Impacts of Tanneries Wastewater on Agronomical & Biochemical Aspects of Lettuce (*Lactuca sativa*)

By

Darakhshanda Iram

Reg# 00000319208

Session 2019-2021

Supervised by

Dr. Tahir Ahmad

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

H-12, Islamabad 44000, Pakistan

2021

Impacts of Tanneries Wastewater on Agronomical & Biochemical Aspects of Lettuce (*Lactuca sativa*)

By

Darakhshanda Iram

Reg# 00000319208

Session 2019-2021

Supervised by

Dr. Tahir Ahmad

A thesis submitted as research project in partial fulfillment of the requirement of the degree of MS industrial Biotechnology

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

H-12, Islamabad 44000, Pakistan

2021

Thesis Acceptance Certificate

Certified that the contents and form of thesis entitled "Impacts of Tanneries Wastewater on Agronomical & Biochemical Aspects of Lettuce (*Lactuca sativa*)" submitted by Darakhshanda Iram, have been found satisfactory for the requirement of the degree.

Supervisor: ______________

Dr. Tahir Ahmad

ASAB, NUST

Head of Department: ______________

Dr. Sadia Andleeb

ASAB, NUST

Principal: ______________

Dr. Hussnain Janjua

ASAB, NUST

Dated: ________________

Declaration

I Darakhshanda Iram, Registration number 00000319208 student of MS Industrial Biotechnology in session 2019-21 hereby declare that the research work printed in thesis titled **"Impacts of tanneries wastewater on agronomical & biochemical aspects of Lettuce (*Lactuca sativa*)"** is my own work and has not been printed, published, and submitted as research work in any from in university, research institution in Pakistan or abroad.

Date ____________________ Student Signature ________________

Certificate of Plagiarism

It is certified that MS thesis entitled "Impacts of Tanneries Wastewater on Agronomical & Biochemical Aspects of Lettuce (*Lactuca sativa*)" submitted by Darakhshanda Iram, Reg no. 00000319208 has been examined by me. I undertake that:

1. Thesis has significant new work/knowledge as compared to already published or are under consideration to be published elsewhere. No sentence, equation, diagram, table, paragraph, or section has been copied verbatim from previous work unless it is placed under quotation marks and duly referenced.
2. The work presented is original and own work of the author (i.e., there is no plagiarism). No ideas, processes, results, or words of others have been presented as author own work.
3. There is no fabrication of data or results, which have been compiled/analyzed.
4. There is no falsification by manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.
5. The thesis has been checked using TURNTTIN (copy of originality report attached) and found within limits as per HEC plagiarism policy and instructions.

____

(Supervisor)

Dr. Tahir Ahmad

Associate professor

ASAB, NUST

Dedication

This study is wholeheartedly dedicated to my beloved Father, who have been my source of inspiration and gave me strength, who continually provide moral, spiritual, emotional, and financial support. To my affectionate mother who always prayed for my success. To my teachers who supported me throughout my studies and lent a helping hand whenever needed. To my brothers, sisters, relatives, mentor, friends, and classmates who shared their words of advice and encouragement to finish this study.

Acknowledgments

In the name of Allah, the Most Gracious and the Most Merciful Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis.

I express my cordial gratitude to Dr. Hussnain Ahmed Janjua, Principal ASAB for providing all kinds of facilities in labs during the course of this research work.

I wish to express deep gratitude to my sincere and respectable supervisor Dr. Tahir Ahmad for his kind help, continued guidance, constructive suggestions and encouragement throughout my studies and research work.

I am very thankful to Soil Survey of Pakistan, Lahore for all kinds of guidance and facilities for biochemical analysis.

I would like to thank my class fellows & lab fellows for their valuable suggestions and support during my research.

I would like to thank my beloved husband who helped me with his valuable suggestion and guidance in various phases of completion of this project.

Finally, I would like to show my greatest appreciation to my parents who gave support in the entire completion of the project who provided their love, wisdom, knowledge, and hard-earned money in making my way to success. With the cooperation and contributions from all parties, the objectives of the project; soft skills, knowledge and experience was gained accordingly.

Table of Contents

List of Figures

List of Tables

List of Abbreviation

BOD	Biological oxygen demand
COD	Chemical oxygen demand
SAR	Sodium absorption ratio
EC	Electrical conductivity
GDP	Gross domestic product
EPA	Environmental protection agency
TDS	Total dissolve solids
WHO	World health organization
OM	Organic matter
RSC	Residual sodium carbonate
CEC	Cation exchange capacity
TKN	Total kjeldahl nitrogen
T_1	25% effluent concentration
T_2	50% effluent concentration
T_3	75% effluent concentration
T_4	100% effluent concentration
ROS	Reactive oxygen specie
KTWMA	Kasur tannery waste management authority
TWTP	Tannery wastewater treatment plant
PHED	Public health engineering department

Abstract

This research study deals with the impacts of tanneries wastewater on agronomical and biochemical aspects of Lettuce (*Lactuca sativa*). To determine the affects of tanneries wastewater on above mentioned plant, wastewater was taken from Kasur tannery treatment plant. The wastewater was found alkaline along with high biological oxygen demand (BOD), chemical oxygen demand (COD), electrical conductivity (EC) & sodium absorption ratio (SAR) along with huge amount of total settleable solids (TSS) and total dissolve solids (TDS). The number of heavy metals in the effluent was quite high. The wastewater was diluted up to four concentrations ranging from 25-100%. Treatment was given to 15 days old seedling. The tanneries effluent affects were investigated against both agronomical and biochemical aspects of lettuce. Plant height, dry & fresh weight of plant, leaf & root decreases with increase in effluent concentration as compared to control. Tanneries wastewater significantly affected the height of plant ($p \leq 0.0001$), had highly significant affect ($p \leq 0.0001$) on dry & fresh weight of whole plant & root, the overall impact on dry & fresh weight of leaves was significant ($p \leq 0.05$). The chlorophyll content and protein content decrease with increase in effluent concentrations. Tanneries wastewater had very significant affect on chlorophyll content of leaves ($p \leq 0.001$)., significantly affected the protein content in lettuce leaves ($p \leq 0.05$). The uptake of heavy metals increases with increase in effluent concentrations & the affect of tanneries wastewater on uptake of trace elements was highly significant ($p \leq 0.0001$). These heavy metals biomagnified at different trophic levels and enter the food chain thus cause serious health problems, also destroy the ecosystem.

Chapter 1

Introduction

1.1 Introduction

Environmental pollution is one of the world's biggest issues, and it is worsening every day as a result of urbanization and industrialization. Chemical use in many human activities has increased dramatically during the last several decades. Current patterns of industrial activity have altered the material flow in its natural state and introduced new toxic substances into the environment. One of the major reasons that has a detrimental influence on humans and the environment is the release of organic compounds and heavy metals that causes toxicity in plants and other biological agents that are constantly being exposed to potentially hazardous heavy metals [1].

Groundwater is the main source of drinking water supply and is also used for agricultural, industrial & irrigation purpose. The pollution of groundwater resources by urbanization and industrialization is becoming more and more serious. Global water contamination as a result of a rise in the number of industries is a significant problem that the world is now dealing with. The discharge of pollutants in water bodies is the main cause of water pollution. These contaminants find their way into aquatic environments including rivers, ponds, and lakes, posing a risk to human health and ecosystems [2, 3].

Almost all industries discharge pollutants along with water at one stage or another in their production processes. The presence of contaminants in the water alters the physical chemistry parameters, causing them to deviate from normal levels. Negative effects on water quality include increased turbidity, nutrient loads & alteration in color as well as the addition of toxic and persistent compounds.

Leather production is a process that uses lot of water and release large amount of wastewater along with chemicals. About 6.5 million metric tons of wet blue leather & fur are produced globally each year, and about 3.5 million metric tons of various chemicals are used in leather processing [4].

Although a great number of chemicals are used in the leather processing, only 20 % of the chemicals are absorbed during the processing by the leather and the rest are discharged along with large amount of water. Tannery wastewater is the most serious pollutant, which mainly comes from high salinity, specific toxic pollutants & high organic load [5, 6].

1.2 Sites of Leather Sectors

Various leather sectors are working throughout the country. There are more than 2,500 registered and unregistered tanneries in Pakistan. Sialkot, Karachi, Kasur, Korangi, Gujranwala, Multan, Lahore, Faisalabad, Sahiwal, Hyderabad, and Peshawar were the major cities where the leather industry was founded. There are 784 leather production units, 461 leather garment production units, 348 gloves production units and 524 footwear production units. Buffalo, cowhide, goatskin & sheep skin are used as the main raw materials for the production of high-quality leather [7].

Kasur occupies 1/3 of total leather industries in Pakistan. As the demand for leather continues to increase, the leather industry is also booming. Tanning can be done in a variety of ways, but the two most common tanning methods are chrome tanning and vegetable tanning. Compared with chrome tanning, vegetable tanning is much less harmful. However, vegetable tanning is expensive and time-consuming. Therefore, chrome tanning is the most common tanning method [8]. After chrome tanning, the chrome compound is applied to raw leather and washed with fresh water. This chromium-rich water is either drained or discharged into the open lands. It seeps from the land into groundwater or flows into oceans, rivers, canals, and lakes and into groundwater. Groundwater is a key source of fresh water that requires regular human consumption and irrigation, and therefore can cause serious environmental and health problems when contaminated with heavy metals [9, 10].

1.3 Economic Importance of Leather Industry in Pakistan

When Pakistan become an independent country, there were a few leather industries in this region. After independence, a number of leather industries were established in Karachi. In 1960, leather industries were set up in the cities of Sahiwal, Kasur, Gujranwala, Multan, and Sialkot. Finished leather production began in Pakistan in 1970. In 1990, the leather industry accounted for 10.41% of the state's total exports & thus making it the country's second largest source of foreign exchange earnings. Pakistan is considered to be a major leather production center. There are 2500 tanneries in Pakistan. Pakistan produces 13 million hides and 47.4 million skin per year. Pakistan's leather industry employed more than half a million people between 2009 and 2010. It accounts for 5 % of GDP and 5.4 % of the country's total export earnings and is considered to be the most important sector. In the international market, Pakistan is famous for producing high quality leather [11].

