59-68.
- Naumczyk J., Rusiniak, M. (2005). Physicochemical and chemical purification of tannery wastewaters. *Polish journal of environmental studies*, **14** (6) : 789-797.
- NEQS. (2000). National Environmental Quality Standards for municipal and liquid industrial effluents. Url: [http://www.emc.com.pk/pdf/3-NEQS\(New\).pdf](http://www.emc.com.pk/pdf/3-NEQS(New).pdf) (Last visited: Sept. 23,2012)
- Newman M C. and A. W. Mchosh editors (1991). *Metal Ecotoxicology, Concepts and Applications*. Lewis Publishers, Chelsea Michigan.
- Nicholas P. C. (1996). *Biotechnology for Waste and Wastewater Treatment*. Weatwood, New Jerary, U.S.A.

- Nicholson R.A, Roth S.H, Zhang A, Zheng J, Brookes J, Skrajny B, Bennington R (1998). Inhibition of respiratory and bioenergetic mechanisms by hydrogen sulphide in mammalian brain. *J Toxicol Environ Health* **54**: 461–507.
- Nies D.H, Silver S. (1999). Ion efflux systems involved in bacterial metal resistances. *J. Ind Microbiol* **14**: 186–199.
- Nourisepehr M. Nasser S. Mazaheri A.M. Yaghmaian K.* (2005). Chromium Bioremoval From Tannery Industries Effluent By *Aspergillus Oryzae*. *Iran. J. Environ. Health. Sci. Eng.*, **2** (4): 273-279.
- Olajumoke A. Oluwatosin A. Olumuyiwa O. Abimbola F. (2010). Impact assessment of brewery effluent on water quality in Majawe, Ibadan, South western Nigeria. *Researcher*. **2** (5): 21-28.
- Olson J.W., Mehta N.S, Maier R.J. (2001). Requirement of nickel metabolism protein HypA and HypB for full activity of both hydrogenase and urease in *Helicobacter pylori*. *Mole. Microbiol* **39**: 176–182. And Proceedings of a workshop hosted by Department of Conservation, 10 – 12 May 2001, Hamilton.
- Owlad M., Aroua, M. K., Daud, W. A. A., Baroutian, S. (2008). Removal of Hexavalent Chromium–Contaminated Water and Wastewater. *Water Air Soil Pollution*. **200**: 59-77.
- Ozdemir G., Ceyhan N., Ozturk T., Akirmak F. and coasar T., (2004). Biosorption of chromium (VI), cadmium (II) and cupper (II) by *Pantoea* spp. *TEM 18*, *Chemical Engineering Journal*. **102**: 249-253.
- Pal A. Choudhuri, P. Dutta, S. Mukherjee, P. K. and Paul, A. K. (2004): Isolation and characterization of nickel-resistant microflora from serpentine soils of Andaman. *World Journal of Microbiology and Biotechnology* **20**: 881-886.
- Pala A., Tokat, E., (2000). Color removal from cotton textile industry wastewater in an activated sludge system with various additives, *Water Res.*, **36**: 2920 - 2925.
- Paul A.K and Pal A., (2004). Aerobic chromate reduction by chromium resistant bacteria isolated from serpentine soil. *Microbiol. Res.* **159** : 347-354.

- Pearson H. W., Mara, D. D. and Bartone, C. R. (1987). Guidelines for the Minimum Evaluation of the Performance of Full-scale Waste Stabilization Ponds. *Wat. Res.*, **21** (9): 1067–1075.
- Pittier P. and Chudoba, J. (1990). Biodegradability of Organic Substances in the aquatic Environment, CRC Press.
- Pizzaro D., Uhlmann, E., & Salovey, P. (2003). Asymmetry in judgments of moral blame and praise: The role of perceived metadesires. *Psychological Science*, **14** : 267–272.
- Rabbani M, Ghafourian H. Sadeghis S and Nazeri Y. (2005). Biosorption of Cr (III) by new bacterial strain (NRC-BT-2). *Int. CongrSer*, **1276**: 268-269.
- Radojevic M, Bashkin V.N (1999). Practical Environmental Analysis. The Royal Society of Chemistry, Cambridge, 466.
- Rafiqullah I. M, Hossain A. M. M, Ilias M, Hoq M. M. (2008). Chromium (VI) Reducing Native Microorganisms for Remediation of Chromium Eco-toxicity in Environment of Bangladesh. *Bangladesh J Sci Ind Res.* **43(4)**: 455-466.
- Rahman M.A (1997). Characteristics of major industrial liquid pollutants in Bangladesh. Masters Project, Department of Civil Engineering, Bangladesh University of Engineering and Technology.
- Rai A.K., S.N. Upadhay, S. Kumar, and Y.D. Upadhyay. (1998). “Heavy metal pollution and its control through a cheaper method: A review”, *Journal IAEM.* **25**: 220-251.
- Rajat Ameta, Jitendra vardia, Pinki B Punjabi and Suresh C. Ameta (2006). Use of semiconducting iron (III) oxide in photocatalytic bleaching of some dyes. *Indian Journal of Chemical Technology.* **13**: 114-118.
- Ramesh B., Bhadrinarayana, N.S., Meera S.B. and Anantharaman, N. (2007). Treatment of tannery wastewater by electrocoagulation. *Chem. Techn. Metallurgy.* **42 (2)**: 201-206.

- Ramteke Pramod W., Thiruneelakantan Srinath and Shilpi Khare (2001). Isolation of hexavalent chromium-reducing Cr-tolerant facultative anaerobes from tannery effluent. *J. Gen. Appl. Microbiol.* **47**: 307–312.
- Rani A, Goel R. (2009). Strategies for crop improvement in contaminated soils using metal-tolerant bioinoculants. In: Khan MS, Zaidi A, Musarrat J, (eds.) *Microbial strategies for crop improvement*, Springer, *Berlin* p. 105–132.
- Rao J. R., Chandrababu., C. Muraidharn, N. B Unni P. G. and Ramasami.T,(2003). Recouping the waste water: a way forward for cleaner leather processing, *J. Cleaner production*, **11**: 591-599.
- Rapoport A.I. and Muter, O.A. (1995). Biosorption of hexavalent chromium by yeasts. *Process Biochem.*, **30** (2): 145-149.
- Ratnaweera H, Degaard Z.H, Fettig J. (1992). Coagulation with pre-polymerized aluminum salts and their influence on particle and phosphate removal. *Water Sci. Tech.* **26**: 12-29.
- Rehman A, Zahoor A, Muneer B, Hasnain S, (2008). Chromium tolerance and reduction potential of a *Bacillus sp. ev3* isolated from metal contaminated wastewater. *Bull Environ Contam Toxicol.* **81**: 25–29.
- Repsyte J. and Simutis, R. (2004). Process Simulator for Wastewater Treatment Plant Informacin_s technology, Kaunas, Technologija, Nr. 3 (32).
- Roane T.M. (1999). Lead resistance in two isolates from heavy metal contaminated soils. *Microbial Ecology.* **37**: 218-224.
- Rodríguez S. Couto S., D. Moldes, A. Liébanas and A. Sanromán (2003). *Biochemical Engineering Journal* **15**: 21.
- Rovan P. H., Berg and Johson G. B. (1998). *Environment 2nd*. ED. Hartcourt Brace colledge publisher, New York.
- Rusal M. G., I. Faisal, K. M. M. Kamal, (2006). Environmental pollution generated from process industries in Bangladesh, *Journal of Enviromental Polution.* **28** (2): 44-161.

- Sachchida Nand Singh., Gaurav Srivastav and Arun Bhatt., (2012). Physicochemical determination of Pollutants in Wastewater in Dheradun. *Current World Environment*. **7 (1)**: 133-138.
- Safiullah S., (2010). Theme Lecture, Bangladesh Chemical Congress, 33rd Annual Conference, 30.
- Saha S.; Singh, R. P.; Verma, J. P.; Jayaraman, J., (2001). Plasmid-borne determinants of colony morphology, pigmentation, antibiotic resistance and antibiosis in *Pseudomonas* species antagonistic to bacterial blight of cotton. *Curr. Sci.*, **79 (9)**: 1384-1385.
- Salt D. E., M. Blaylock, P. B. Ananda Kumar, V. Dushenkov B. D. Ensley, I. Chet and I. RASKIN. (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.* **13**: 468 -474.
- Sambrook J.; Fritsch, E. F.; Maniatis, T., (1989). *Molecular cloning: A laboratory manual*, 2nd. Ed. Cold spring harbour, NY: Cold spring harbor laboratory press.
- Sanders P.M, Bui A.Q, Weterings K, McIntire K.N, Hsu Y.C, Lee P.Y, Truong M.T, Beals T.P, Goldberg R.B. (1999). Another developmental defects in *Arabidopsis thaliana* male-sterile mutants. *Sex Plant Reprod.* **11**: 297-322.
- Sankar M., Sekaran, G., Sadulla, S., Shanmugasundaram, K. A. and Mariappan, M. (1998). Recycling of tannery effluent through photo-oxidation method, *Indian J. Environ. Prot.*, **18 (3)**: 206-210.
- Santosa S., Siswanta, D., Sudiono, S. and Utarianingrum, R. (2008). Chitin-humic acid hybrid as adsorbent for Cr (III) in effluent of tannery wastewater treatment. *Appl. Surf. Sci.*, **254**: 7846-7850.
- Saravanabhavan P., Thanikaivelan, J., Raghava, R. and Balachandran, U. N. (2005). A one bath chrome tanning together with wet-finishing process for reduced water usage and discharge. *Clean Techn Environ. Policy* **7**: 168-176.
- Sayler G. S., Hooper, S. W., Layton A. C. and Henryking, J. M. (1990). Catabolic plasmid of environmental and ecological significance. *Microb. Ecol.* **19**: 1-20.

- Schrank S. G., Jose, H. J. and Moreira, R. F. P. M. and Schroder, H. F. (2004). Comparison of different Advance Oxidation Process to reduce toxicity and mineralisation of tannery wastewater. *Water Sci. Technol.* **50** (5): 329-334.
- Seabloom R. W. and Buchanan, J. R. (2005). Aerobic Treatment of Wastewater and Aerobic Treatment. National Decentralized Water Resources Capacity Development Project. University of Arkansas, Fayetteville, A.R.
- SEHD (1998). Bangladesh Environment. Facing 21st century, First Edition. Society for environment and Human development (SEHD) publications, Dhaka, Bangladesh.
- Sekaran G., Chitra, K., Mariappan, M. and Raghavan, K. V. (1996). Removal of sulfide in anaerobically treated tannery wastewater by wet air oxidation. *J. Environ. Sci. Heal. A*, **31** (3): 579-98.
- Seki H., Suzuki, A., Maruyama, H., (2005). Biosorption of chromium (VI) and arsenic (V) onto methylated yeast biomass. *J. colloid Interf. Sci*, **281**: 261-266.
- Sengil A., Kulac, S. and Ozacar, M. (2009). Treatment of tannery liming drum wastewater by electrocoagulation. *J. Hazar. Mater.*, **167**: 940-946.
- Shamas K. M., G. Tichy, M. Sager, T. Peer, A. Bashar, M. Jozic (2009). Soil contamination from tannery wastes with emphasis on the fate and distribution of tri- and hexavalent chromium, *Water, Air and Soil Pollution* **199**: 123-137.
- Shams K. M., G. Tichy, A. Fischer, K. Filip, M. Sager, A. Bashar, T. Peer, and M. Jozic, *Geophys* (2008). *Res. Abstr.* 10.
- Shanker A.K, Cervantes C, Loza-Tavera H, Avudainayagam S (2005). Chromium toxicity in plants. *Environ Int.* **31**: 739-753.
- Sharma D.C, Sharma C.P, Tripathi R.D (2003). Phytotoxic lesions of chromium in maize. *Chemosphere* **51**: 63-68.
- Sharma D. C., Srivastava, P. C., Johri, B. N. & Chandra, P. (1996). Environmental Impact of Tannery Effluents on Heavy Metal Phytotoxicity and Health Hazards. *Pollution Research*, **15**: 267-269.

- Sharma R. K., Agrawal, M., and Marshall, F. (2007). Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicol. Environ. Safety*. J. Doi: 10.1016/j.ecenv.
- Shen T. T. (1999). *Industrial Pollution Prevention*, 2nd ed. Springer, pp 40.
- Shouli A.S, Bewtra J. K, Biswas N. (1992). Effectiveness of coagulation and flocculation processes in removal of selected volatile organic contaminants from wastewaters. *Int. J. Environ. Stud.* **40**:2-7.
- Shukla O.P, Juwaokar A.A, Singh S.K, Khan S. Rai U.N. (2011). Growth responses and metal accumulation capabilities of woody plants during the phytoremediation of tannery sludge. *Waste Manag.* **31 (1)**: 115-123.
- Silver S. and Misra, T.K. (1988). Plasmid mediated metals resistance. *Annual Review of Microbiology.* **42**: 717-743.
- Sinha S, Saxena R, Singh S. (2002). Comparative studies on accumulation of Cr from metal solution and tannery effluent under repeated metal exposure by aquatic plants: its toxic effects. *Environ Monit Assess* **80**: 17–31.
- Sirianuntapiboon N., Ungkaprasatcha, O., (2007). Removal of Pb^{2+} and Ni^{2+} by bio-sludge in sequencing batch reactor (SBR) and granular activated carbon – SBR (GAC-SBR) systems, *Bioresource Technology.*, **98**: 2749-2757.
- Smita S. Borchate, Dr. G. S. Kulkarni, S. V. Kore, V. S. Kore (2012). Application of coagulation–floculation for vegetable tannery wastewater. *International Journal of Engineering Science and Technology (IJEST).* **4 (5)**: 1944-1948.
- Song C. J. Williams and R. G. J. Edyvean. (2003). Tannery waste water treatment. *Env.Eng.Sci*, Vol.**20** N o.6.
- Song Z., Williams, C. J., Edyvean, G. J. (2004). Treatment of tannery wastewater by chemical coagulation, *Desalination.* **164**: 249-259.
- Song Z., Williams, C. J. and Edyvean R. G. J. (2000). Sedimentation of Tannery Wastewater. *Water Res.* **34 (7)**: 2171-2176.