Pakistan's leather clothing accounted for 6.17%, leather gloves accounted for 10.76%, shoes accounted for 0.28%, in the world market, and Pakistan is ranked at 11th position in world

Market. In 2014, Pakistan had a market share of $300 million. In 2014, Pakistani glove production ranked first on the international market. Footwear exports accounted for US $131.2 million (2014-2015). Pakistan exports leather goods to Germany, Italy, Portugal, the United Kingdom, Canada, Norway, Spain, South Korea, Sweden, Denmark, Chile, Japan, South Africa, Belgium, Singapore, and Australia [11, 12, 13]. Pakistan's leather and leather-made-ups sector is very important to the country's economy. Export Performance of Pakistan in the year 2019-2020 is given in figure 1.1[14].

1.4 Pakistan's Status in World Market

Pakistan's leather products are of great variety and quality and enjoy a high reputation in the international market. Pakistan occupies 11th position in the world market for leather production, with 6.17 % leather clothing, 1.12 % leather and fur, 10.76 % leather gloves, 0.28 % leather shoes and 0.21 % leather goods [7]. List of top Leather producers in year 2020 is given in table 1.1

Figure 1.1: Percentage of export performance of Pakistan for Leather & Leather products in year (2019-2020) [14].

Table 1.1: List of top leather producers across the globe in year 2020, Pakistan occupies 11th position in the world market [13, 14].

Rank	Country	Production (mil of sq.ft.)	Share in global Production
1	China	6,170	25%
2	Brazil	23,60	9.5%
3	Russia	1652	7%
4	India	1560	6.4%
5	Italy	1521	6.3%
6	Korea	1140	4.8%
7	Mexico	642	2.7%
8	US	719	3%
9	Argentina	804	3.4%
10	Turkey	529	2.2%
11	Pakistan	541	2.43%
	All the rest	6,704	27.2%
	World Combined	23,976	100%

1.5 Impact on Environment

Water pollution is a major concern at the local, regional & global levels because of its impact on public health. The purpose of this study was to assess the impacts of tannery industrial wastes on water and the environment. The harmful impacts of leather industry on ecosystem, particularly on water, are caused by harmful industrial waste and is a matter of concern. Discharge of tanneries industrial waste on large scale into water, contaminate water sources, rendering it unfit for human consumption because it contains toxic chemicals and pose serious impacts on the surrounding environment, including humans, animals, birds, and aquatic organisms. This wastewater has a negative impact on agricultural land and groundwater.

A wide variety of world-renowned & high-quality products are produced by Kasur district of Pakistan. Leather and leather products of district Kasur in Pakistan are famous worldwide due to the production of high quality and large range of leather goods. This business directly or indirectly is an important source of income for hundreds of families, but the effluent from the tanneries also severely affects the population. Because developing countries such as Pakistan do not have the proper infrastructure to treat industrial wastewater, it is discharged into public sewer systems and to the other surrounding bodies of water [15].

The lack of modern leather processing technology is the main cause of water pollution and excessive water use in tanneries. Compared with modern technology, most tanneries still use centuries-old traditional processes that require more chemicals and consume an average of 40-70 % of the water. Due to excessive use of water, the wastewater ratio is very high and has a negative impact on the environment. Chemicals used in leather processing in tanneries are a major source of water pollution. More than 170 of these chemicals are reported, they include sodium chloride, calcium oxide, sodium bisulfate, chromium sulfate, ammonium sulfate, ammonium chloride, Sodium bicarbonate, sodium hypochlorite, sodium sulfate, formic acid, fat emulsion, lime, fungicides, fat, non-ionic wetting agents, soda ash, synthetic tanning agents, formaldehyde, polyurethane, various dyes, and vegetable tannins. During the procedure, a large amount of water is used, and the chemicals are then discharged from the tanneries. The ecosystem is contaminated by a variety of elements that are caused directly or indirectly by numerous events in our everyday lives. According to many studies, industrial waste is the most prevalent and harmful form of water pollution, that is increasing every year as a result of increased industrialization. Leather tanneries in the Kasur region are wreaking havoc on the environment, particularly the water supply. In this regard, harmful substances were first detected in the groundwater of Kalalanwala, Kasur district, in July 2000, following media reports of strange skeletal abnormalities among the local population [15].

Many investigations have finally confirmed the facts about the state of water and the environment in Kasur and their impact on environmental variables & on public health. The presence of highly toxic and destructive substances in the water is a major source of numerous illnesses and environmental instability in the region, as well as a possible threat throughout the process, leather processing units use water, various colors and chemicals. These chemicals and pigments eventually contaminate the water, making it dirty and unfit for aquatic life and human consumption. Industrial wastewater has the potential to contaminate groundwater as well as the fertility of the land surrounding the contaminated water. In many stages of converting raw leather into finished leather, leather processing units require a large amount of water and a wide variety of chemicals. In this case, the whole process produces a large amount of highly polluted wastewater, which is the main problem. The conversion of raw leather to leather requires a great deal of water, about 4,000 gallons of water per ton. This is a major problem in states like Pakistan, where substantial portions of the population already face water shortages. Leather production is another direct threat to water resources in this regard, since it not only uses enormous amounts of water but also pollutes the remaining reservoirs as well [15,16].

Every day, a large number of chemicals are used, to pollute water resources, such as ponds, streams, rivers, & even groundwater, from tanneries. According to a study, the catastrophic consequences of a typical tannery can have a negative impact on humans, animals, birds, and aquatic life within a 7-8 km radius. Stomach, respiratory, ulcers, high blood pressure, kidney stones, cardiac arrest, genetic mutations, liver, kidney, heart diseases are just a few of the diseases that can be caused by contaminated groundwater. In the chemical process of microbial decomposition, dissolved organic waste in tannery wastewater absorbs a large amount of oxygen from the water body. Tannery wastewater in this way reduces the amount of oxygen in the water. Since fish, plants and other aquatic organisms cannot live in such water without oxygen, it cannot meet the needs of aquatic organisms [16].

Due to the high content of dyes and chemical extracts in tanning water, the transparency of the water will be damaged because the pollutants will block the sunlight from reaching the water. The infrared rays of sunlight are absorbed by dyes & other chemical extracts on the water surface. Because light cannot penetrate water, the life cycle of aquatic organisms is disrupted, causing additional disruption to other organisms. In order to reduce the harmful effects of industrial wastewater, it should be treated before discharge. However, even in the developed country, wastewater treatment processes that discharge wastewater require significant expenditures and operating costs to meet the standards of the environmental protection agency (EPA). In underdeveloped countries, such as Pakistan, the situation is quite different because there is insufficient infrastructure to treat industrial wastewater, which is discharged into public sewerage systems and other adjacent water resources. Leather mills, despite their destructive affects and hazardous chemicals, play an important role in the regional and national economies. In such cases, their negative effects should be minimized in order to increase their efficiency by minimizing their disastrous effects of industrial wastewater on the environment [17].

As a part of biosphere pollution, plant growth is also faced with potential decline in the process of development. Plants growing in heavily polluted areas are contaminated with toxic metals. High metal accumulation affects plant growth & metabolism & increases the production of ROS in plants. Leather industry is one of Pakistan’s oldest cottage industries and now dominates the country’s economy. The industry is considered to be a major polluter of the environment, with a high risk of soil & water pollution due to the release of untreated wastewater [18].

Before being turned into flattened leather, the raw skin is subjected to a series of chemical processes. This includes soaking, liming, depilation, softening, degreasing, and pickling. Ammonium chloride, sodium sulfite, hydrogen peroxide, salt format, sodium, hydrogen carbonate, chromate, and chloride are all hazardous compounds utilized in these stages. As a result of many processes, which include the use of a variety of chemicals, the leather processing industry is one of the most ecologically damaging industry [18]. The interaction between wastewater and the environment is quite fast. The volume of wastewater produced per kilogram of treated skin is between 30-50 liters. From the total volume of liquids, nearly 10 % corresponds to the tanning stage and the other treatment process i.e., depilation, pickling, neutralization, dyeing and cleaning. The environmental challenges posed by the kind and quality of the chemicals utilized, as well as the amount of waste created and released, all play a role in leather processing. Sulfides, phenolic compounds, magnesium, sodium, potassium, chromium, mineral salts, colors, and solvents are all released by the leather industry. The detrimental impacts of tannery wastewater on the environment have become a prominent problem in Pakistan. The quantity of water utilized in tanneries varies considerably depending on the procedure, the raw materials used, and the goods produced. Large volumes of fresh water, on the other hand, are needed for leather production, releasing a variety of potentially hazardous substances like chromium, synthetic tannins, petroleum, resins, biocides, & detergents. The careless disposal of untreated tannery waste causes air and water pollution and releases greenhouse gases such as methane [19].

The possibility of using plant biota to clean environment is drawing the attention of environmental scientists against the traditional expensive site cleanup technologies. In recent years, there has been great interest in using diluted wastewater to irrigate crops. This is a method of safely treating sewage for irrigation without a negative impact on the environment and human health. As a low-cost management approach, smaller volumes of sludge are modified using agricultural soil to improve productivity and thus remediate waste waters. The roots and root hairs of plants produce large surface areas through which organic matter can be extracted from contaminated soil & water. Irrigating plants with 50-100% raw effluents has adverse effects on plant development and growth. Changes in growth rate and metabolic activities are linked to physiological processes in plant cells due to the toxicity of pollutants. Respiration, photosynthesis, and mitotic activity are all impacted significantly. Tannery effluent has a high contamination potential, which causes phytotoxic effects on plant. Plant stress is caused by a high concentration of heavy metals [20].

The use of sewage as a source of irrigation is inevitable where there is a shortage of irrigation water and excess wastewater generated by industry. The new strategy to solve this problem is to use the dilution form of tannery wastewater to promote the growth of plants. Different amounts of wastewater have been used to produce diverse plant crops in a variety of experiments, and biochemical alterations have been observed [21].