- Soyaslan I. I., Karaguzel, R., (2007). Investigation of water pollution in the Yalvacbasin into Egirdir Lake, Turkey. *Environ. Geol.* Doi: 10.1007/s00254-007-1074-2.
- SPSS Inc (2008) SPSS 15.0 for Windows. SPSS Inc., Chicago.
- Sreeram K. J., Ramasami, T., (2003). Sustaining tanning process through conservation, recovery and better utilization of chromium. *Resourc. Conserv. Recycling* **38** (3): 185–212.
- Stephenson R. J, Duff S. B. J. (1996). Coagulation and precipitation of a mechanical pulping effluent: Removal of carbon, color and turbidity. *Water Res.*, **30**: 781.
- Stoyanova R., Querec, T., Brown, T., Patriotis, C., (2004). Normalization of single-channel DNA array data by principal component analysis. *Bioinformatics* **20**: 1772–1784.
- Strong P. J, Burgess J. E. (2008). Treatment methods for wine-related ad distillery wastewaters: a review. *Journal of Bioremediation* **12**: 70–87.
- Sumathi M. and S. Umagowrie (2012). Biotreatment to reduce the phytotoxicity of tannery effluent on *Arachis hypogea* and *Sesamum indicum* *INT J CURR SCI*, 105-111.
- Sumeet Gandhi, Byung-Taek. O. H., Jerald. L., Schnoor and Pedro, Alvarez. J. J, (2002). Degradation of TCE, Cr (VI), sulfate, and nitrate mixtures by granular iron in flow-through columns under different microbial conditions. *Water Research*, **36** (8): 1973-1982.
- Sweeton F. H., Baes, C. F., Jr., (1970). The solubility of magnetite and Hydrolysis of ferrous ion in aqueous solution at elevated temperature. *J. Chem. Thermodynamics* **2**.
- Szpryokowicz L. and Zillio, G. F. (1995). Electrochemical treatment of tannery wastewater using Ti/Pt and Ti/Pt/Ir electrodes. *Wat. Res.* **29** (2): 517-524.
- Tang Hong-Xiao and Werner Stumm., (1987). The coagulating behaviors of Fe (III) polymeric species-II., Preformed polymers in various concentrations. *Water Research*, **21** (1): 123-128.

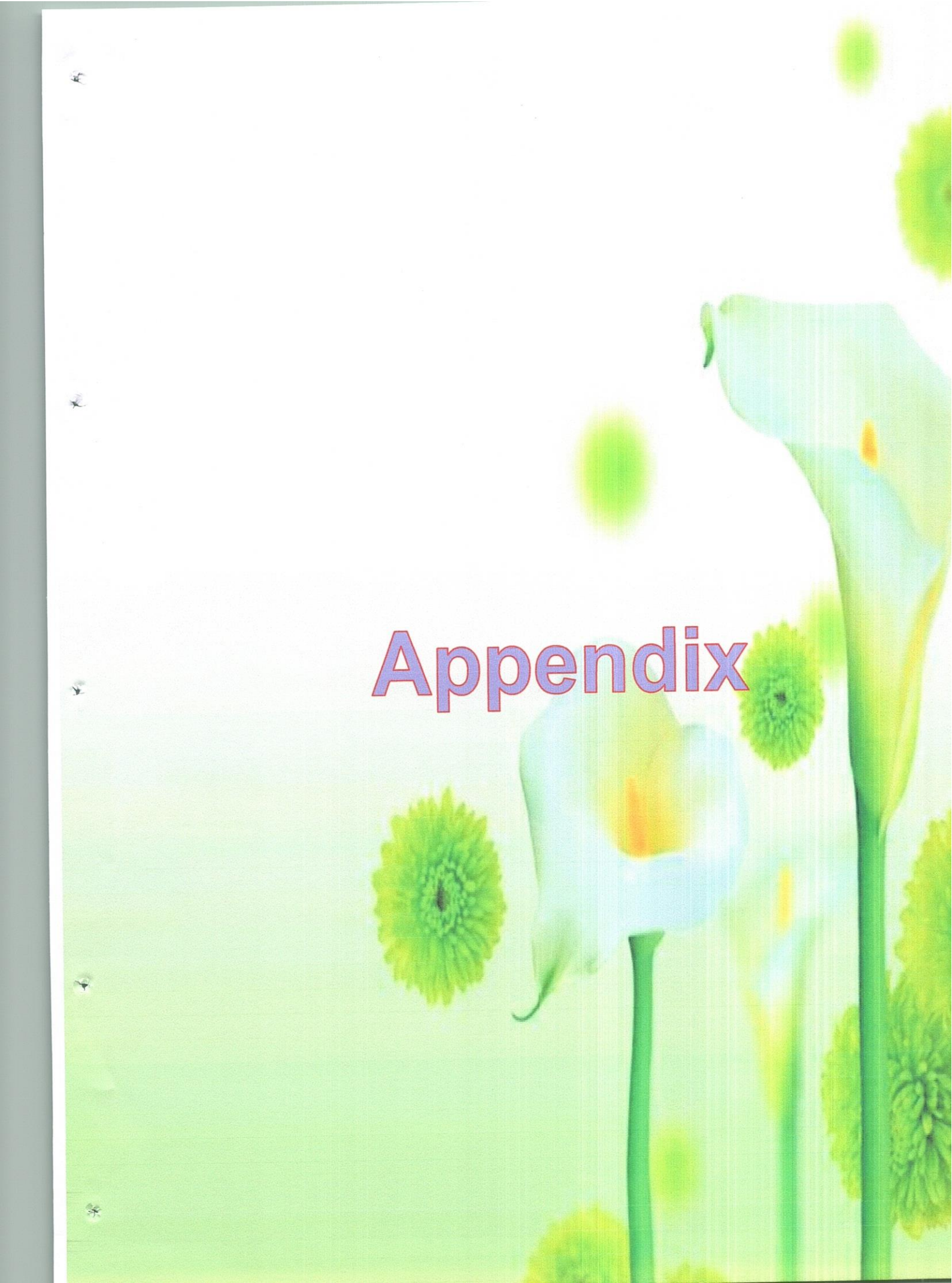
- Tang X.Y. Zhu, Y.G. Cui, Y.S. Duan, J. and Tang, L. (2006): The effect of ageing on the bio-accessibility and fractionation of cadmium in some typical soils of China. *Environment International* **32**: 682-689.
- Tariq S. R., Shah, M. H., Shaheen, N., Khalique, A., Manzoor, S., & Jaffar, M. (2005). Multivariate analysis of selected metals in tannery effluents and related soil. *Journal of Hazardous Materials, A* **122**: 17-22.
- Tariq S. R., Shah, M. H., Shaheen, N., Khalique, A., Manzoor, S. and Jaffar, (2006). Multivariate analysis of trace metal levels in tannery effluents in relation to soil and water- A case study from Peshawar, Pakistan. *Journal of Environmental Management* **79**: 20-9.
- Tauriainen S., Virta, M. and M. Karp. (2000). Detecting Bioavailable Toxic Metals and Metalloids from Natural Water Samples Using Luminescent Sensor Bacteria. *Water Research*, **34** (10): 2661-2666.
- Taylor M., Dieeendorf E. J. (1997). Extraction of Value Added Byproducts from the Treatment of Chromium Containing collagenous Leather Industry waste. *JSLTC*. **81**: 5.
- Thacker U., Parikh, R., Shouche, Y., Madamwar, D., (2007). Reduction of chromate by cell-free extract of *Brucella* sp. isolated from Cr (VI) contaminated sites. *Bioresource Technology* **98**: 1541-1547.
- Thorsten R. and Martin, J. (1997). Dissolved organics in tannery wastewaters and their alteration by a combined anaerobic and aerobic treatment. *Wat. Res.*, **31** (5): 1035-1046.
- Tiglyene S., Jaouad, A. and Mandi, L. (2008). Treatment of Tannery Wastewater by Infiltration Percolation: Chromium Removal and Speciation in Soil. *Environ. Technol.*, **29** (6): 613 – 624.
- Tiravanti G., Petruzzelli, D. and Passino, R. (1997). Pretreatment of Tannery wastewaters by an ion exchange process for Cr (III) removal and recovery. *Water Sci. Tech.*, **36** (2-3): 197-207.

- Tiwari S., Rai, R. Agrawal, M. (2008). Annual and seasonal variations in troposphere ozone concentrations around Varanasi. *International Journal of Remote Sensing* **29**: 4499-4514.
- Tobin J. M. and Roux, J. C. (1998). *Mucor* biosorbent for chromium removal from tanning effluents. *Wat.Res.*, **32 (5)** : 1407-1416
- Tresner H. D. and Danga F. F., (1958). Hydrogen sulphide production by *Streptomyces* as a criterion for species differentiation. *Journal of Bacterial*. **76 (3)**: 239-244.
- Tripathi A. K., Nirmal S. K. H., Nutangupha, (2007). Fungal treatment of industrial effluents, A mini review. Ecology and environment division, India.
- Tripathi R. D, Smith S. (1996). Effect of chromium (VI) on growth, pigment content photosynthesis, nitrate reductase activity, metabolic nitrate pool and protein content in duckweed (*Spirodela polyrrhiza*), pp. 159. In: Yunnus M. (ed.), ICPEP, 1996. Book of Abstracts, India.
- Trivedy R. K. and Goel, P. K. (1986). Chemical and Biological Methods for water pollution studies. Environmental Publication, Karad.
- Tudunwada I. Y.1 E. U. Essiet, S. G. Mohammed (2007). The effects of tannery sludge on heavy metals concentration in cereals on small holder farms in Kano, Nigeria *Not. Bot. Hort. Agrobot. Cluj*, **35 (2)**.
- Tudunwada I. Y., (2006). The effects of some soil amendment materials on heavy metal concentrations in sorghum and millets in the soils of Challawa Industrial Area, Kano State, Unpublished Ph. D. Thesis, Department of Geography, Bayero University, Kano.
- Tunay O., Orhon D., Kabdasli I. (1994). Pretreatment requirements for leather tanning industry wastewaters. *Water Sci Tech* **29 (9)**: 121-128.
- Turnland J. R (1988). Copper nutrition, Bioavailability and influence of dietary factors. *J. Am. Dietetic Assoc.*, **1**: 303-308.
- Turpeinen R. (2002). Interactions between metals, microbes and plants- bioremediation of arsenic and lead contaminated soils. Dissertation, Department of Ecological and Environmental Sciences, University of Helsinki, Lahti, p. 10-11

- Umrana V.V. (2006). Bioremediation of toxic heavy metals using acidothermophilic autotrophes. *Biores Technol* **97**: 1237–1242.
- UNEP (1991). Tanneries and the Environment: a technical guide to reducing the environmental impact of tannery operations. United Nations Environment Programme, Industry and Environment, Programme Activity Centre (UNIDO, IE/PAC), Paris.
- UNEP (1999). Technical Guidelines on Hazardous Wastes physico-Chemical Treatment, Biological Treatment. Basel Convention Series/SBC No: 99/007, Geneva.
- UNIDO (2000). Pollutants in Tannery Effluents. Regional Program for Pollution Control in the Tanning Industry in South-East Asia. UNIDO.
- UNIDO (2005). United Nations Industrial Development Organization, Cost of Tanned Waste Treatment, 15th Session of the Leather and Leather Products Industry Panel, Leon, Mexico.
- US EPA (2004). Primer for Municipal Wastewater Treatment Systems. Office of Wastewater Management, Washington DC.
- Usman Aftab, Muhammad Riaz Khan, Muhammad Mahfooz, Musadiq Ali, Salik Hassan, Aslam and A. Rehman, (2011). Decolourization and degradation of textile azo dyes by *Corynebacterium sp.* Isolated from industrial effluent. *Pakistan J. Zool.* **43** (1): 1-8.
- Vajpayee P, Sharma CS, Tripathi DR, Rai UN, Yunus M (1999). Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo Nucifera* Gaertn. *Chemosphere* **39** (12): 2159–2169.
- Valeika V., Beleska, K. and Valeikiene, V. (2006). Oxidation of sulphides in tannery wastewater by use of Manganese (IV) oxide. *J. Environ. Stud.*, **15** (4): 623-629.
- Van Groenestijn, J.W., Hazewinkel, J.H.O., Nienoord, M., Bussmann, P.J.T., (2002). Energy aspects of biological hydrogen production in high rate bioreactors operated in the thermophilic temperature range. *International Journal of Hydrogen Energy*, **27**: 1141-1147.