The presence of heavy metals in the environment can be hazardous to humans, especially if they are exposed and absorbed to high levels. While some metals, such as copper, iron, manganese, and zinc, are required for life processes, others, such as cadmium, nickel, and mercury, have no physiological role but can cause detrimental diseases when present in high concentrations [22]. Exposure of workers to chemicals used in industrial processes can cause various forms of harmful impacts on their health, because they are unaware of the toxic effects of chromium, hydrogen sulfide, lead, zinc, cadmium, formaldehyde and other compounds, their entry into biological systems can lead to disorders of organs such as the liver, lungs, and kidney. Workers associated with tanneries have been found to have a range of health problems over the years. Workers in tannery industries are exposed to these chemicals in a variety of ways during the tanning process. They breathe in air-borne substances such as gases, dust, steam, aerosols, and smoke when eating and drinking in the workplace or absorbed or transferred from contaminated hands and skin through pores or wounds on unprotected hands and arms, are some of the possibilities for exposure to hazardous chemicals in the workplace. Skin contact with chromium can cause skin ulcers, allergic reactions and swelling of skin. Thus, the leather industry has acquired a negative image of society in terms of its pollution potential due to the release of large quantities of chemicals & threats posed by unsafe discharge of tannery effluents into the biosphere [23, 24].

1.6 Study Area

Kasur, a city near the Indian border, is 55 km from Lahore. There used to be 180 tanneries in this area at the time of independence, however according to the latest official figures, their number has increased to 272. According to unofficial figures, there are more than 300 tanneries, mainly in the city of Kasur. The area covers a total area of 3,995 km. As the leather industry is concentrated in the city of Kasur, the industrial density of the city is only 0.06. The formula for industrial density is the total number/area of industries. According to the Kasur tannery waste management authority, the tanneries discharge 892.70 cubic meters of wastewater per hour. In addition to liquid waste, industrial production produces more than 300 tons of solid waste per day. Although the government has built the sewage treatment plant in Kasur, due to certain irregularities, compared with the large amount of sewage generated, the treatment capacity is reduced, unable to treat all sewage. From the outset, the waste was discharged into the open space around the tannery. The tannery wastewater remained in the open spaces around the city in the form of sewage ponds in the open fields around the city before the construction of wastewater drain and water treatment plant. These pools use to cover 327 acres of land. In addition to this area, the area of sewage treatment increased during the monsoons, with almost 311 acres of cultivable land is occupied with this wastewater. That is an unmanageable burden on the region's current capacity to properly handle such a huge waste. As a result, the environmental degradation procedure has created many problems for local residents [25].

1.7 Environmental Conditions

The weather in the region is extreme in summer and winter. The summer stretched from April to September. The hottest summer month in the region is June, with average highs and lows of between 40 and 27 degrees Celsius. The winter months are from November to March, with the mean maximum and minimum temperatures ranging from 20 to 6 degrees Celsius. The monsoon season begins at the end of June and lasts until mid-September, with unpredictable weather. Low pressure in the west also brought rain in January, February, and March. In winter, rainfall is maintained between 23 and 31 millimeters. Industrial and domestic activities contribute significantly to the generation of waste in this region. In Kasur, the environmental situation is quite discouraging as the waste from the tannery and the animal waste from the carts/ carriages that play on the urban roads are the main causes of pollution. Today, Kasur faces serious problems on a daily basis, such as the toxic effluent from the tannery sitting in a cesspool and the long running Rohi Nullah, which carries all of the city's sewage and household waste. Untreated tannery effluents are discharged into fresh water and open areas, worsening environmental conditions [26].

Before the formation of the Kasur tannery waste management authority (KTWMA), about 400 acres of arable land were turned into stagnant pools. From the 1990s to 2000, the city was notorious for its foul-smelling pools of wastewater and tannery waste. Originally, tanneries used a vegetable tanning process to tan leather, which was later replaced by a chrome tanning process. The chrome tanning process consumes a large amount of toxic chemicals. Industry thrives on the southern and southeastern edges of the river and tends to dump waste into the river. But after the Indus water treaty, the river dried up permanently and its land was used for farming. After the river was permanently abandoned, tanneries began dumping waste into the abandoned river. Therefore, the discharge of sewage has become a permanent environmental problem in the region. The tannery was later expanded from Din Garh to Kot Abdul Qadir and Younis Nagar. According to the latest available data from KTWMA, there are 272 tanneries in Kasur that discharges more than 21,425 cubic meters of wastewater per day. The average daily yield of lacy leather selected for wet salting is estimated by the Din Garh Association at 380 tons. The daily consumption of cowhide and buffalo hides is 18,000.

Between 24,000 and 30,000 sheep and goat skins are used on the daily base for leather production in this area. In addition, the sanitary conditions in this city are very poor. Frame of work for Kasur tanneries waste management project is given in figure 1.2 [25].

1.8 Location of Tanneries in Kasur

As mentioned earlier, the leather industry is located in south and southeastern corner of Kasur City in the form of clusters. Only a few leather units are located other than that cluster. Such units are located along the wastewater outfall outside the city as individual units.

1.9 Kasur Tannery Pollution Control Project

The government of Pakistan, with the assistance of United Nations Development Program (UNDP), developed a plan for the treatment of tanneries wastewater. To address the pollution problems caused by discharge of effluent by the tanneries, the major objectives of the project are:

i. Construction of a shared drainage system to collect tannery wastewater
ii. Construction of the Sewage treatment
iii. Construction of in-house chromium recovery plant
iv. Construction solid waste disposal facilities
v. The final outfall of the construction works will discharge the treated water into the Pandoki leakage drainage channel

The project is implemented through the waste management authority of the Kasur tannery established by the Punjab government’s ministry of housing, urban development, and public health works. The authorities are planning to construct a drainage channel from Kasur to Pandoki so that the sewage pool at the Rohi Nullah can be discharged into the Pandoki drainage channel. Until the new 6000 ft drainage ditch has been dug, the current excavation work has been shelved due to some disputes with local farmers, from the drainage ditch through the land [25].

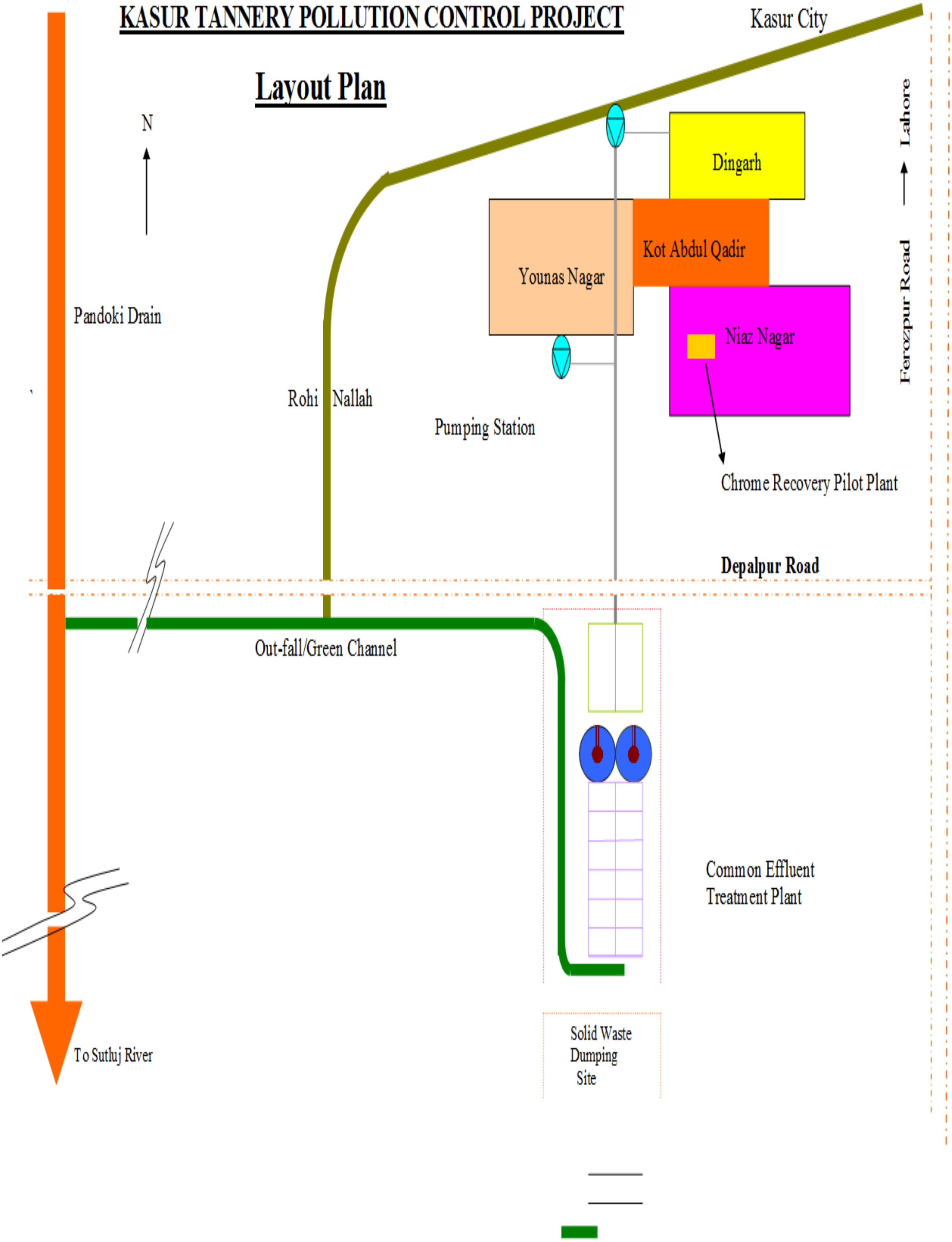

Figure 1.2: Frame of work for Kasur tanneries waste management project

1.10 Waterborne and Hygiene Related Diseases in District Kasur

The number of patients suffering from water-related and sanitation-related diseases is very high in Kasur district. Data on water-borne and sanitation-related diseases are collected from all government health agencies in the region. The general directorate of health services in Punjab has developed a computer-based system to record and manage data in the name of the health management information system. At least 96,079 patients go to government health facilities in the region for treatment, according to the office of the globally harmonized system, and the actual number is bound to be higher. Diarrhea or dysentery in children under five years of age accounts for a large proportion of water-and sanitation-related patients. A total 43849 patients under the age of five years have diarrhea/ dysentery. Patients having acute flaccid paralysis were 05, disease related to worm infections were diagnosed in 11110 patients. The second most common water-borne and sanitation-related disease in Kasur is peptic ulcer, affecting 40,820 people with peptic ulcer. The number of patients with cirrhosis was 186. There were 109 cases of nephritis/kidney disease who had been to a government health facility. The total number of patients treated in these institutions was 75,382. According to the type of diarrheal disease in children under the age of 05, 21,808 patients received government treatment. Only 03 patients with acute mobility difficulties received medical care. Peptic ulcer treated 4,291 worm infections and 20,252 worm infections. Health facilities were provided to 105 patients with cirrhosis of the liver and 16 with nephritis from government health institutions [26].

1.11 Water Supply of Kasur City by PHED

The water supply currently comes from groundwater and 43 tube wells. The government water supply system covers 70 % of the urban population and serves 75 % of city. The government water network consists of seven elevated reservoirs, six with a capacity of 50,000 gallons and one with a capacity of 60,000 gallons. The total capacity of these reservoirs is 360000 gallons. The public health engineering department (PHED) has developed a water supply plan as part of the overall environmental improvement project in Kasur district. The scope of the project includes the replacement and installation of pipelines, 31 hydrochlorination units and the establishment of a water quality testing laboratory [26].

1.12 Sewerage and Drainage

Sewage treatment facilities is available to only 01% of the total population and covers only 03% of total area. The existing sewerage system including the Pacca waterway built by the local group are being disposed in the Rohi Nullah, but there is no classification of urban and industrial waste. All tannery wastewater is discharged to open sewer (except for the four-sewage treatment now connected to a conventional sewage pretreatment plant) then the waste is eventually disposed of in a leaky drainage, this leads to Pandoki seepage drain where the waste is ultimately disposed of. PHED has been carrying out sewerage improvement and drainage works in Kasur, including planned improvement of drains and a 200-acre sewage treatment plant [26].

1.13 Statement of Problem

The establishment of a tanning unit is prohibited in Kasur by EPA. United States environmental protection agency estimates that in 1994, wastewater containing heavy metals & hazardous chemicals were released resulting in three stagnant pools of wastewater covering an area of around 400 acres. Approximately 150 tons of hazardous solid waste from the tanning industries is believed to be dumped into the open land around these pools every day (EPA, 1994). After the sewage treatment was built, the sewage was treated on a small scale and discharged into rivers, but the topsoil on the land reclaimed by the evacuated ponds was highly toxic. In the tanning process, huge amounts of chemicals and heavy metals such as chromium, nickel, and manganese are consumed, which are carcinogenic and cause a variety of illnesses among employees and populations living and working near tanning plants [27].

The soil of Kasur is primarily soft alluvium, with a lot of sand from the neighboring Sutlej River. The groundwater level is high, and hazardous wastewater leakage contributes significantly to groundwater contamination. The level of heavy metal in tannery effluent is considerable. Metals are categorized as "heavy metals" if their specific gravity in their standard condition exceeds 5 grams per cubic centimeter. There are 60 heavy metal bands that are well-known. Heavy metal buildup in soil and plants over time has a detrimental impact on plant including physiological processes, resulting in reduced plant growth, dry matter accumulation, and yield. Tannery effluent that comes into contact with soil and water increases the hazardous metal burden in these environments. Chromium and other heavy metals are key components of the chemicals used in tanning [27].

In compliance with afore mentioned problems caused by tanneries effluents the present experiment was designed in order to investigate the effect of tanneries effluent on agronomical and biochemical aspects of Lettuce (*Lactuca sativa*).

1.14 Scope of Study

The prime concern of this research is to assess the impact of leather tanning industries on agronomical and biochemical aspects of lettuce. The impact of heavy metals discharged from tannery effluents on lettuce plant is assessed. The research also discusses the influence of polluted water along with heavy metals on the public health in the area. A brief discussion on the possible measures to treat the polluted water along with suitable suggestions is included.

1.15 Aims and Objective

The specific objectives of the study include:

i. To characterize the physiochemical properties of the tannery effluent
ii. To analyze the potable water samples used in industrial area
iii. To study the profile of effluent contaminated soil
iv. To investigate certain agronomic characteristics
v. To study the impacts of tanneries effluents on soluble proteins and chlorophyll contents in leaves of plant.
vi. To determine the degree of uptake of heavy metals especially Cr, Zn, Mn, Fe, Co, Ni, Mn and Cu by various concentrations of effluents.

Chapter 2

Literature Review

2.1 Environmental Pollution

The quality of soil and groundwater is influenced by the discharge of untreated industrial and residential waste into the environment. From the past few decades, environmental pollution has become a major problem. The contamination of our environment by a variety of chemicals employed in numerous industrial activities is causing an increasing sense of global urgency. The survival of plants and animals has been severely influenced since the advent of mining and smelting, metallurgical industry, sewage treatment, and tanning. Soil, water, and biodiversity are all important components of ecosystems, and they're the focus of a lot of agricultural, ecological, biological, and hydrological study. Arable land that is treated with agrochemicals to produce the upper layer of the soil, makes up a major percentage of ecosystems. Water from farmed soils leaches large amounts of chemical elements, which then enter into the animal and human food chain and disturb the ecosystem. The quality of life on earth is inextricably linked to the overall quality of the environment. There are two basic pollution-related problems: the treatment of the ever-increasing volume of waste and to remove the toxic compounds that have accumulated in dumping sites in soil and water bodies over the past decades. Pollution is defined in various ways. It is believed to be the release of large quantities of harmful substances into the environment, damaging health or the resources themselves. With the increasing use of industrial and agricultural chemicals, heavy metal pollution to the environment is a serious threat [27, 28, 29].

2.2 Leather Tanning Process

Tanning is the conversion of raw skin or skin proteins into a form of stable material, non-corrosive, suitable for all kinds of terminal applications. Chrome tanning and vegetable tanning are the two most common forms of tanning. Chromium is the most commonly used tanning material. It is softer than vegetable-tanned leather and does not discolor or lose form in water. The chromium compounds are used to protect leather and skin from decay, making them more durable, resistant to moisture and aging. Chromium interacts with raw/skin fibers during a bath, and the tanned skin is then rubbed dry for finishing.

The leather-forming process in a tannery consists of four main sub-processes. These include:

i. Beam house processes
ii. Tan yard processes
iii. Re-tanning
iv. Finishing

However, the tanning process differs for each end product, and the quality and quantity of effluent generated might vary significantly. The following is a detailed description of each of the four leather-making sub-processes [27].

2.2.1 Hides and Skins Storage and Beam-House Operations

Skin can be categorized, trimmed, cured (where raw materials can't be handled right away), and kept in the beam house pending operation. Soaking, de-haring, liming, fleshing (mechanical scraping out of superfluous biological material), and splitting are common procedures carried out in a tannery's beam house [28]. The soaking and liming processes in the beam house result in a significant concentration of sulphides, lime, ammonium salts, and protein in the effluent. Beam house effluent accounts for roughly 55-65 % of total tannery effluent and 35-45 % of tanning process effluent. Suspended solids, filth, manure, pesticides, blood stuck to hides and skins, and chlorides are all found in soak liquor [30]. Lime liquors include suspended particles, dissolved lime, sodium sulphide, high ammoniac nitrogen, and organic materials, making them extremely alkaline. Fatty fleshing substance is suspended in the un-hairing and fleshing effluent. As a result, soaking, liming, degreasing, pickling, and tanning operations account for almost 70% of BOD, COD, and (TDS) pollution loads [31].

2.2.2 Tan Yard Operations

De-liming, bating, pickling, and tanning are included in the tarn yard's processes. Tanned leather and fur can be traded as an intermediary commodity once they've been transformed into non-degradable materials (wet blue). Because of the inorganic salts, chloride, ammonia, chromium, and sulphate in the tan yard effluent, it is severely polluted. Bating liquors account for the presence of soluble skin proteins and ammonium salts carrying substantial organic matter, whereas deliming liquors convey a considerable BOD load. The pickling solution is acidic and contains a lot of salt, whereas the chromium liquor has a lot of chromium compounds and neutral salts in it [30, 31].

2.2.3 Re-Tannings

Following the tanning process, this sub-step includes washing away the acids that remain in the leather. The leather is re-tanned (to improve the feel and handle of leathers), dyed with water-soluble dye stuffs (to produce even colors over the entire surface of each hide and skin), fat liquored (to achieve product-specific characteristics and to re-establish the fat content lost in the previous procedures), and finally dried, depending on the desired leather type. Dyes, lipids, fat liquor, tans, and chromium are among the major water contaminants released at this level. The effluent from the sections of neutralization, re-tanning, dyeing, and fat liquoring contributes very little pollution to overall pollution load [30, 31, 32].

2.2.4 Finishing Operations

The purpose of finishing leather is to make it as thin, smooth, and appealing as possible while maintaining its recognized qualities like as look and breathability. The goal of this procedure is to treat the surface in order to get the desired end result. The overall objective of finishing is to improve the look of leather while also providing suitable color, gloss, and handling qualities [33, 34]. Various steps for conversion of raw hides to pure fine leather are elaborated in Figure 2.1. Pictorial Explanation for different stages of tannery operations is given in Figure 2.2 to 2.6.