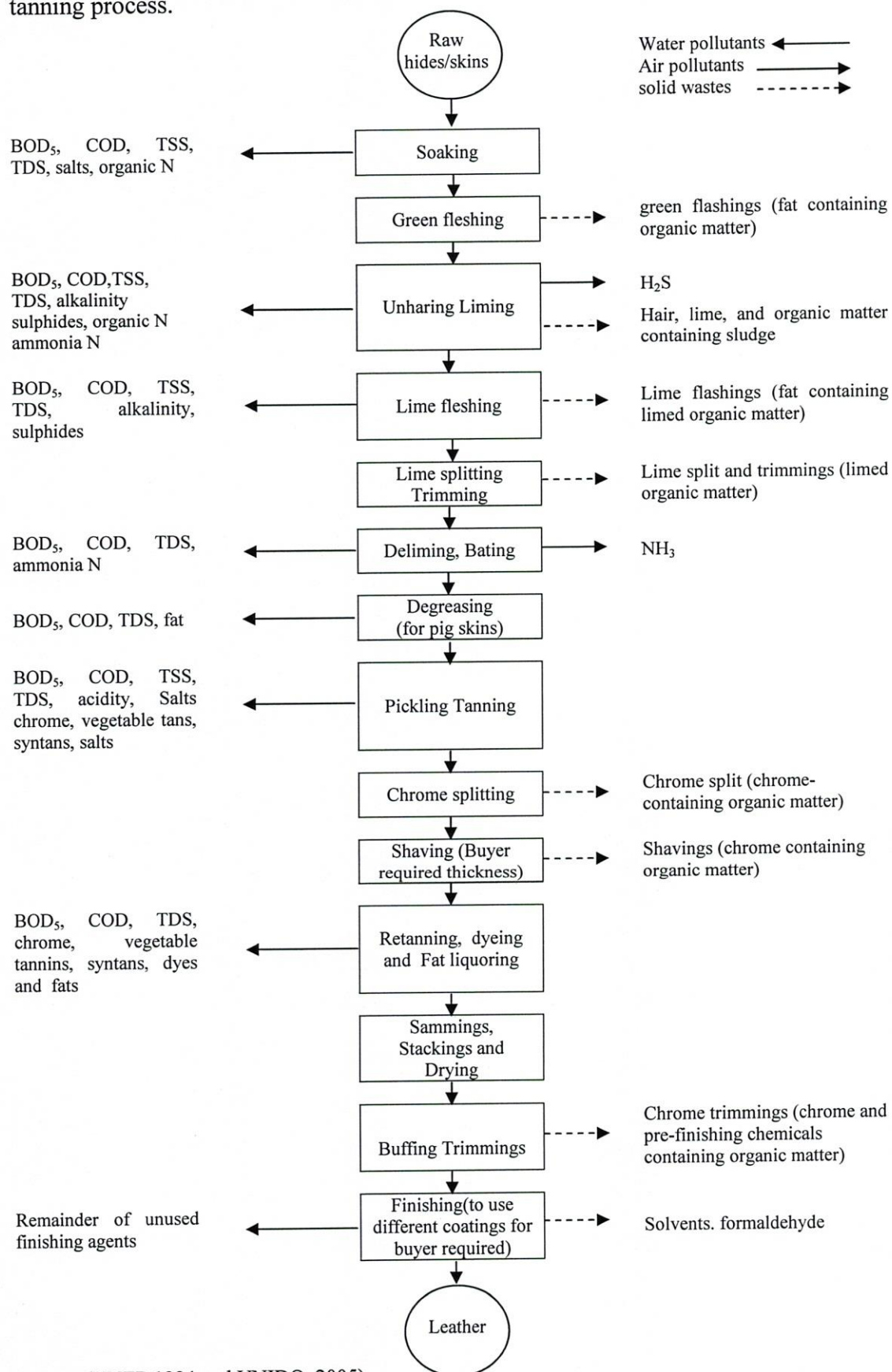
- Vasudevan S., Lakshmi J. and. Ganapathy S. (2011). Effects of alternating and direct current in electrocoagulation process on the removal of cadmium from water. *J. Hazard. Mater.*, **192**: 26-34.
- Venkatesh V., Bala, H. (2008), "Technology Acceptance Model 3 and a Research Agenda on Interventions", *Decision Sciences* **39** (2): 273–315.
- Verma T. Srinath, T. Gadpayle, R. U. Ramtake, P. W. Hans, R. K. and Garg, S. K. (2001). Chromate tolerant bacteria isolated from tannery effluent. *Bioresource Technology* **78**: 31-35.
- Verma T., Ramteke, P. W., Garg, S. K. (2008). Quality Assessment of Treated Tannery Wastewater with Special Emphasis on Pathogenic *E. coli* Detection through Serotyping. *Environ Monit Assess.*, **145**: 243-249.
- Vidali M. (2001). Bioremediation: an overview. *Pure Appl Chem*, **73**: 1163–1172.
- Wang Y.-T. and Shen, H. (1995). Bacterial reduction of hexavalent chromium. *J Ind Microbiol* **14**: 159–163.
- Welch E. B. (1980): Ecological effect of wastewater press syndicate of the University of Cambridge 377pp.
- Weng C.H. and Y.F. Pan, (2007). Adsorption of a Cationic dye (Methylene Blue) onto spent activated clay. *J. Hazard Materials*. **144**: 355-362.
- Wiegant W., Kalker, T. J., Sontakke, V. and Zwaag, R. (1999). Full scale experience with tannery water management: An integrated approach. *Wat. Sci. Tech.* **39** (5): 169-176.
- Wiesmann U., Choi, I. S. and Evamaria, D. (2007). Fundamentals of biological wastewater treatment. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Romania.
- World Bank (1998). Pollution Prevention and Abatement: Tanning and Leather Finishing. Draft Technical Background Document. Washington, D. C.
- Wu H. and Liu L. (1989). Comparison of the performance of polyferric sulphate and other inorganic flocculants. *Technol. of Water Treatment* **15**: 290 - 294.
- Xiong Y., Strunk, P., Xia, H., Zhu, X., Karlsson, H., (2000). Treatment of dye wastewater containing acidic orange II using a cell with three-phase three dimensional electrodes, *Water Res.*, **35** (17): 4226-4230.

- Yajun S. (2006). Treatment of tannery waste water by nano -TiO₂ photocatalytic oxidation. *Huagong Huanbao*, **26** (1): 13-1.
- Yee C. J., HSU Y. and Shieh W. K. (1992). Effects of microcarrierpore characteristics on methanogenic fluidized bed performance. *Water research*. **26** (8): 119-1125.
- Yoochatchaval W., Ohashi, A., Harada, H., Yamaguchi, T. and Syutsubo, K. (2008). Characteristics of granular sludge in an EGSB reactor for treating low strength wastewater. *Int. J. Environ. Res.*, **2** (4): 319-328.
- Zaidi A, Khan M. S, Wani P. A, Ahemad M. (2009). Bioremediation of heavy metals by plant growth promoting rhizobacteria. In: *Microbes in sustainable agriculture*, Khan MS, Zaidi A, Musarrat J, (eds.) Nova Science Publishers, New York, USA, p. 55-90.
- Zakaria Z. A., Z. Zakaria, S. Surif and W.A. Ahmad (2007). Hexavalent chromium reduction by *Acinetobacter haemolyticus* isolated from heavy-metal contaminated wastewater. *J. Hazard. Materials* **146** (1-2): 30-38.
- Zarraa M. A., (1995). A study on the removal of Cr (VI) from waste solutions by adsorption on to sawdust in stirred vessels, *Adsorption Science and Technology*. **12** (2) : 129-138.
- Zhi X., Qingzhi, F. and Weilei, Z. (2009). Research on orthogonal coagulated setting and coagulation-flotation test of tannery wastewater. *J. Environ. Sci. Supp.* S158-S161.
- Zouboulis A. I., Moussas P. A., Vasilakou F., (2007). Polyferric Sulphate: Preparation, characterization and application in coagulation experiments, submitted for publication in *Journal of Hazardous Materials*.
- Zouboulis A. I. N. D. Tzoupanos and P. A. Moussas (2007). Inorganic pre-polymerized coagulants: current status and Future trends. *Proc. of the 3rd IASME/WSEAS Int. conf. on Energy. Environment, Ecosustems and Sustainable Development*, Agios Nikolaos, Greece, July **24-26**: 292-300.
- Zupancic G. and Jemec, A. (2010). Anaerobic digestion of tannery waste: Semi-continuous and anaerobic sequencing batch reactor processes, *Bioresource Technol.*, **101**: 26- 33.

The background of the page features a soft-focus illustration of several white calla lilies with yellow centers, and several bright green, pom-pom-like flowers. The scene is set against a light green background that transitions into a white sky at the top. The overall aesthetic is clean and fresh.

Appendix

Appendix 1.1: Modified schematic diagram indicating type of pollutants during the tanning process.



Source: (UNEP 1994 and UNIDO, 2005).

Appendix 1.2: Represents a breakdown on this solid waste and its key characteristics with comments

Type of Solid Wastes	Rate of Generation	Characteristics of Solid Waste	Comments
Dusted Salt	0.1 kg/skin	Contains around 120 gm/kg of moisture, 120 gm/kg of volatile matter, and 450 gm/kg of salt.	Contaminated with blood, hair, dirt and bacteria. Partly reused in curing and the rest is indiscriminately dumped in undeveloped lands near the tanneries.
Raw Trimmings	0.024 kg/skin	Proteins	The skins are trimmed (especially at legs, belly, neck, and tail parts) in order to give them a smooth shape. The trimmings are usually sold to soap and poultry feed production
Fleshings	0.25 kg/skin	Contains around 240 gm/kg of proteins, 200 gm/kg of fats, 3 gm/kg of sulphide.	This is the flesh material of limed skins. It is usually sold to soap and poultry feed makers.
Wet Trimming or Wet shaving	0.14 kg/skin	Contains around 240 gm/kg of proteins, 30 gm/kg of fats, 15 gm/kg of chromium oxide	After chrome tanning, skins or split hides are shaved to proper thickness. This operation produces solid waste containing chrome. Secondary users including poultry feed makers; usually collect these shaving from the tanners.
Dry Trimmings or Dry Shaving Buffing Dust	0.06kg/skins	Contains around 300 gm/kg of proteins, 130 gm/kg of fats, 30 gm/kg of chromium oxide.	Secondary users, including poultry feed makers, collect cuttings and dry trimmings and buffing dust of the leather from the tanneries.
Assorted Refuse	No consistent quantity.	Primarily cartons, bags, drums, etc.	This is normally sold separately (in bulk) in the retail market.

Source: ETPI, (1998). Survey

Appendix 1.3: Pollution Levels in Leather Industrial Effluents

Parameters	Raw effluents	Standard value NEQS in BDS-ISW*
pH	3.5-13.5	6-9
TSS (mg/L)	820-7000	500
TDS(mg/L)	2000-51000	2100
BOD ₅ (mg/L)	714-14827	250
COD(mg/L)	2000-43000	450
Turbidity (FTU)	204-800	-
SO ₄ (mg/L)	800-6480	1000
Cl ⁻ (mg/L)	1260-6000	600
Cr(mg/L)	5-1675	2(total Cr)

The quantity of raw sheep & goat skin-finished materials is 12000 kg/day then volume of wastewater is 600m³/day. Another think, the quantity of raw cow hides - finished materials is 55000kg/day then volume of wastewater is 814m³/day and wetblue sheep and goatskin-finished materials quantity would be 10000kg/day, then wastewater volume is to be 110m³/day.

Source: Jeved *et al.*, (2005); ETPI survey, ECR – 97: National Environmental quality Standards in Bangladesh for inland surface water.