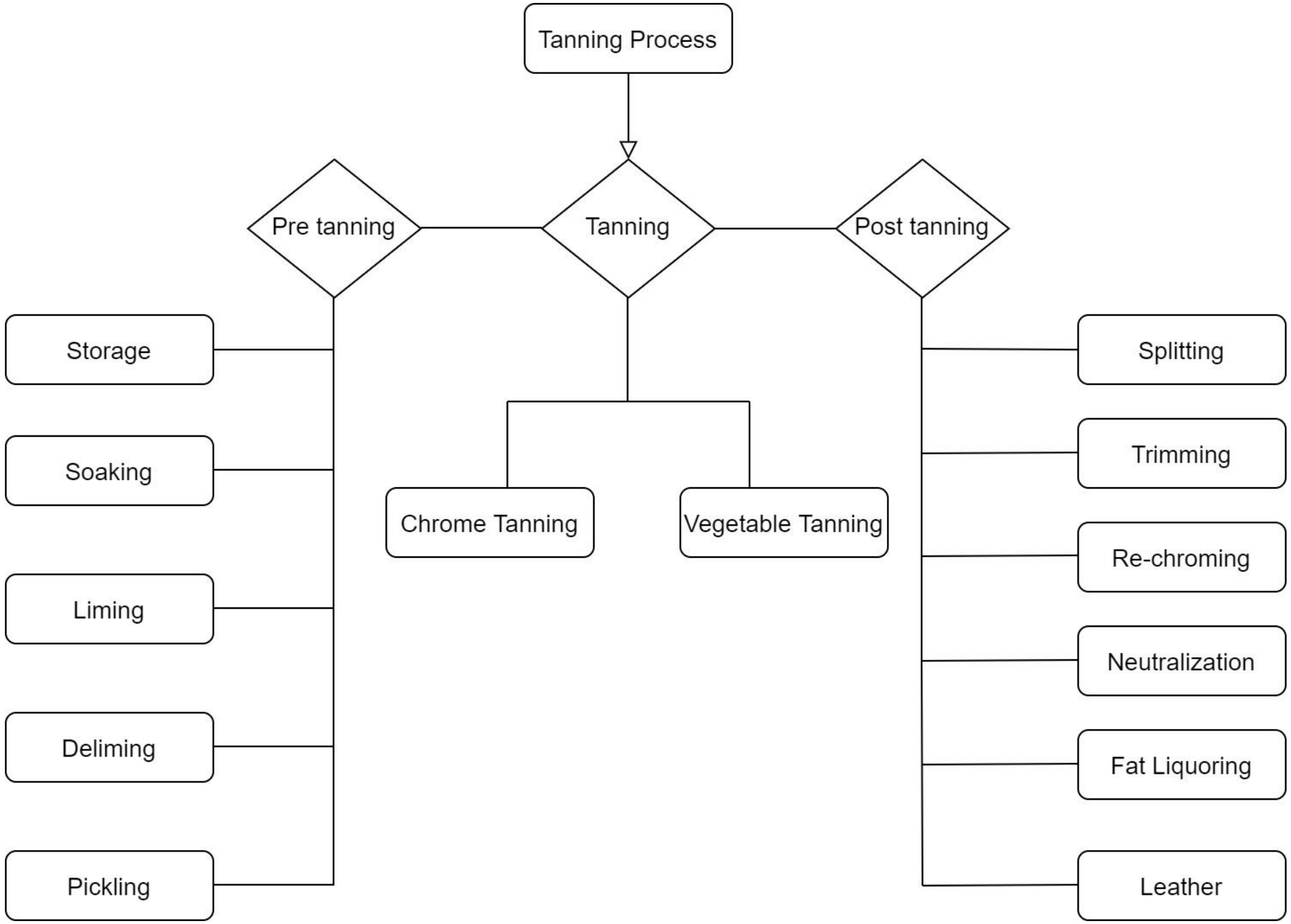

Figure 2.1: A Flowchart summary representing various stages for conversion of raw hides to pure fine leather [38].

2.3 Pictorial Explanation of Different Stages of Tannery Operations

2.3.1 Collection of Raw Hides

Figure 2.2: Collection of raw hides for leather production

2.3.2 Soaking of Hides

Figure 2.3: Soaking of raw hides in alkali solution for unhairing

2.3.3 Liming

Figure 2.4: Liming process done in a drum for unhairing

2.3.4 Tanning

Figure 2.5: Coloring of leather with desired color by heavy metal

2.3.5 Drying/Finishing

Figure 2.6: Finished, glazed leather product

2.4 Chemicals Used in Leather Processing

Leather processing uses around 3.5 million metric tons of different chemicals. More than 170 of these chemicals have been reported for their harmful impact. Some of these chemicals are listed in table 2.1.

Table 2.1: List of hazardous chemicals employed in different stages of leather processing grouped into different levels based on their toxicity [38, 39].

High Potential Hazardous Group	
i.	Acetic acid
ii.	Formaldehyde
iii.	Hydrogen peroxide
iv.	Sodium hydroxide
v.	Ammonia
vi.	Formic acid
vii.	Oxalic acid
viii.	Sulphuric acid
ix.	Calcium hydroxide
x.	Glutaraldehyde Hydrochloric acid
xi.	Sodium chloride
xii.	Sulphides and hydrosulphide
Moderate Potential Hazardous Group	
xiii.	Aluminum sulphate
xiv.	Amyl alcohol
xv.	Benzyl alcohol
xvi.	Isopropyl per chloroethene
xvii.	Chromium salts
Low Potential Hazardous Group	
xviii.	Alum
xix.	Gelatin
xx.	Casein
xxi.	Paraffin
xxii.	Ferrous acetate
xxiii.	Sodium citrate
xxiv.	Sodium sulphite
xxv.	Sodium acetate
xxvi.	Sodium bicarbonate
xxvii.	Titanium salts
xxviii.	Ferrous sulphate
xxix.	Acetone
xxx.	Calcium Chloride

2.5 Pollutants Released by Tanneries

Tannery wastewater is characterized by excessive salinity, high organic load, and the presence of particular hazardous contaminants. According to United Nations industrial development organization report, following pollutants are released by tannery industry [36].

2.5.1 Solids

The solids detected in tannery effluent are divided into various groups based on their nature. The details of these groups are given below:

i. Suspended Solids

The amount of insoluble material in wastewater is referred to as the suspended solid component. When released from one location, these insoluble compounds produce a range of issues; in reality, they are made up of solids with two distinct characteristics.

ii. Settleable Solids

Suspended matter is visible when the departing sample is shaken, while settleable matter is visible when the sample is stationary. The majority of these solids settle in 5 to 10 minutes, but very tiny particles might take up to an hour to settle. Fine leather particles, leftovers from different chemical emissions, and reagents from various waste alcohols are among the solids generated from all stages of leather production. During the beam chamber processing, a significant amount of volume is produced.

The quantity of sludge that develops as particles settle down is the major difficulty if the wastewater is to be treated in sewage treatment or traditional wastewater treatment. This sludge must be collected, transported, dehydrated, dried, and precipitated in its whole, putting undue strain on facilities, equipment, and resources. If wastewater is released into surface water, the flow rate determines how far the material travels before being deposited in a river or riverbed. Even a tiny layer of silt can form a blanket, preventing oxygen from reaching portions of a river or lakebed.

iii. Semi-Colloidal Solids

Semi-colloidal solids are very fine solids that, in all practical applications, do not precipitate from sewage samples even after a considerable period of time, however, they can be filtered out of the solution. Together with more readily precipitating solids, they constitute suspended solids that can be measured analytically for the effluent. The bulk of these solids are protein residues from cabin operations, mostly from liming procedures; however, substantial quantities of protein residues are also created owing to poor absorption by the tanning process, and another cause is poor absorption during the re-tanning process. Sludge is not directly caused by semi-colloidal substances. Bacteria may digest and break them down over time, resulting in solids that ultimately precipitate out.

iv. Gross Solids

Because gross solids are too big for a sample equipment to handle, they aren't measured. Their presence, on the other hand, is undeniable, and the risks they represent are well understood. Large bits of leather, trims and coarse shavings, skin residues, solid hair debris, and paper bag remnants are common waste components that cause this problem. Coarse bar screens installed in the wastewater flow may readily remove them. If they leave the workplace, on the other hand, they quickly settle in. If these items settle in the pipework, they can cause major difficulties by causing clogs. When blockages form in inaccessible pipes, the consequences can be disastrous. Replacing a burnt-out engine or a broken rotor is expensive. If trash is thrown into a river, it quickly accumulates, blocking the channel and creating stagnation [36].

2.5.2 Oxygen Demand

Bacteria break down many sewage components into smaller components. To live and break down their components, these bacteria (aerobic bacteria) require oxygen. This breakdown might be quick or take a long period, depending on their makeup. The delicate equilibrium maintained in the water will be overwhelmed if high-oxygen effluent is released straight into surface water. Water is depleted of oxygen, resulting in the death of oxygen-dependent plants, microorganisms, and fish. As a result, non-oxygen-dependent (anaerobic) microorganisms colonize the environment, resulting in hazardous water conditions. Low oxygen demand is tolerated by a healthy river. Tanneries, on the other hand, are frequently overburdened, and sewage must be cleaned before being released. This is generally accomplished by the use of bacteria in a functioning sewage treatment system: a procedure that requires a high quantity of oxygen [37].

2.5.3 Chemical Oxygen Demand

The quantity of oxygen required to oxidize organic materials with a potent chemical oxidant is known as chemical oxygen demand. The permissible limit for COD in water is 250 mg l^{-1}. If the value of COD is more than this permissible limit than water contain a high number of organic pollutants [37].

2.5.4 Nitrogen

High amount of nitrogen in water causes redox reaction and is responsible for production of nitrogen monoxide. Several different components of tannery wastewater contain nitrogen. Various forms of nitrogen in tannery effluent are discussed below:

i. Total Kjeldahl Nitrogen (TKN)

Some components of tannery wastewater contain nitrogen in their chemical structure. The most common among them are ammonia & nitrogen (from de-liming/depilation operations) from protein materials. These nitrogen sources pose two immediate problems.