Appendix 1.4: Common health hazards caused due to metals released from leather industry

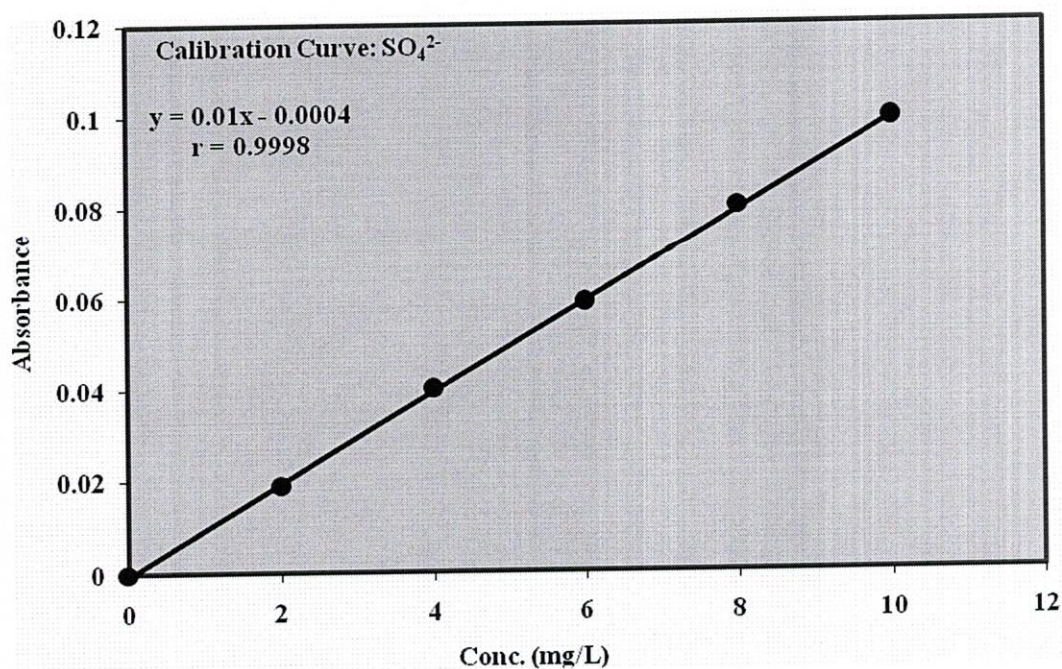
Metals	Health hazards
Cadmium	Inhibits functioning of enzymes, affects gastro intestinal tract, lungs and bones, causes renal problems
Chromium	Carcinogenic, leads to kidney disorders, ulcer, nervous disorder
Lead	Anemia, abdominal pain, damage to nerves, convulsion, hypertension human carcinogen, immune system attacks its own cells, joint diseases and ailment of the kidneys, circulatory system and neurons
Arsenic	High levels of arsenic in groundwater can cause serious human health problems if imbibed for a long time (from 5 to 15 years); including skin ailments, damage to internal organs, Liver damage ulcers, kidney problems, dermatological disorders, a skin and lung cancers and eventual death.
Copper	Mental stress, coma, uremia, anemia, liver and kidney damage; stomach and intestinal irritation.
Zinc	Kidney problems, pain in legs, vomiting, zinc fumes have corrosive effect on skin, causes damage to nervous membrane.
Nickel	Decreases body weight, damages heart and liver, causes skin irritation
Sodium	Salinity increase, heart disease and high blood pressure.
Calcium	Scale formation
Magnesium	Magnesium may cause vomiting and diarrhoea. High doses of magnesium in medicine and food supplements may cause muscle slackening, nerve problems, depressions and personality changes
Cobalt	High concentrations of cobalt are : vomiting and nausea, vision problems, heart problems and thyroid damage
Manganese	Inhalation or contact causes damage to central nervous system.

Appendix 2.1: Manufacturing Unit Wise wastewater or effluents generated by a Leather Tanning Industry

Operational stage in tanning Industry	Discharged effluents (m ³ /t raw-hide)
Soaking	7-9
Liming	9-15
De-liming and Bating	7-11
Pickling and tanning	3-5
Post-tanning	7-13
Finishing	1-3

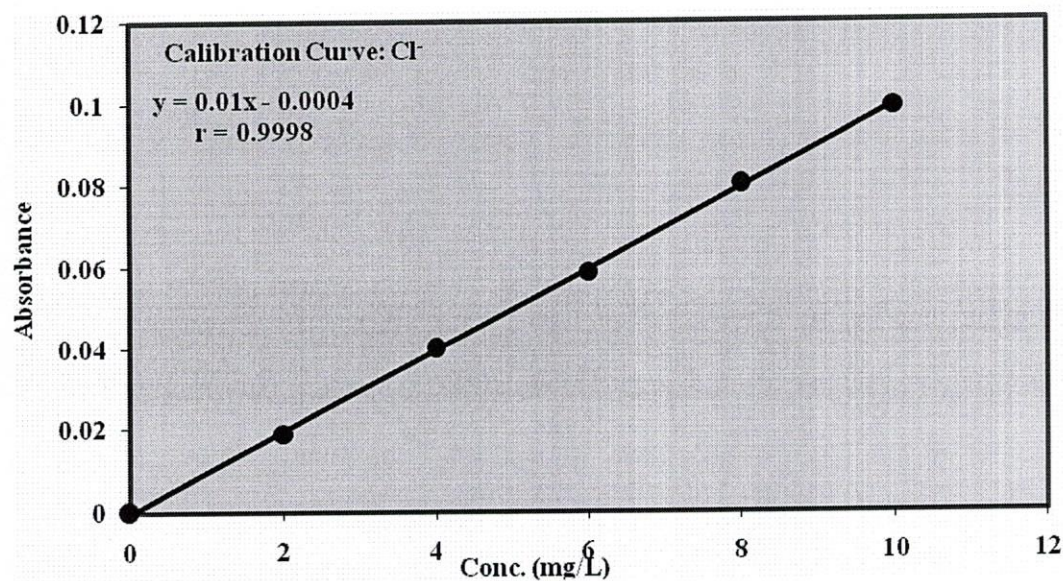
Source: World Bank, (1998).

Appendix 2.2:

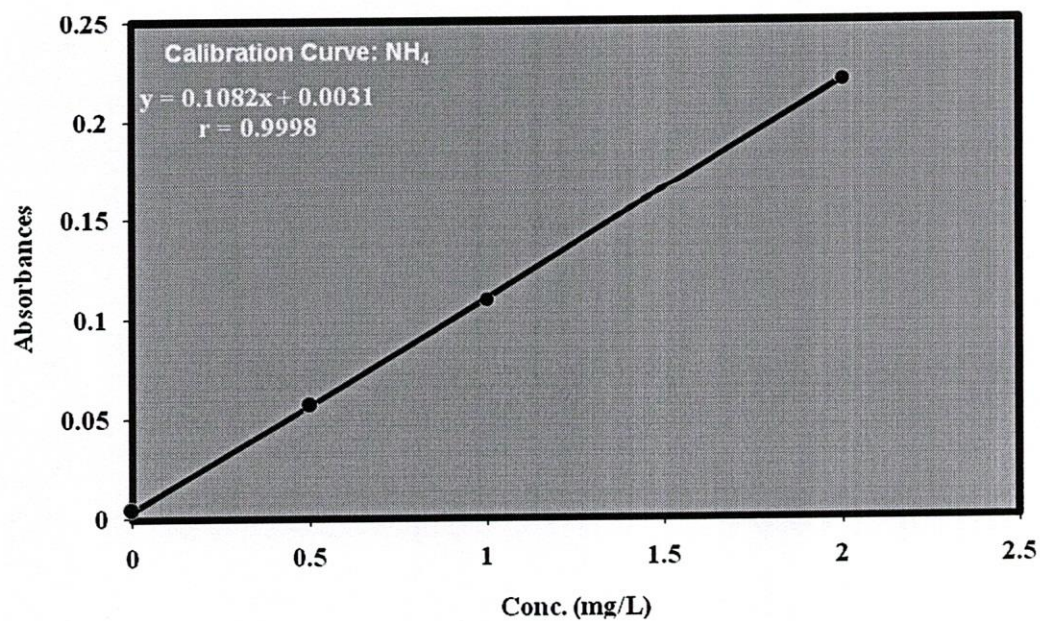


Calibration curve of SO_4^{2-}

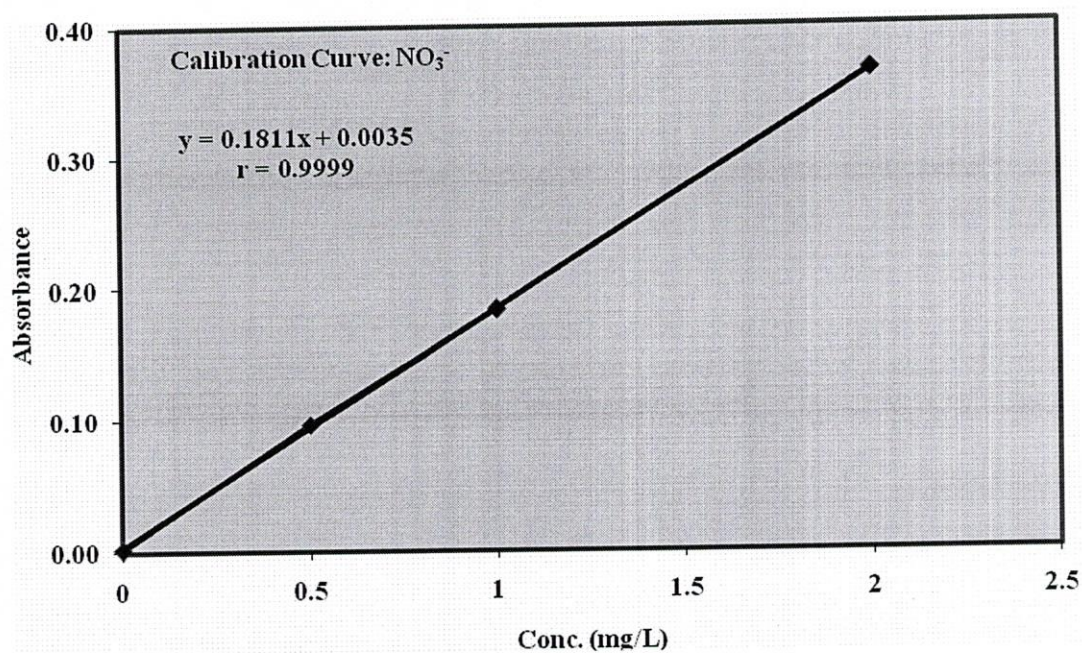
Appendix 2.3:

Calibration curve of Cl^-

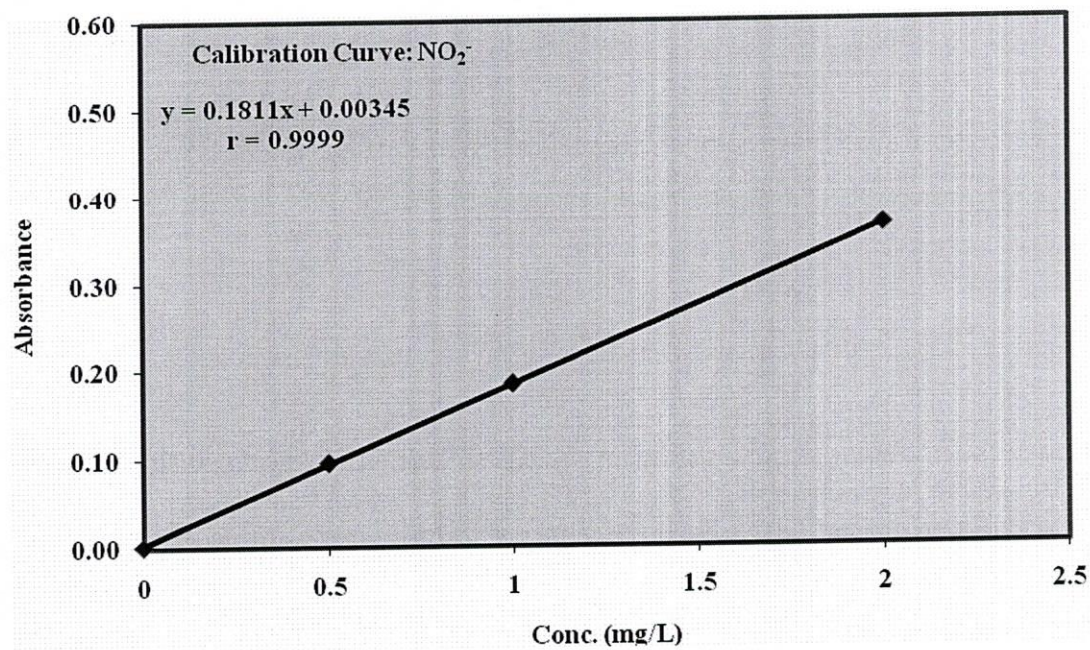
Appendix 2.4:

Calibration curve of NH_4^+

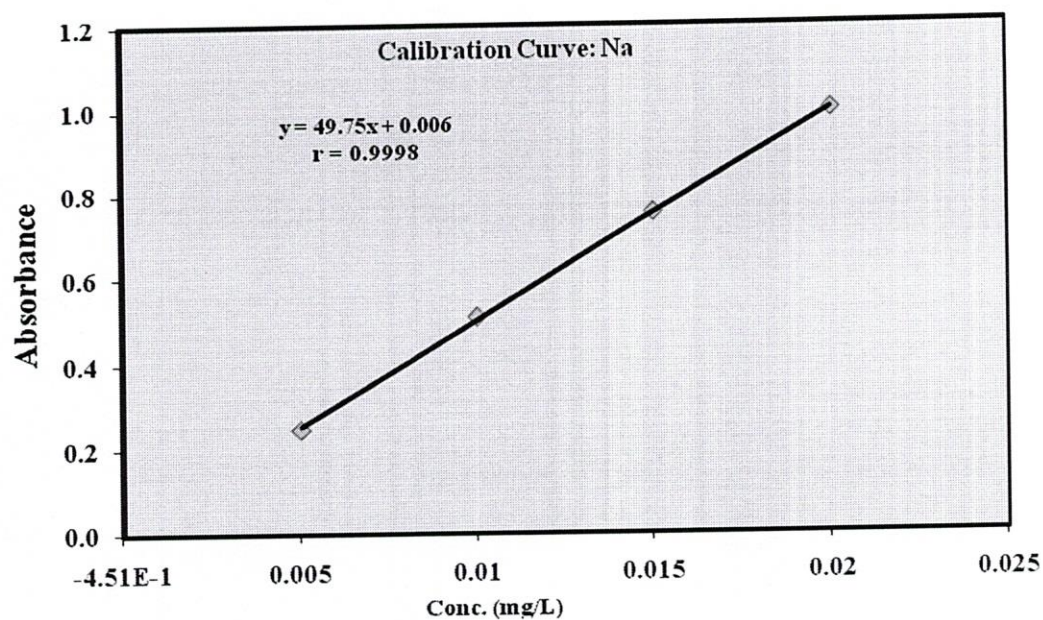
Appendix 2.5:

Calibration curve of NO_3^-

Appendix 2.6:

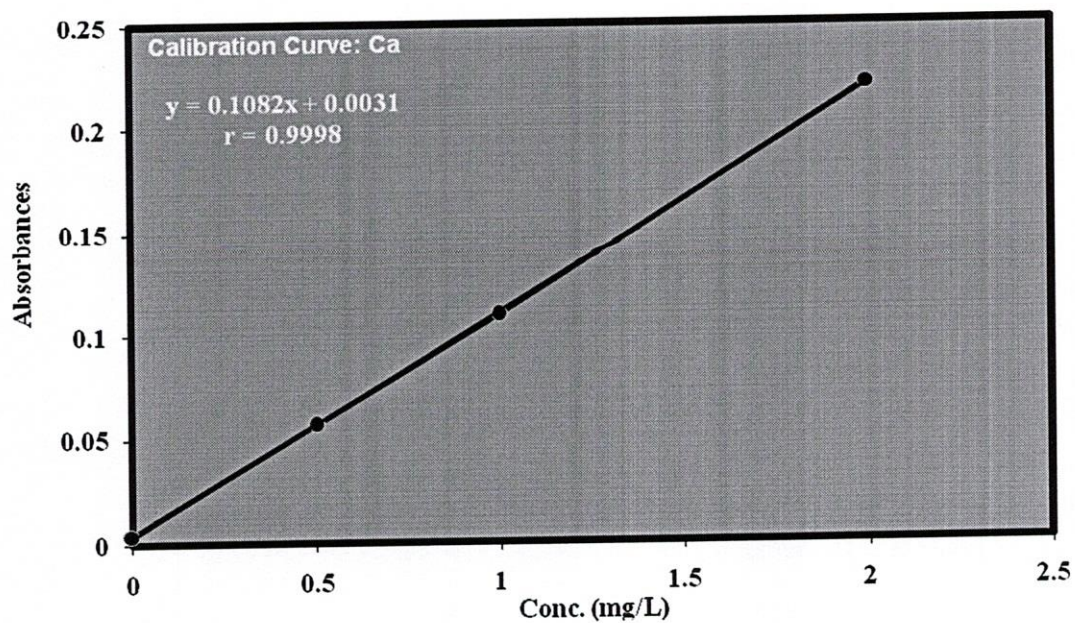
Calibration curve of NO_2^-

Appendix 2.7:



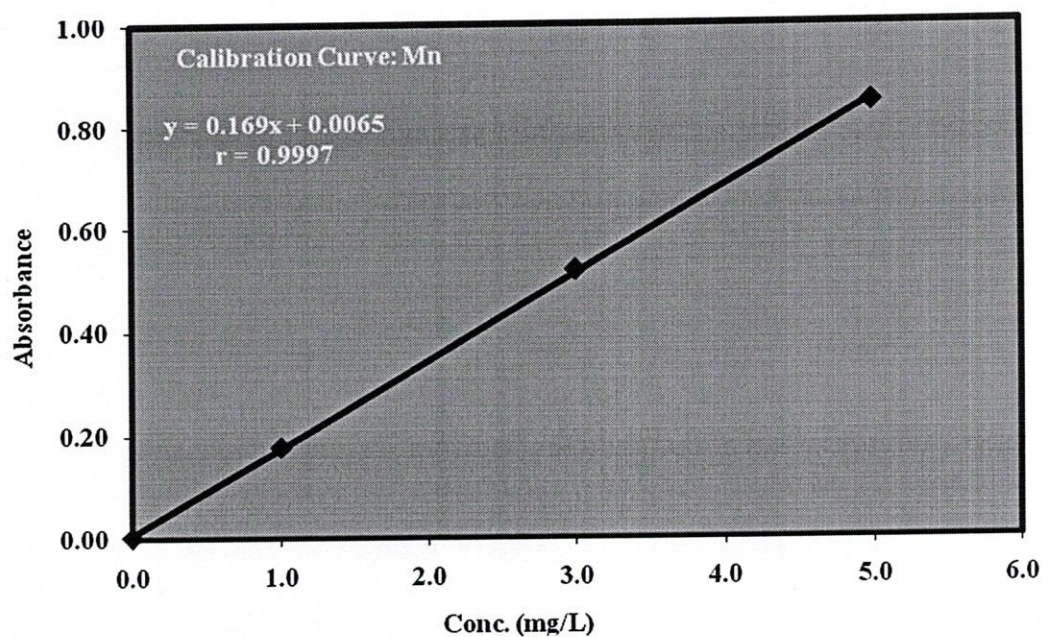
Calibration curve of Na

Appendix 2.8:



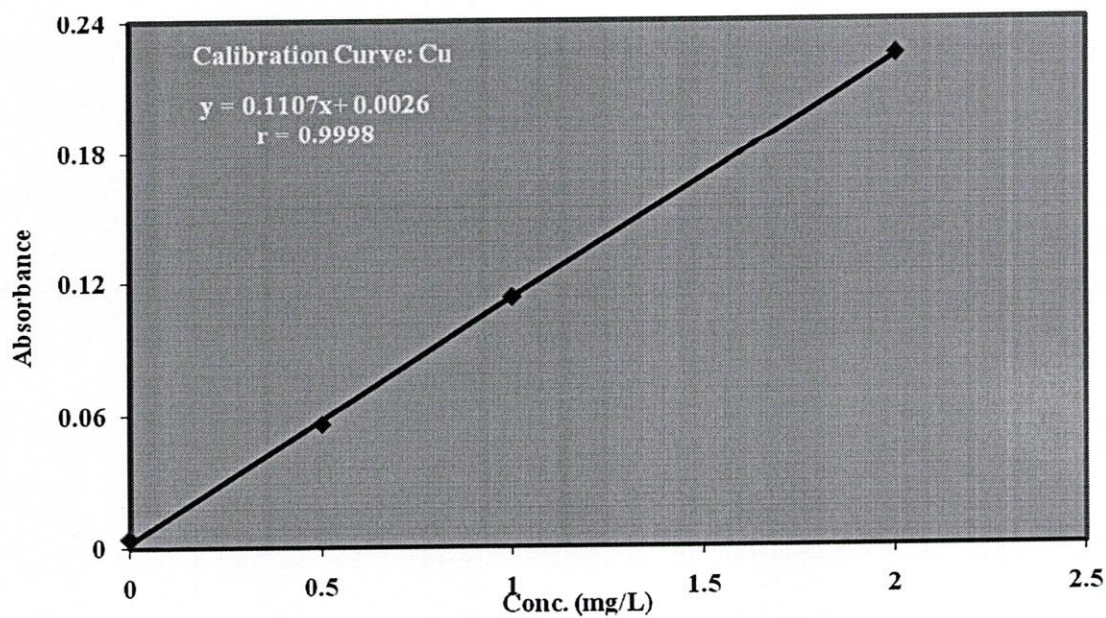
Calibration curve of Ca

Appendix 2.9:



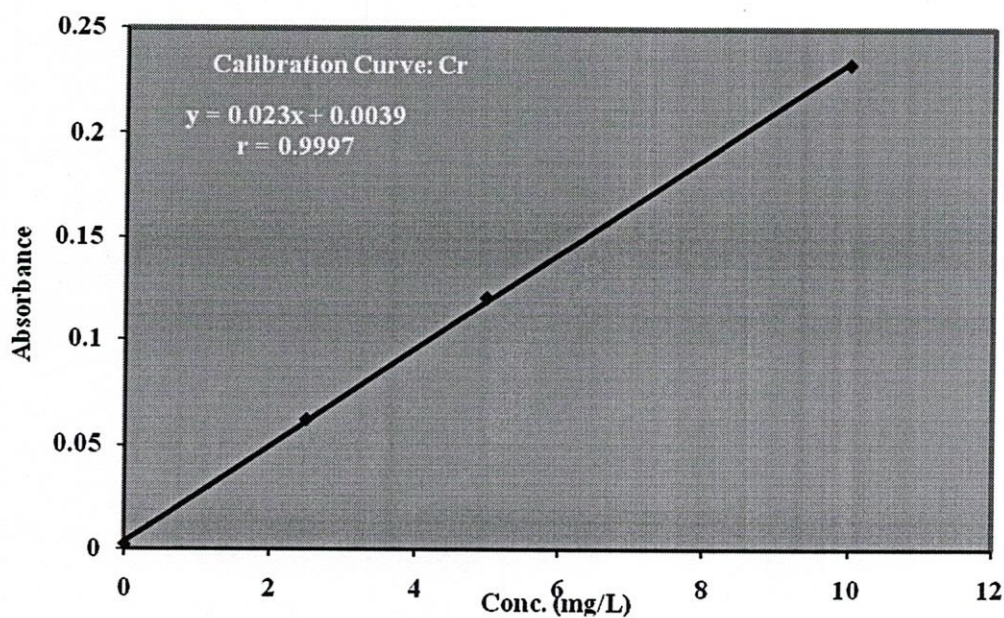
Calibration curve of Mn

Appendix 2.10:



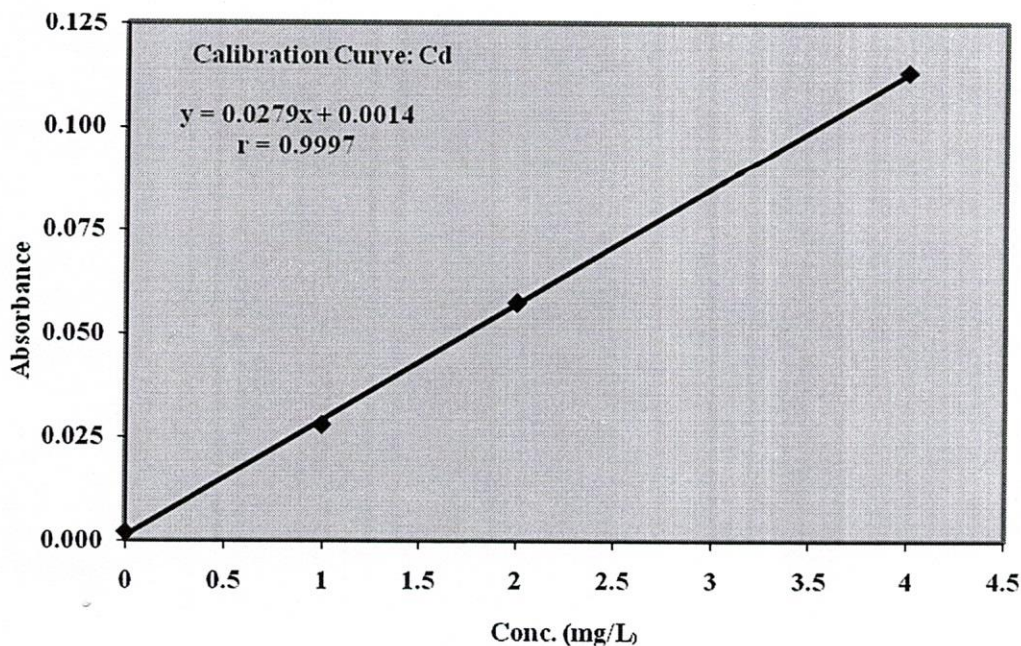
Calibration curve of Cu

Appendix 2.11:



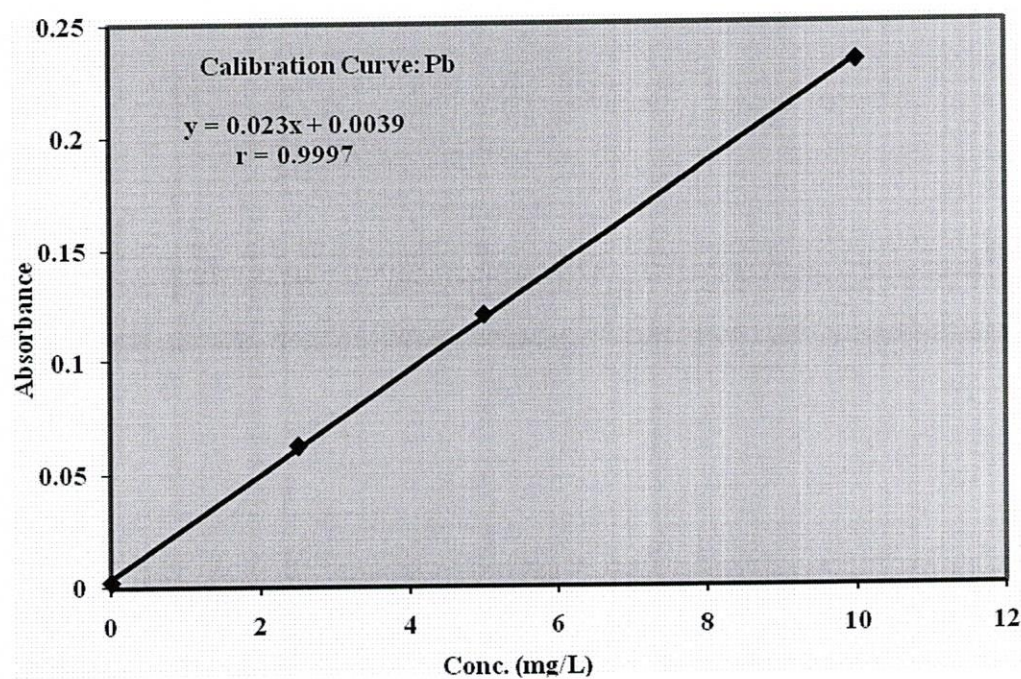
Calibration curve of Cr

Appendix 2.12:



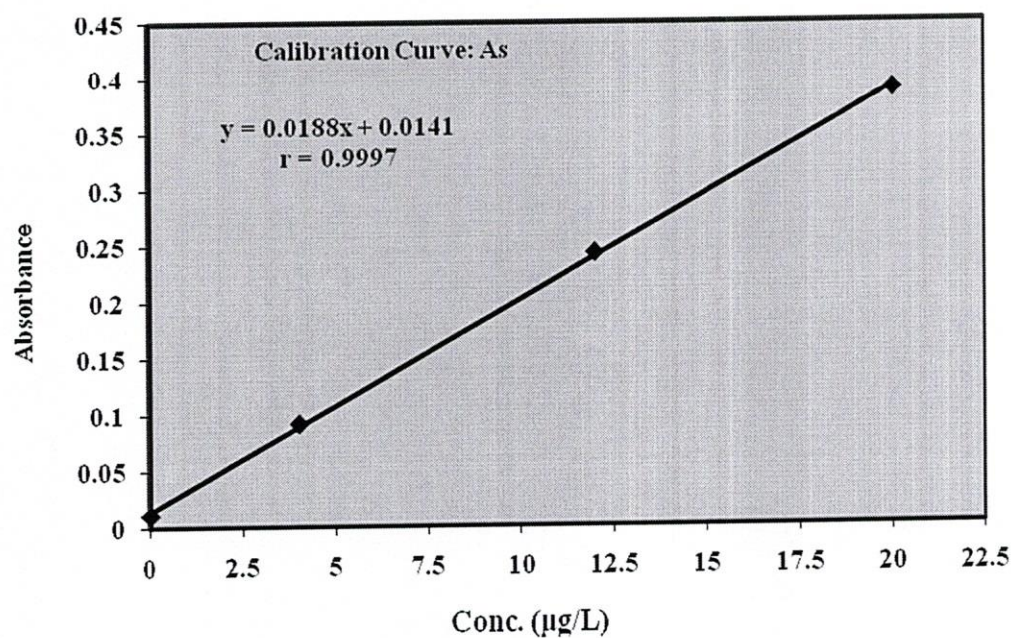
Calibration curve of Cd

Appendix 2.13:



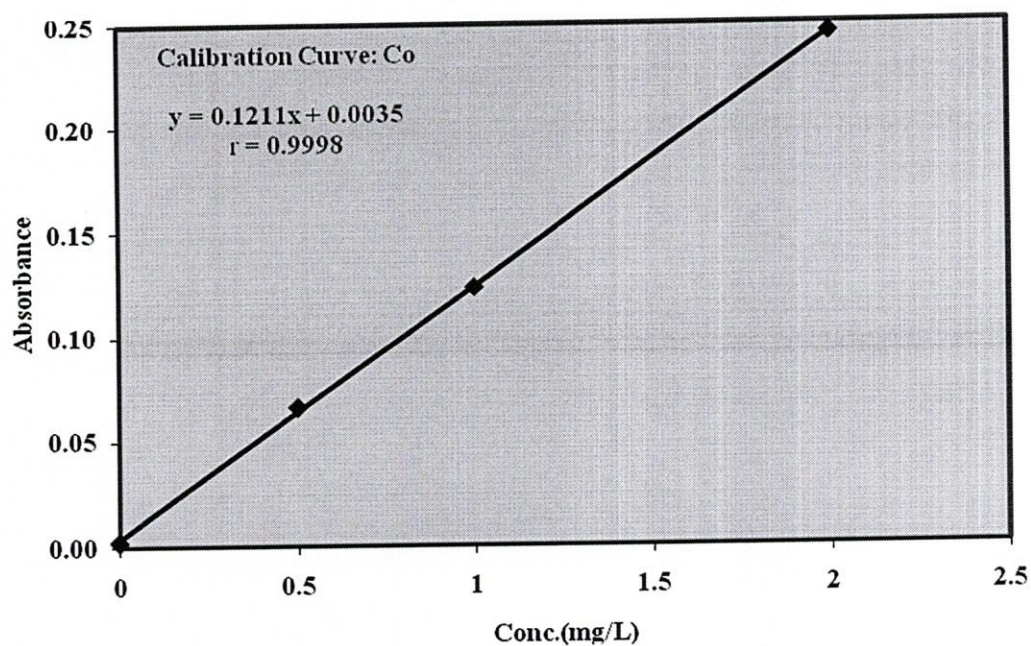
Calibration curve of Pb

Appendix 2.14:



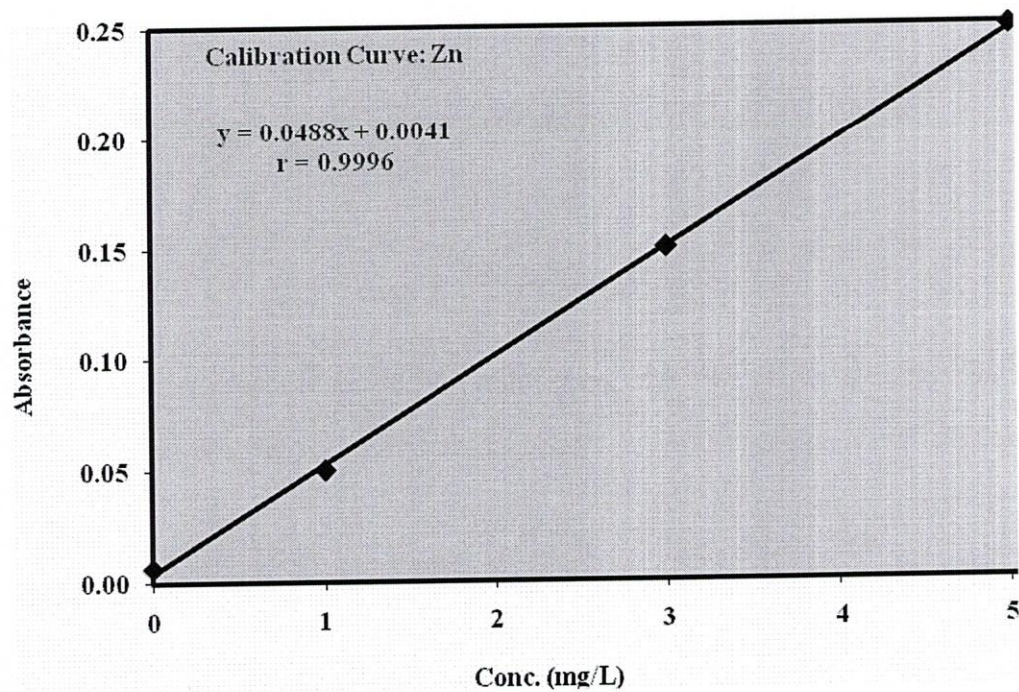
Calibration curve of As

Appendix 2.15:



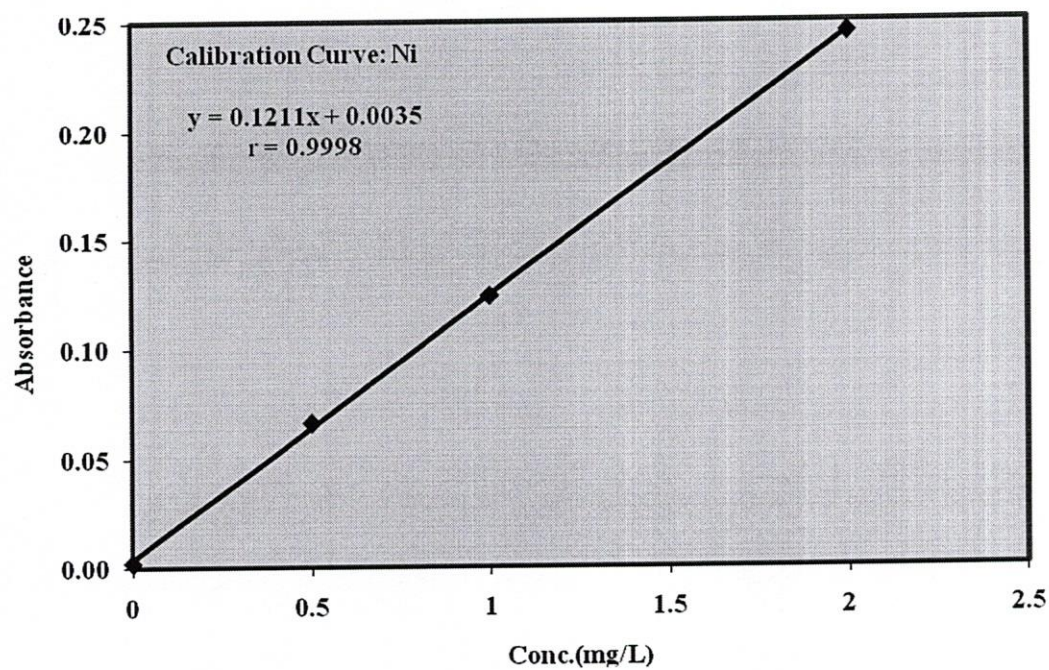
Calibration curve of Co

Appendix 2.16:



Calibration curve of Zn

Appendix 2.17:



Calibration curve of Ni

Appendix 4.1:

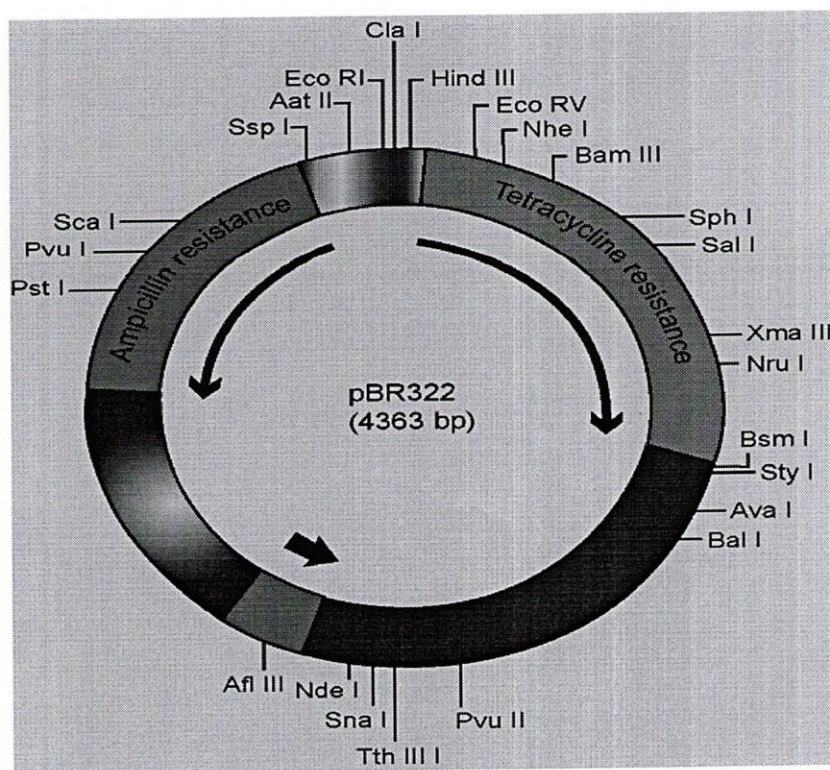


Figure (4.1): A typical structure of plasmid pBR322

Appendix 4.2: Lineage Report of Bacterial DNA Sequencing

```

Bacteria [bacteria]
. Enterobacteriaceae [enterobacteria]
. . Enterobacter [enterobacteria]
. . . Enterobacter sp. NCCP-287 ----- 1368 1 hit
[enterobacteria] Enterobacter sp. NCCP-287 gene for 16S rRNA,
partial sequen
. . . Enterobacter sp. NCCP-280 ..... 1368 1 hit
[enterobacteria] Enterobacter sp. NCCP-280 gene for 16S rRNA,
partial sequen
. . . Enterobacter cloacae ..... 1368 13 hits
[enterobacteria] Enterobacter cloacae strain FR 16S ribosomal RNA
gene, part
. . . Enterobacter sp. EMB19 ..... 1368 1 hit
[enterobacteria] Enterobacter sp. EMB19 16S ribosomal RNA gene,
partial sequ
. . . Enterobacter cloacae subsp. dissolvens ..... 1364 7 hits
[enterobacteria] Enterobacter dissolvens strain AGYP1 16S ribosomal
RNA gene
. . . Enterobacter sp. enrichment culture clone HSL19 .. 1364 1 hit
[enterobacteria] Enterobacter sp. enrichment culture clone HSL19 16S
ribosom
. . . Enterobacter sp. enrichment culture clone HSL10 .. 1364 1 hit
[enterobacteria] Enterobacter sp. enrichment culture clone HSL10 16S
ribosom
. . . Enterobacter sp. I5.TYA ..... 1362 1 hit
[enterobacteria] Enterobacter sp. I5.TYA 16S ribosomal RNA gene,
partial seq
. . . Enterobacter sp. SB2-GLA ..... 1362 1 hit
[enterobacteria] Enterobacter sp. SB2-GLA partial 16S rRNA gene,
strain SB2-
. . . Enterobacter sp. A732 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. A732 16S ribosomal RNA gene,
partial sequ
. . . Enterobacter sp. A715 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. A715 16S ribosomal RNA gene,
partial sequ
. . . Enterobacter sp. NCCP-293 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. NCCP-293 gene for 16S rRNA,
partial sequen
. . . Enterobacter sp. NCCP-289 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. NCCP-289 gene for 16S rRNA,
partial sequen
. . . Enterobacter sp. NCCP-285 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. NCCP-285 gene for 16S rRNA,
partial sequen
. . . Enterobacter sp. NCCP-282 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. NCCP-282 gene for 16S rRNA,
partial sequen
. . . Enterobacter sp. NCCP-281 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. NCCP-281 gene for 16S rRNA,
partial sequen
. . . Enterobacter sp. NCCP-267 ..... 1362 1 hit
[enterobacteria] Enterobacter sp. NCCP-267 gene for 16S rRNA,
partial sequen