1. Plants need nitrogen to grow, but high concentrations of nitrogen released by nitrogen-containing substances overstimulate plant growth. Hydrophytes and algae grow too fast, blocking waterways and damaging water currents. As the plant dies, a disproportionate amount of organic material must also be broken down. Plants, fish, and aerobic microorganisms perish when the load surpasses the river's natural supply of oxygen, resulting in anaerobic conditions.
2. Nitrogen is released as ammonia as a result of protein breakdown and deliming, before being released into the atmosphere, it can be transformed by bacteria in multiple steps. Decomposition products is nontoxic, but a lot of oxygen is needed in the process. Toxic anaerobic conditions can develop rapidly if the demand of oxygen is greater than the natural supply of the waterway. Nitrogenous compounds can be decomposed by enhanced anoxic & anaerobic biological treatment. The demand for oxygen is high, resulting in high operating and energy costs. The results show that about 40% of the oxygen demand is used to remove nitrogen components from the typical tannery wastewater [37].

2.5.5 Ammonium

As nitrogen content (N) is frequently mistaken with this term TKN. The difficulties related with fast plant development and oxygen consumption are the same since the ammonium component is part of TKN. Most of these compounds are the result of the deliming process and are produced in relatively small yields [37].

2.5.6 Sulphide

The usage of sodium sulphide and sodium hydrogen sulphide, as well as hair loss during the depilation process, contribute to the sulphide concentration of tannery effluent. Sulphides cause many problems: under alkaline conditions, most of the sulphides remain in the solution.

When the pH of sewage is below 9.5, hydrogen sulphide evolves from sewage: the lower the pH, the faster the evolution. The smell of rotten eggs can cause serious odor problems. The toxic equivalent of hydrogen cyanide, even at low concentrations, can cause headaches, nausea and possibly eye damage. At higher levels, deaths can occur rapidly, recording numerous deaths caused by the build-up of sulfides in sewage systems. The hydrogen sulfide gas is also quite soluble. When absorbed, weak acids form and cause corrosion, this weakened the metal roof, and the building's support. In sewers, the main problem may be corrosion of metal fittings, structural rubbers, and pipes. If released into surface water, even at very low concentrations, it can be toxic. Sulfides can be oxidized by certain bacteria in rivers to nontoxic compounds, but this creates a need for oxygen, which in excess can harm aquatic life [37, 38].

2.5.7 Neutral Salts

Two common types of salts to be found in tannery effluent are:

i. Sulphates

Sulfate is a component of tannery wastewater. It is produced from products with sulfuric acid or high (sodium) sulfate content. Sodium sulfate, a by-product of many auxiliary chemicals, is produced in the production process. Another source is the removal of sulfides from sewage through ventilation, since the oxidation process produces a range of substances, including sodium sulfate. These sulfates can be precipitated by calcium-containing compounds to form calcium sulfate with low solubility. However, there are two main reasons for the problem of soluble sulfates:

a. Sulfate cannot be removed completely from the solution by chemical means. Under certain biological conditions, sulfate can be removed from the solution and sulfur can be combined with microorganisms. In general, however, sulfates are either present in the form of sulfates or are broken down by anaerobic bacteria to produce malodorous hydrogen sulfide. This process occurs very quickly in sewage treatment, sewage systems and waterways, if the sewage remains static. The bacteria's conversion to hydrogen sulfide in sewage systems leads to corrosion of metal parts.
b. If decomposition does not occur, there is a risk of an increase in the total concentration of salts in surface water and groundwater [35].

ii. Chlorides

The chloride in tannery wastewater is introduced in the form of sodium chloride due to the use of a large amount of salt in tannery wastewater during leather preservation or pickling. They are highly soluble and stable, immune to sewage treatment and natural conditions, and therefore remain a burden on the environment. A considerable amount of salt is produced industrially and can rapidly rise to the highest acceptable level of drinking water. The increasing salinity of groundwater, especially in areas with high industrial density, is becoming a serious environmental hazard.

Chlorides inhibit the growth of bacteria, plant & fish in surface water, too much of them can lead to the collapse of cell structure. If the water is used for irrigation purposes, the surface salinity increases due to evaporation and crop yields decline. When washed out of the soil by rain, the chloride re-enters the ecosystem and may end up in groundwater. High salinity is only acceptable when sewage is discharged into the tidal/marine environment [36].

2.5.8 Oils and Grease

During the manufacture of leather, natural oils are released from the skin. If the fat solution is exhausted, the wastewater may produce some fatty substances when mixed. Floating grease and fat particles clump together to create "mats," which then mix with other contaminants, potentially blocking wastewater treatment systems. The quantity of oxygen transferred from the atmosphere is lowered when surface waters are polluted with grease or a thin coating of oil. Due to their biodegradability, when these fatty compounds are emulsified, they produce a very high oxygen demand [37].

2.5.9 pH Value

The pH of wastewater discharged into surface waterways and sewers must be between 5.5 and 10.0. While restrictions are frequently imposed, higher pH levels are typically tolerated since CO_2 from biological activities in the environment or in healthy surface water systems is highly efficient at bringing pH to a neutral condition. Sensitive Fish and plants are vulnerable if the pH of the surface water is too far from the 6.5-7.5 range. Municipal and general treatment plants tend to discharge more alkalinity as it reduces the corrosive effect on concrete. Metals tend to be insoluble and inert, limiting the formation of hydrogen sulphide. When biological activities are included in the therapy, carbon dioxide is used to lower pH levels to more neutral values [37].

2.5.10 Chromium Compounds

Metal compounds are not biodegradable, therefore, they can be considered as long-term environmental components, because they can also have cumulative properties, they are the object of close attention. Two forms of chromium are associated with the tanning industry and their properties are often confused.

i. Cr (III)

Chromium mainly exists in the waste produced in the chrome tanning process. As part of the re-tanning system, it is discharged from the leather during re-tanning and dyeing. This chromium is discharged from the process in a soluble form; however, it reacts very quickly when mixed with tannery wastewater from other processes, especially if protein is present. It also forms a very fine colloid, and then stabilizes the chrome-effect. As a result, these components are highly resistant to biodegradation and inhibit biological processes in surface water and treatment plants units. Chromium hydroxide precipitates after successful decomposition and stays in the environment for a long time. If too much chromium is released, it may end up in the solution. It can be harmful to water fleas even at low doses, affecting the fish feeding chain and perhaps limiting photosynthesis [37].

ii. Chrome (VI)

Tannery wastewater is unlikely to contain this form of chromium. Dichromate is toxic to fish because it penetrates rapidly through cell walls. They're mostly absorbed by the gills, although they can build up. Analysis requires a high level of expertise. The study is based on colorimetric measurements at 670 nm, and the predicted concentrations are often quite low [38].

2.5.11 Other Metals

Aluminum and zirconium are two other metals that may be emitted by tanneries and are subject to legislative limitations. The toxicity of these metals varies depending on the chemical species, and it is also impacted by the pH of other organisms, complexing agents, and water. Green algae, in particular, appear to be inhibited by aluminum, and crustaceans are sensitive to low levels of aluminum. Cadmium is a very poisonous metal that is occasionally used in yellow colors. It is cumulative and has a long-term impact on a variety of species. It can cause brittle bones if it is found in drinking water [37].

2.5.12 Solvents

Degreasing and finishing procedures produce solvents that are released into surface water and produce a micro-film on the water's surface, preventing oxygen uptake. Solvents degrade in a variety of ways, with some suppressing bacterial activity and remaining in the environment for longer [37].

2.6 Bioaccumulation of Heavy Metals in Plants Grown with Tanneries Wastewater

Tannery wastewaters induce phytotoxic effects, and the biomagnification of heavy metals causes stress to the plants such as salt stress, which seriously affects the metabolic processes of the plants, and leads to the decline of the nutritional status of the plants and the inhibition of the growth and development of the plants. It has great influence on photosynthesis, respiration, mitotic activity and germination of embryo, tannery wastewater also increases the production of reactive oxygen species (ROS). Excess chromium, chlorides, dissolved solids, sulphides, and high BOD and COD levels in wastewater over the tolerance limit hinder seed germination and seedling development. More than 80% of high effluent concentrations were found to be fatal to plant development at both the vegetative and reproductive phases [40, 41].

2.7 Local Studies

According to a study, the wastewater from the Muridke Firdaus tannery is highly salty and sodic. It had been used to irrigate the fields near the tannery, but there was a wasteland where the water was not acceptable due to the high elevation. The vegetation of the wasteland and irrigated land was studied. The effect of sewage treatment on soil properties under irrigation condition was studied and compared with that of wasteland soil. These soil samples were taken from the surface and from depths of 5,10 and 20 cm. Soil water content, water-stable aggregates, cation exchange capacity, water holding capacity, organic matter content and exchangeable sodium content in irrigated land were higher than those in wasteland soil. The electrical conductivity, pH value, calcium, magnesium, soluble sodium, chloride, sulfate content, sodium absorption rate and exchangeable sodium content of the abandoned soil were significantly higher than those of the irrigated soil. From the surface to a depth of 20 cm in the soil, these data indicate a declining tendency at each site [42].

A study conducted on tannery wastewater showed that toxic heavy metals were accumulated in soil and groundwater during tanning. Toxic contaminants from heavy metals rendered the fresher ground water unfit for human consumption, because fresh water is in short supply for irrigation and residential use, heavy metal bioaccumulation in edible plants and vegetables contaminates the food chain and constitutes a severe hazard to human health [43].

The results of a study conducted on tannery wastewater showed that the effluent quality is high salt and low sodium. The results of this study clearly show that germination is significantly reduced by high effluent treatment. The results of biochemical analysis showed that the concentration of wastewater was unfavorable to the growth of sunflower seedlings.

Sewage treatment has adverse effects on chlorophyll, protein, and carbohydrate contents. At higher effluent concentration, the photosynthetic matter decreased, making the plant appear yellowish. High concentration of wastewater reduced biomass accumulation and reproductive growth of sunflower cultivars. The seed yield was reduced by more than 70% under the higher sewage dosage [44].

In another study harmful impacts of Leather industry on environment are stated as:

2.7.1 Air pollutants

Various air pollutants such as H_2S, NH_3, SO_2, CO_2, Cl_2, formic acid fume, and volatile organic compounds are released into the environment during leather manufacturing. In the leather finishing operations of liming, deashing, and pickling, H_2S, NH_3, and Cl_2 are generated.

2.7.2 Impacts on Worker

H_2S is a colorless gas that smells like rotten eggs. In tanneries, workers work without masks and inhale H_2S. If the H_2S level is above 900 ppm in a minute, it can cause a coma. Moderate exposure to H_2S (50-100 mg/L) causes olfactory tiredness, respiratory irritation, and keratoconjunctivitis, but high levels (250-500 mg/L) cause olfactory paralysis, pulmonary edema, severe lung, and eye irritation. NH_3 and its oxides are corrosive and alkaline, causing irritation and damage to the skin, eyes, and airways. Gaseous chlorides are toxic and can cause respiratory complications. Excessive inhalation of concentrated chlorine gas can cause loss of consciousness. Long-term exposure of leather workers to leather dust, lead, nitrogen dioxide, sulfur dioxide and hydrogen sulfide leads to higher morbidity and mortality [45, 46].

2.7.3 Solid Wastes

During leather processing, only 150 kg of the 1,000 kg of raw leather is converted into finished leather, while 850 kg is generated as various forms of solid waste. Solid waste contains nitrogen, sodium chloride, dichloromethane, sulfide, calcium, iron, cadmium, chromium, and other pollutants [46].

2.7.4 Chromium and Cadmium Impact

The histopathology changes of spleen and kidney and the changes of myocardium and myogastric histopathology in *Coturnix japonicus* after chromium poisoning were observed. These changes include myocardial longitudinal division, myocardial degeneration, myocardial necrosis, myocardial pigmentation, myocardial and gastric nuclear dislocation and so on. The presence of cadmium in tissues leads to a decrease in serum iron and hemoglobin levels, which eventually leads to hematopoietic stimulation. Cadmium poisoning also led to growth retardation and had adverse effects on testicular function [47].

2.7.5 Water Pollution

The leather industry is associated with high water consumption, requiring up to 4,000 liters of water per ton of suntanned skin. During the leather processing, a large number of chemicals are used and discharged untreated into streams, ponds, and rivers, causing groundwater pollution. A single tannery can contaminate groundwater within a 7-8 km radius, with devastating effects on aquatic life, birds, animals, and humans. The elements found in contaminated groundwater are sodium, calcium, magnesium, chloride, sulfate ions and Cr (VI), which contribute to the hardness of the water and thus to the kidney's stones, stomach problems and heart problems. Elevated levels of sodium and chloride ions in groundwater can lead to cardiac arrest, high blood pressure, and asthma. Sulfate ions can cause gastrointestinal distress and dehydration. Ingestion of Cr (VI) dissolved in groundwater can cause serious health problems such as ulcers, genetic mutations, respiratory diseases, and liver and kidney damage [48].

The wastewater from the leather industry also contains ammonium nitrogen and germanium. Elevated levels of nitrogen in drinking water can lead to nitrate interference in the baby's Methemoglobinemia. High intakes of germanium ions bind to proteins, leading to lung cancer, atrophy of the nasal membranes, and nosebleeds. Contaminated groundwater near tanneries can cause different health problems, such as skin diseases, allergies, tumors, and gastrointestinal problems. High acidity wastewater and heavy metals combined with amino acid sulfhydryl group can lead to corrosion of intestinal mucosa and inhibition of essential enzyme activity [26].

2.7.6 Workers Health

The exposure rate of tannery workers to chromium salt, arsenic, benzene, formaldehyde, ethanol, toluene, ethylene solvent and other carcinogenic compounds is very high. Chromium chemicals are the main compounds used in the tanning process, in tanneries, chromium exists in the form of sulfate, inorganic and protein combination, which is called leather dust. Ingestion of these compounds can lead to a variety of cancers, including bladder, lung, kidney, oral, pancreatic, nasal cancers, soft tissue sarcomas, skin cancers, ulcers, and respiratory diseases [21]. Various types of cancer among tannery workers due to exposure to leather dust are discussed below:

i. **Skin Cancer**

Exposure to leather dyes and fats causes skin cancer in leather workers. Skin melanoma and skin cancer rates are higher among women than among men in leather factories. Skin cancer is usually caused by direct exposure to Cr (VI) [21].

ii. **Buccal Cavity and Pharynx Cancer**

This type of cancer is observed in those workers who do their jobs in tanning and liming workshop. Exposure to variety of heavy metals for more than 10 years cause oral cancers. Heavy metals accumulate through air passageways and cause cancer [21].

iii. **Pancreatic Cancer**

Tanning workers are more exposed to formaldehyde, which can lead to the development of pancreatic cancer. Cadmium is also considered to be the cause of pancreatic cancer because this metal accumulates in human pancreas [21].

iv. **Bladder Cancer**

Aromatic amines and phenyl dyes are considered to be carcinogens. Long-term exposure to these dyes can cause bladder cancer in tanning workers. Researchers pointed out that the prevalence of bladder cancer differed by gender, with women having a considerably lower chance of developing the disease than men [21].

v. **Testicular Cancer**

An excess risk of testicular cancer incidence was observed among leather tanners from the finishing department of the tannery and were exposed to dimethylformamide, a substance known to cause testicular damage. Prolonged exposure to acetone can also cause testicular damage. The workers in the leather processing department suffer with this type of cancer. Compared to the rest of the population, the rate of testicular cancer was 7.2% [21].

vi. **Soft Tissue Sarcoma**

This uncommon malignancy was shown to be substantially more common in two different tannery investigations. Both researchers believe that the chlorophenols used in the pre-tanning and tanning processes are to blame for the malignancies. The rare cancer was caused by exposure to chlorophenols in tannery workers [21].

2.7.7 Disease Pattern in Kasur

The district health information system health records provided by the district headquarters hospital Kasur demonstrate that there is a well-known link between common illnesses and environmental pollution caused by the tanning industry. In children, asthma, acute (upper) respiratory infections, and diarrhea/dysentery are the most frequent diseases; in adults, typhoid fever, high blood pressure, dermatitis, neuropsychiatric disorders, eye disease, nephritis, and chronic cough are the most common diseases. During the years 2008 and 2009, the district health information software (DHIS) data clearly reveals that respiratory illnesses (induced by air pollution: smoking and polluted soil dust) and diarrhea/dysentery (caused by drinking water contamination) were the most prevalent ailments among the recorded patients. In comparison to other Kasur sites and control samples, soil in regions exposed to tannery effluents had high amounts of Cr, Mn, and Ni. In comparison to far-flung places such as government girls' high school on haji freed road and college road near Rohi Nullah, Din Ghar and Kot Haleem Khan contain high levels of Cr, Mn, and Ni. As tannery effluent is cleaned and released onto untreated fields, fields close Chromium levels rise. Due to the natural flow of the drain due to the soil gradient, farms surrounding Kamal Chishti and Shah Abdul Khalak colony have no high levels of Cr. Annually, 40,000 tons of basic chemicals and 15,000 tons of chromium sulphate are projected to be utilized, with much of it being released as residual waste in wastewater that may be collected, and repurposed [49].

According to studies, air pollution from drinking water, soil, and the tanning industry causes illnesses in the majority of metropolitan populations. Despite all of these dangers, occupational health regulations, the avoidance of hazardous chemical exposure, and workplace safety have received little attention. Chemicals have been improperly managed and handled, exposing employees and the general public to dangerous compounds [49].

In Lahore, Kasur, and Kala sha Kaku, groundwater is a major source of drinking water and irrigation. The dumping of untreated industrial effluents into water bodies has contaminated groundwater quality. The results of the study revealed a high level of metal contamination at the industrial area in Lahore, Kasur, and Kala Shah Kaku. All the metals have been amplified in concentration. The average contents of metal elements in groundwater showed a decreasing trend (Mn > Zn > Cu > Cd > Cr > Pb > Ni). Table 2.2 shows descriptive statistical data of groundwater samples along industrial zones from research done in three areas: Lahore, Kasur, and Kala Shah Kaku [50].

Table 2.2: Descriptive statistical data of groundwater samples along three different tannery industrial zones.

Parameters	**Mean**	**Max**	**WHO**
pH	7.16	7.71	6.5-8.5
TDS (mg/ml)	738.59	999	1400
EC (mg/l)	1367.08	2280	1000
Na (mg/l)	9.73	17.49	200
HCO_3 (mg/l)	9.48	16.1	350
Cl (mg/l)	2.40	5.30	250
Cr (mg/l)	0.18	1.30	0.05
Zn (mg/l)	1.25	4.50	3
Mn (mg/l)	1.28	7.50	0.5
Cu (mg/l)	0.799	5.10	1.2
Cd (mg/l)	0.018	0.132	0.003
Pb (mg/l)	0.12	0.688	0.01
Ni (mg/l)	0.12	1.320	0.07

Another study conducted to assess the ecotoxicity potential of tannery effluent wastewater and its chromium-based components, such as potassium dichromate and chromium sulphate, the researchers used a variety of bioassays. The concentration of chromium in tanneries effluent was determined by particle-induced X-ray emission (PIXE), and then equivalent amounts of Cr (VI) and Cr (III) were acquired for this investigation. The eco-toxicological potential of varied concentrations of tanneries effluent, was investigated using cytotoxicity tests, artemia biological toxicity tests, and phytotoxicity analysis. In these analysis, potassium dichromate and chromium sulphate showed concentration-dependent cytotoxic effects. The results clearly shows that varied dilutions of potassium dichromate caused considerably greater damage to vero cell, brine shrimp, and maize seed germination than the other two materials. Surprisingly, the overall toxicity effects of the tannery wastewater treated groups came after the potassium dichromate treated group. Based on the biological evidence presented in the article, it was concluded that hexavalent chromium, potassium dichromate and tanneries wastewater have significant eco-damaging potential, clearly elaborating that the environmental burden in Kasur is numerous, and high levels of chromium pose a significant risk to the human population, aquaculture, and agricultural industry, which can obliterate [51].