```


. . . Enterobacter sp. 61(2011) 1362 1 hit
[enterobacteria] Enterobacter sp. 61(2011) 16S ribosomal RNA gene,
partial s

. . . uncultured Enterobacter sp. 1362 9 hits
[enterobacteria] Uncultured Enterobacter sp. clone SL22 16S
ribosomal RNA ge

. . . Enterobacter sp. enrichment culture clone HSL90B . 1362 1 hit
[enterobacteria] Enterobacter sp. enrichment culture clone HSL90B
16S riboso

. . . Enterobacter sp. enrichment culture clone HSL90A . 1362 1 hit
[enterobacteria] Enterobacter sp. enrichment culture clone HSL90A
16S riboso

. . . Enterobacter sp. enrichment culture clone HSL81A . 1362 1 hit
[enterobacteria] Enterobacter sp. enrichment culture clone HSL81A
16S riboso

. . . Enterobacter sp. DQ11(2010) 1362 1 hit
[enterobacteria] Enterobacter sp. DQ11(2010) 16S ribosomal RNA gene,
partial

. . . Enterobacter sp. IBP-VN4 1362 1 hit
[enterobacteria] Enterobacter sp. IBP-VN4 16S ribosomal RNA gene,
partial se

. . . Enterobacter sp. IBP-VN1 1362 1 hit
[enterobacteria] Enterobacter sp. IBP-VN1 16S ribosomal RNA gene,
partial se

. . . Enterobacter sp. DIC9JA 1362 1 hit
[enterobacteria] Enterobacter sp. DIC9JA 16S ribosomal RNA gene,
partial seq

. . . Enterobacter sp. il 1362 1 hit
[enterobacteria] Enterobacter sp. il 16S ribosomal RNA gene, partial
sequence

. . . Enterobacter sp. Fw17a 1362 1 hit
[enterobacteria] Enterobacter sp. Fw17a 16S ribosomal RNA gene,
partial sequ

. . . Enterobacter sp. BSRA2 1362 1 hit
[enterobacteria] Enterobacter sp. BSRA2 16S ribosomal RNA gene,
partial sequ

. . . Enterobacter sp. IPPW-27 1362 1 hit
[enterobacteria] Enterobacter sp. IPPW-27 16S ribosomal RNA gene,
partial se

. . . Enterobacter sp. VTT E-073086 1362 1 hit
[enterobacteria] Enterobacter sp. VTT E-073086 16S ribosomal RNA
gene, parti

. . . Enterobacter ludwigii 1362 1 hit
[enterobacteria] Enterobacter ludwigii strain CaR 16S ribosomal RNA
gene, pa

. . . Enterobacter sp. GJ1-11 1362 1 hit
[enterobacteria] Enterobacter sp. GJ1-11 16S ribosomal RNA gene,
partial seq

. . . Enterobacter sp. sa 1362 1 hit
[enterobacteria] Enterobacter sp. sa 16S ribosomal RNA gene, partial
sequence

. . Salmonella sp. enrichment culture clone CL107 ----- 1368 1 hit
[enterobacteria] Salmonella sp. enrichment culture clone CL107 16S
ribosomal

. . Pantoea sp. T55 1362 1 hit
[enterobacteria] Pantoea sp. T55 16S ribosomal RNA gene, partial
sequence

. . Pantoea sp. PYPB15 1362 1 hit
[enterobacteria] Uncultured Enterobacter sp. clone SL22 16S
ribosomal RNA ge

. . Pantoea sp. XJ3 1362 1 hit
 [enterobacteria] Pantoea sp. XJ3 16S ribosomal RNA gene, partial sequence
 . . Pantoea sp. enrichment culture clone S28b 1362 1 hit
 [enterobacteria] Pantoea sp. enrichment culture clone S28b 16S ribosomal RNA
 . . Pantoea agglomerans 1362 15 hits
 [enterobacteria] Pantoea agglomerans strain 3I2 16S ribosomal RNA gene, part
 . . Enterobacteriaceae bacterium M406 1362 1 hit
 [enterobacteria] Enterobacteriaceae bacterium M406 gene for 16S rRNA, partia
 . . Enterobacteriaceae bacterium M405 1362 1 hit
 [enterobacteria] Enterobacteriaceae bacterium M405 gene for 16S rRNA, partia
 . . Pantoea sp. NJ-32 1362 1 hit
 [enterobacteria] Pantoea sp. NJ-32 16S rRNA gene, strain NJ-32
 . . Pantoea sp. J1-17-2a 1362 1 hit
 [enterobacteria] Pantoea sp. J1-17-2a 16S ribosomal RNA gene, partial sequen
 . . Pantoea sp. DAP17 1362 1 hit
 [enterobacteria] Pantoea sp. DAP17 16S ribosomal RNA gene, partial sequence
 . . Enterobacteriaceae bacterium ZHS050721 1362 1 hit
 [enterobacteria] Enterobacteriaceae bacterium ZHS050721 16S ribosomal RNA ge
 . bacterium VNS3-1-2 ----- 1364 1 hit
 [bacteria] Bacterium VNS3-1-2 16S ribosomal RNA gene, partial sequence
 . uncultured bacterium 1362 11 hits
 [bacteria] Uncultured bacterium clone 4-31015 16S ribosomal RNA gene,
 . activated sludge bacterium GC R2A S 14 1362 1 hit
 [bacteria] Activated sludge bacterium GC R2A S 14 16S ribosomal RNA ge
 . endophytic bacterium S02 1362 1 hit
 [bacteria] Endophytic bacterium S02 16S ribosomal RNA gene, partial se
 . bacterium G33-1 1362 1 hit
 [bacteria] Bacterium G33-1 16S ribosomal RNA gene, partial sequence
 . bacterium RHA-3-18 1362 1 hit
 [bacteria] Bacterium RHA-3-18 16S ribosomal RNA gene, partial sequence
 . bacterium SFS2-2-1 1355 1 hit
 [bacteria] Bacterium SFS2-2-1 16S ribosomal RNA gene, partial sequence

Appendix 4.3:

Bacterial Organism Report

Enterobacter sp. NCCP-287 [enterobacteria] taxid 1050385
 gi|338858342|dbj|AB641896.1| Enterobacter sp. NCCP-287 gen...
 1368 0.0

Enterobacter sp. NCCP-280 [enterobacteria] taxid 1050378
 gi|338858335|dbj|AB641889.1| Enterobacter sp. NCCP-280 gen...
 1368 0.0

<u>Enterobacter cloacae</u> [enterobacteria] taxid 550				
<u>gi 334852731 gb JF894166.1 </u>	Enterobacter	cloacae	strain	FR...
1368 0.0				
<u>gi 329665845 gb JF489149.1 </u>	Enterobacter	cloacae	strain	SF...
1368 0.0				
<u>gi 209361530 gb FJ194527.1 </u>	Enterobacter	cloacae	strain	EB...
1368 0.0				
<u>gi 339959025 gb JF513137.1 </u>	Enterobacter	cloacae	strain	S1...
1362 0.0				
<u>gi 307816515 gb HQ154578.1 </u>	Enterobacter	cloacae	strain	R1...
1362 0.0				
<u>gi 307816489 gb HQ154552.1 </u>	Enterobacter	cloacae	strain	R2...
1362 0.0				
<u>gi 299562114 gb HM131220.1 </u>	Enterobacter	cloacae	strain	MS...
1362 0.0				
<u>gi 253508763 gb FJ608260.1 </u>	Enterobacter	cloacae	strain	F2...
1362 0.0				
<u>gi 241897664 gb FJ976579.1 </u>	Enterobacter	cloacae	strain	LC...
1362 0.0				
<u>gi 195547657 gb EU849019.1 </u>	Enterobacter	cloacae	strain	FR...
1362 0.0				
<u>gi 155382459 gb EU048321.1 </u>	Enterobacter	cloacae	strain	CM...
1362 0.0				
<u>gi 146742373 gb EF551364.1 </u>	Enterobacter	cloacae	strain	Rs...
1362 0.0				
<u>gi 131054073 gb EF446901.1 </u>	Enterobacter	cloacae	strain	GW...
1362 0.0				
<u>Enterobacter sp. EMB19</u> [enterobacteria] taxid 998095				
<u>gi 326375384 gb JF281095.1 </u>	Enterobacter	sp.	EMB19	16S rib...
1368 0.0				
<u>Salmonella sp. enrichment culture clone CL107</u> [enterobacteria]				
taxid 946044				
<u>gi 319433526 gb HQ686272.1 </u>	Salmonella	sp.	enrichment	cult...
1368 0.0				
<u>Enterobacter cloacae subsp. dissolvens</u> [enterobacteria] taxid 69219				
<u>gi 326885703 gb HQ336043.1 </u>	Enterobacter	dissolvens	strain...	
1364 0.0				
<u>gi 343206386 ref NR_044978.1 </u>	Enterobacter	cloacae	subsp.	...
1364 0.0				
<u>gi 2584748 emb Z96079.1 </u>	Enterobacter	dissolvens	LMG 2683	...
1364 0.0				
<u>gi 333952663 gb JF797605.1 </u>	Enterobacter	cloacae	subsp.	di...
1362 0.0				
<u>gi 319997288 gb HQ651841.1 </u>	Enterobacter	cloacae	subsp.	di...
1362 0.0				
<u>gi 310780838 gb HQ434623.1 </u>	Enterobacter	cloacae	subsp.	di...
1362 0.0				
<u>gi 116241164 gb DQ988523.1 </u>	Enterobacter	cloacae	subsp.	di...
1362 0.0				
<u>Enterobacter sp. enrichment culture clone HSL19</u> [enterobacteria]				
taxid 887605				
<u>gi 307713820 gb HM461143.1 </u>	Enterobacter	sp.	enrichment	cu...
1364 0.0				

Enterobacter sp. enrichment culture clone HSL10 [enterobacteria]
taxid 887598
gi|307713810|gb|HM461133.1| Enterobacter sp. enrichment cu...
1364 0.0

bacterium VNS3-1-2 [bacteria] taxid 478499
gi|158514398|gb|EU195905.1| Bacterium VNS3-1-2 16S ribosom...
1364 0.0

Pantoea sp. T55 [enterobacteria] taxid 1048428
gi|342209918|gb|JN029950.1| Pantoea sp. T55 16S ribosomal ...
1362 0.0

Enterobacter sp. I5.TYA [enterobacteria] taxid 1054065
gi|340343756|gb|JN089363.1| Enterobacter sp. I5.TYA 16S ri...
1362 0.0

Enterobacter sp. SB2-GLA [enterobacteria] taxid 1054552
gi|339895672|emb|HE572588.1| Enterobacter sp. SB2-GLA part...
1362 0.0

Enterobacter sp. A732 [enterobacteria] taxid 1048699
gi|339283268|gb|JF946808.1| Enterobacter sp. A732 16S ribo...
1362 0.0

Enterobacter sp. A715 [enterobacteria] taxid 1048698
gi|339283263|gb|JF946803.1| Enterobacter sp. A715 16S ribo...
1362 0.0

uncultured bacterium [bacteria] taxid 77133
gi|339282818|gb|JN033080.1| Uncultured bacterium clone 4-3...
1362 0.0
gi|339282770|gb|JN033032.1| Uncultured bacterium clone 3-4...
1362 0.0
gi|339282768|gb|JN033030.1| Uncultured bacterium clone 3-4...
1362 0.0
gi|339282763|gb|JN033025.1| Uncultured bacterium clone 3-4...
1362 0.0
gi|322187087|gb|JF201682.1| Uncultured bacterium clone ncd...
1362 0.0
gi|327342283|gb|JF417768.1| Uncultured bacterium clone QQS...
1362 0.0
gi|253770944|gb|GQ264478.1| Uncultured bacterium clone WW3...
1362 0.0
gi|196475524|gb|EU915224.1| Uncultured bacterium clone 103...
1362 0.0
gi|72067413|emb|AJ852327.1| Uncultured bacterium partial 1...
1362 0.0
gi|72067408|emb|AJ852322.1| Uncultured bacterium partial 1...
1362 0.0
gi|72067393|emb|AJ852309.1| Uncultured bacterium partial 1...
1362 0.0

Enterobacter sp. NCCP-293 [enterobacteria] taxid 1050390
gi|338858347|dbj|AB641901.1| Enterobacter sp. NCCP-293 gen...
1362 0.0

Enterobacter sp. NCCP-289 [enterobacteria] taxid 1050387

gi|338858344|dbj|AB641898.1| Enterobacter sp. NCCP-289 gen...
1362 0.0

Enterobacter sp. NCCP-285 [enterobacteria] taxid 1050383
gi|338858340|dbj|AB641894.1| Enterobacter sp. NCCP-285 gen...
1362 0.0

Enterobacter sp. NCCP-282 [enterobacteria] taxid 1050380
gi|338858337|dbj|AB641891.1| Enterobacter sp. NCCP-282 gen...
1362 0.0

Enterobacter sp. NCCP-281 [enterobacteria] taxid 1050379
gi|338858336|dbj|AB641890.1| Enterobacter sp. NCCP-281 gen...
1362 0.0

Enterobacter sp. NCCP-267 [enterobacteria] taxid 1050372
gi|338858329|dbj|AB641883.1| Enterobacter sp. NCCP-267 gen...
1362 0.0

Enterobacter sp. 61(2011) [enterobacteria] taxid 980069
gi|322366712|gb|HQ880677.1| Enterobacter sp. 61(2011) 16S ...
1362 0.0

uncultured Enterobacter sp. [enterobacteria] taxid 238202
gi|310007437|gb|HQ264082.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|306450665|gb|HQ148907.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257074426|gb|GQ418145.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257074420|gb|GQ418139.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257074404|gb|GQ418123.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257074400|gb|GQ418119.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257074360|gb|GQ418079.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257072316|gb|GQ416035.1| Uncultured Enterobacter sp. cl...
1362 0.0
gi|257072212|gb|GQ415931.1| Uncultured Enterobacter sp. cl...
1362 0.0

Pantoea sp. PYPB15 [enterobacteria] taxid 988770
gi|326885698|gb|JF346898.1| Pantoea sp. PYPB15 16S ribosom...
1362 0.0

Enterobacter sp. enrichment culture clone HSL90B [enterobacteria]
taxid 887647
gi|307713897|gb|HM461220.1| Enterobacter sp. enrichment cu...
1362 0.0

Enterobacter sp. enrichment culture clone HSL90A [enterobacteria]
taxid 887646
gi|307713896|gb|HM461219.1| Enterobacter sp. enrichment cu...
1362 0.0

Enterobacter sp. enrichment culture clone HSL81A [enterobacteria]
taxid 887640

gi|307713887|gb|HM461210.1| Enterobacter sp. enrichment cu...
1362 0.0

Enterobacter sp. DQ11(2010) [enterobacteria] taxid 886502
gi|307135747|gb|GU377060.1| Enterobacter sp. DQ11(2010) 16...
1362 0.0

Enterobacter sp. IBP-VN4 [enterobacteria] taxid 867214
gi|301322443|gb|HM587331.1| Enterobacter sp. IBP-VN4 16S r...
1362 0.0

Enterobacter sp. IBP-VN1 [enterobacteria] taxid 867213
gi|301322440|gb|HM587328.1| Enterobacter sp. IBP-VN1 16S r...
1362 0.0

Enterobacter sp. DIC9JA [enterobacteria] taxid 671183
gi|257815244|gb|FJ668829.1| Enterobacter sp. DIC9JA 16S ri...
1362 0.0

Enterobacter sp. i1 [enterobacteria] taxid 691329
gi|269115997|gb|GQ478379.1| Enterobacter sp. i1 16S riboso...
1362 0.0

Pantoea sp. XJ3 [enterobacteria] taxid 690876
gi|265679080|gb|GU140078.1| Pantoea sp. XJ3 16S ribosomal ...
1362 0.0

activated sludge bacterium GC R2A S 14 [bacteria] taxid 689315
gi|263201318|gb|GU136506.1| Activated sludge bacterium GC_...
1362 0.0

Pantoea sp. enrichment culture clone S28b [enterobacteria] taxid 660050
gi|255684713|gb|GQ288411.1| Pantoea sp. enrichment culture...
1362 0.0

Enterobacter sp. Fw17a [enterobacteria] taxid 658126
gi|253741312|gb|GQ247734.1| Enterobacter sp. Fw17a 16S rib...
1362 0.0

Pantoea agglomerans [enterobacteria] taxid 549
gi|197253901|gb|EU721614.1| Pantoea agglomerans strain 3I2...
1362 0.0
gi|220967119|gb|FJ603033.1| Pantoea agglomerans strain se8...
1362 0.0
gi|220967117|gb|FJ603031.1| Pantoea agglomerans strain sd3...
1362 0.0
gi|220067266|gb|FJ546348.1| Pantoea agglomerans strain ES ...
1362 0.0
gi|162532571|gb|EU306608.1| Pantoea agglomerans strain LHL...
1362 0.0
gi|126742078|gb|EF428997.1| Pantoea agglomerans strain GIS...
1362 0.0
gi|109942132|emb|AM184266.1| Pantoea agglomerans partial 1...
1362 0.0
gi|109942130|emb|AM184264.1| Pantoea agglomerans partial 1...
1362 0.0
gi|109942129|emb|AM184263.1| Pantoea agglomerans partial 1...
1362 0.0

gi|109941960|emb|AM184212.1| Pantoea agglomerans partial 1...
1362 0.0
gi|51493565|gb|AY691544.1| Pantoea agglomerans strain ChDC...
1362 0.0
gi|32455047|gb|AY315453.1| Pantoea agglomerans strain CC-8...
1362 0.0
gi|32455046|gb|AY315452.1| Pantoea agglomerans strain CC-8...
1362 0.0
gi|32455045|gb|AY315451.1| Pantoea agglomerans strain CC-8...
1362 0.0
gi|32455044|gb|AY315450.1| Pantoea agglomerans strain CC-8...
1362 0.0

Enterobacter sp. BSRA2 [enterobacteria] taxid 638658
gi|226897923|gb|FJ868806.1| Enterobacter sp. BSRA2 16S rib...
1362 0.0

Enterobacter sp. IPPW-27 [enterobacteria] taxid 599692
gi|223953600|gb|FJ646658.1| Enterobacter sp. IPPW-27 16S r...
1362 0.0

Enterobacteriaceae bacterium M406 [enterobacteria] taxid 563412
gi|222427461|dbj|AB461749.1| Enterobacteriaceae bacterium ...
1362 0.0

Enterobacteriaceae bacterium M405 [enterobacteria] taxid 563411
gi|222427460|dbj|AB461748.1| Enterobacteriaceae bacterium ...
1362 0.0

Enterobacter sp. VTT E-073086 [enterobacteria] taxid 501739
gi|167507814|gb|EU438971.1| Enterobacter sp. VTT E-073086 ...
1362 0.0

Enterobacter ludwigii [enterobacteria] taxid 299767
gi|216263297|gb|FJ462703.1| Enterobacter ludwigii strain C...
1362 0.0

endophytic bacterium S02 [bacteria] taxid 564406
gi|207667185|gb|FJ205677.1| Endophytic bacterium S02 16S r...
1362 0.0

bacterium G33-1 [bacteria] taxid 547011
gi|194267680|gb|EU841417.1| Bacterium G33-1 16S ribosomal ...
1362 0.0

Pantoea sp. NJ-32 [enterobacteria] taxid 418636
gi|193878263|emb|AM421978.1| Pantoea sp. NJ-32 16S rRNA ge...
1362 0.0

Pantoea sp. J1-17-2a [enterobacteria] taxid 545156
gi|193508277|gb|EU816767.1| Pantoea sp. J1-17-2a 16S ribos...
1362 0.0

Pantoea sp. DAP17 [enterobacteria] taxid 490223
gi|163931342|gb|EU302842.1| Pantoea sp. DAP17 16S ribosoma...
1362 0.0

Enterobacter sp. GJ1-11 [enterobacteria] taxid 471867
gi|157420081|gb|EU139848.1| Enterobacter sp. GJ1-11 16S ri...
1362 0.0

Enterobacter sp. sa [enterobacteria] taxid 409488
gi|117582136|gb|EF030721.1| Enterobacter sp. sa 16S riboso...
1362 0.0

bacterium RHA-3-18 [bacteria] taxid 301781
gi|56122761|gb|AY822545.1| Bacterium RHA-3-18 16S ribosoma...
1362 0.0

Enterobacteriaceae bacterium ZHS050721 [enterobacteria] taxid
362301
gi|83702216|gb|DQ313392.1| Enterobacteriaceae bacterium ZH...
1362 0.0

bacterium SFS2-2-1 [bacteria] taxid 478492
gi|158514395|gb|EU195902.1| Bacterium SFS2-2-1 16S ribosom...
1355 0.0

Appendix 4.4: Bacterial Taxonomy Report

Bacteria	103 hits
53 orgs [root; cellular organisms]	
. Enterobacteriaceae	86 hits
46 orgs [Proteobacteria; Gammaproteobacteria; Enterobacteriales]	
. . Enterobacter	60 hits
34 orgs	
. . . Enterobacter sp. NCCP-287	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-280	1 hits
1 orgs	
. . . Enterobacter cloacae	20 hits
2 orgs [Enterobacter cloacae complex]	
. . . . Enterobacter cloacae subsp. dissolvens	7 hits
1 orgs	
. . . Enterobacter sp. EMB19	1 hits
1 orgs	
. . . environmental samples	14 hits
6 orgs	
. . . . Enterobacter sp. enrichment culture clone HSL19 ..	1 hits
1 orgs	
. . . . Enterobacter sp. enrichment culture clone HSL10 ..	1 hits
1 orgs	
. . . . uncultured Enterobacter sp.	9 hits
1 orgs	
. . . . Enterobacter sp. enrichment culture clone HSL90B .	1 hits
1 orgs	
. . . . Enterobacter sp. enrichment culture clone HSL90A .	1 hits
1 orgs	
. . . . Enterobacter sp. enrichment culture clone HSL81A .	1 hits
1 orgs	
. . . Enterobacter sp. I5.TYA	1 hits
1 orgs	
. . . Enterobacter sp. SB2-GLA	1 hits
1 orgs	
. . . Enterobacter sp. A732	1 hits
1 orgs	

. . . Enterobacter sp. A715	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-293	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-289	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-285	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-282	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-281	1 hits
1 orgs	
. . . Enterobacter sp. NCCP-267	1 hits
1 orgs	
. . . Enterobacter sp. 61(2011)	1 hits
1 orgs	
. . . Enterobacter sp. DQ11(2010)	1 hits
1 orgs	
. . . Enterobacter sp. IBP-VN4	1 hits
1 orgs	
. . . Enterobacter sp. IBP-VN1	1 hits
1 orgs	
. . . Enterobacter sp. DIC9JA	1 hits
1 orgs	
. . . Enterobacter sp. i1	1 hits
1 orgs	
. . . Enterobacter sp. Fw17a	1 hits
1 orgs	
. . . Enterobacter sp. BSRA2	1 hits
1 orgs	
. . . Enterobacter sp. IPPW-27	1 hits
1 orgs	
. . . Enterobacter sp. VTT E-073086	1 hits
1 orgs	
. . . Enterobacter ludwigii	1 hits
1 orgs	
. . . Enterobacter sp. GJ1-11	1 hits
1 orgs	
. . . Enterobacter sp. sa	1 hits
1 orgs	
. . Salmonella sp. enrichment culture clone CL107	1 hits
1 orgs [Salmonella; environmental samples]	
. . Pantoea	22 hits
8 orgs	
. . . Pantoea sp. T55	1 hits
1 orgs	
. . . Pantoea sp. PYPB15	1 hits
1 orgs	
. . . Pantoea sp. XJ3	1 hits
1 orgs	
. . . Pantoea sp. enrichment culture clone S28b	1 hits
1 orgs [environmental samples]	
. . . Pantoea agglomerans	15 hits
1 orgs	
. . . Pantoea sp. NJ-32	1 hits
1 orgs	
. . . Pantoea sp. J1-17-2a	1 hits
1 orgs	

. . . Pantoea sp. DAP17	1 hits
1 orgs	
. . unclassified Enterobacteriaceae (miscellaneous)	3 hits
3 orgs [unclassified Enterobacteriaceae]	
. . . Enterobacteriaceae bacterium M406	1 hits
1 orgs	
. . . Enterobacteriaceae bacterium M405	1 hits
1 orgs	
. . . Enterobacteriaceae bacterium ZHS050721	1 hits
1 orgs	
. unclassified Bacteria (miscellaneous)	6 hits
6 orgs [unclassified Bacteria]	
. . bacterium VNS3-1-2	1 hits
1 orgs	
. . activated sludge bacterium GC_R2A_S_14	1 hits
1 orgs	
. . endophytic bacterium S02	1 hits
1 orgs	
. . bacterium G33-1	1 hits
1 orgs	
. . bacterium RHA-3-18	1 hits
1 orgs	
. . bacterium SFS2-2-1	1 hits
1 orgs	
. uncultured bacterium	11 hits
1 orgs [environmental samples]	

BLAST is a registered trademark of the National Library of Medicine.