Tannery industrial units discharge dissolved salts, BOD, COD, TDS and TSS, heavy metals, and a variety of other hazardous chemicals into the drainage system [52]. Wastewater used for irrigation is potentially hazardous to animals, crops and human health [53]. Wastewater containing heavy metals such as chromium, zinc, arsenic, cadmium, and lead is phytotoxic and easily transported through vascular tissues. The accumulation of toxic metal elements in plant tissues leads to abnormal plant metabolic activities and affects plant growth and development [51, 54]. Excessive heavy metals can produce ROS and cause serious damage to biofilms. ROS can cause oxidative stress, membrane lipid peroxidation, DNA and RNA damage, chloroplast breakdown, enzyme inhibition, amino acid oxidation and protein degradation [55, 56]. Chromium enters the environment mainly in the form of Cr (III) & (VI). Cr (VI) is more toxic and mobile. However, the important role of Cr in tannery wastewater in the process of human metabolism and its carcinogenic effect have attracted wide attention in recent years [57, 58, 59].

The effects of tannery effluent on the growth and yield of three sunflower varieties showed that the wastewater contained high content of mineral elements and heavy metals, so it was not suitable for irrigation [60, 61]. In addition, the effluent is highly alkaline, with high values of EC, BOD, COD and SAR. manganese, iron, zinc & chromium are also fairly high. Sewage treatment has adverse effects on plant growth and yield, especially high-dose sewage from seedling stage to mature stage, which leads to the decrease of biomass and seed yield compared with control. In the Kasur area, tannery effluent used for irrigation purposes is toxic to the growth and yield of this important crop [62].

2.8 Regional Studies

The contamination of metals is a serious environmental issue, particularly in the aquatic environment. Dyes and tannins disturb the transparency of water and block sunlight from entering into water [63].

Residual metals in polluted sediments from the tannery industry's environment may accumulate in microorganisms and enter the food chain, posing a health risk to humans. Furthermore, even after secondary treatment, organic contaminants in tannery wastewater cannot be fully destroyed, allowing pathogenic bacteria such as E. coli, streptococcus, staphylococcus, anaerobic bacillus, and other hazardous germs to thrive. Together with tannery effluent, these pathogens quickly infiltrate the aquatic environment and the surrounding ecosystem, posing severe health risks to living species [64].

Different heavy metal powders were used in the re-tanning process of leather to prepare antibacterial leather. It was concluded that the leather wastes had antibacterial activity [65]. The results of the Study conducted by a researcher in [66] also show that tannery waste after lather treatment sample has antibacterial activity against almost all living organisms. Chromium has carcinogenic and genotoxic effects in humans and animals, as well as immunotoxin effects. Chromium interferes with the absorption, transport, and accumulation of calcium, potassium, magnesium, phosphorus, boron, and copper at the top of the plant, and by interfering with iron metabolism, it exacerbates iron shortage and chlorosis. Plants respond to sewage in different ways at different phases of growth, and the degree of toxicity varies depending on the plant type. Chromium toxicity weakens photosynthesis and inhibits chloroplast synthesis. The chromium concentration of plants near the emission unit was higher, the leaves were necrotic and chromium content in the leaves was higher. It is well known that it induces the production of reactive oxygen species and oxidation of lipids. The high concentration of chromium in irrigation water will reduce the uptake of many essential elements such as iron, zinc, and copper [67].

Also, the use of these wastewater for irrigation poses a serious threat to plants because of the excessive accumulation of metals in their bodies, the effects of which have been extensively studied worldwide, When Vigna radiata was watered with high quantities of tannery effluent, the carotenoid content decreased. The degradation of chlorophyll and carotenoid is the most common response of plants to increased exposure to various heavy metals. Zinc and iron are important components of various enzyme systems for energy production, protein synthesis and growth regulation. Supplemental zinc and iron can enhance the plant's superoxide detoxification mechanism by producing hydrogen peroxide. The growth of these metals under adverse conditions will produce ROS, destroy the photosynthetic mechanism, and may catalyze the degradation of proteins by oxidative modification and increasing the hydrolytic activity of proteins [68].

Another Study conducted on chicken that were made to feed on skin cut waste of dry hides clearly demonstrate heavy metals accumulation in chicken. Contaminated chickens can further transmit these heavy metals to humans through consumption. Therefore, consumption of contaminated chicken poses a vital risk to human health [69].

Tanneries are the main source of environmental pollution, mainly chromium pollution. India has about 2,500 or more tanneries, the vast majority of which (80 per cent) are chrome-based tanneries. Chromate is the main raw material for conversion of skin to leather in leather-making process. Wastewater containing rich organic matter, phenolic heavy metal and tannin will cause serious environmental pollution. Chromium-contaminated environments affect soil biodiversity and, once in the food chain, lead to serious human diseases, ranging from minor affects such as skin irrigation to lung cancer in humans and other animals [70].

In a study, the effects of tannery wastewater on seed germination were observed in a culture dish for rice cultivation. Seedling growth was measured by root length, shoot length, vigor index, tolerance index, photosynthetic pigment content and Carotenoid content. Rice seeds from the Chinsurah Rice Research institute of west Bengal were selected and planted in Petri dishes irrigated with different sewage concentrations control (0%), 20%, 25%, 50%, 75% and 100%]. In the soaking process, the lower the concentration of wastewater, the higher the imbibition rate. With the increase of the concentration, the imbibition rate gradually decreased. In the diluent of less than 50%, the effluent had growth promoting effect, but at higher concentration (more than 75%), the effluent inhibited germination, inhibited the decrease of root length, vigor index and tolerance index, levels of photosynthetic pigments (chlorophyll and Carotenoid) also declined [71].

2.9 Global Studies

It is obvious that every step of leather production will produce waste and pollution. The leather industry's environmental impacts are comparable to "pollution created by 1,000-4,000 citizens per every ton of processed animal hide."[72].

Starting with 2,200 pounds of raw leather, about 550 pounds of leather will be produced, along with 3,962-13,208 gallons of wastewater and 992-1,609 pounds of solid trash [73]. Heavy metals are released into water, high COD, gaseous emissions (H_2S, NH_3) are the primary pollutants generated during leather manufacturing [74].

If not handled properly, these emissions can cause “Significant toxic affects e.g. ecotoxicity, aquatic acidification, non-carcinogens & aquatic eutrophication, and may pollute groundwater further”. This industry is considered “One of the most polluted in the world and is responsible for 40 % of global chromium pollution.” [75].

Inefficient and unsustainable tanning methods contribute significantly to the wasteful use of water and chemicals, and to the release of significant pollutants and the low productivity of the tanning industry. The use of chemicals results in toxic substances in wastewater, which are harmful to the environment and human health. Chlorophenols and chromium were shown to be strongly linked to waste from tanneries and other sources. Chlorinated phenolics, such as 3,5 dichlorophenol, have been discovered to be extremely hazardous cellular molecules that damage the organisms exposed to waste as organic pollutants in the leather industry. Olfactory paralysis, severe lung and eye irritation, pulmonary edema, and loss of consciousness occur in humans after prolonged exposure to 250-500 mg/l. High concentration of Cr (VI) is toxic, mutagenic, carcinogenic & teratogenic. It mainly exists in soluble and highly toxic anions in nature. Moreover, tanneries, which use sodium chloride as a raw material, release high concentrations of chloride and nitrate as the end products of nitrogen oxides, carbonic acid, sodium bicarbonate, carbonic acid, sodium chloride and calcium chloride cause soil to alkalize during tanning, to increase the pH of the soil [76].

Tannery effluents are a large-scale environmental problem because they degrade the quality and color of the water. The main problem with these organics is the reduction of dissolved oxygen in river water due to microbial decomposition. The primary impact is a loss of dissolved oxygen, which is harmful to aquatic life and stimulates anaerobic activity, which results in the production of poisonous gases. The effect of sewage irrigation water on plant development and production is significant. Toxic heavy metals are biomagnified at various nutritional levels throughout the food chain. Tannery wastewater has phytotoxic effects on plants, resulting in the accumulation of heavy metals and has a significant impact on respiration, photosynthesis, germ sprouting, and mitotic activity, as well as increasing reactive oxygen production. However, the amount of accumulation is determined by the plant species, elements, bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature, and root secretion. Excessive chromium, dissolved solids, chloride, sulphide, high BOD, and high COD in the effluent exceeded the tolerance limit, which is detrimental to crop development and inhibits seed germination and seedling growth at low dilutions [77]. Similar studies by a researcher [78] reported tannery wastewater has the same impact on maize, soybean, and wheat plant development.

Particulate matter, polycyclic aromatic hydrocarbons, lead, ground-level ozone, heavy metals, sulphur dioxide, benzene, carbon monoxide, nitrogen dioxide, hydrogen sulphide, and ammonia waste from tanning are the primary contaminants in the air we breathe. The air is impacted during the soundless liming and softening processes [79].

Long-term exposure from 5 months to 14 years for leather factory workers is a risk factor for disease development associated with genetic damage. Since the toxic substances such as chromium, lead, hydrogen sulfide, zinc, formaldehyde & cadmium released by tanneries, may react temporarily, such as headaches, irritation of the eyes, dizziness, skin or lungs, poisoning of the liver, kidney or nervous system, allergic reactions, exhaustion due to lack of oxygen, & long-term diseases such as ulcers, occupational asthma, Bronchitis, dermatitis & genetic defects may occur [77]. Chromium is an essential metal element for glucose metabolism in humans and animals, but its hexavalent chromium has strong toxicity, mutagenicity, and carcinogenicity [80].

Hexavalent chromium is highly mobile in most environments, mainly because of its solubility, and has a negative impact on the environment due to its outstanding solubility, mobility, and reactivity. The soil and groundwater environment are the main sources of Cr (VI) pollution, mainly from spills, illegal disposal, and unattended stocks of new technologies for chromium. Cr (VI) is very easy to get into organisms through a variety of pathways, such as ingestion, near the epidermis, inhalation, and absorption (in the case of plant and root ages) Cr (VI) affects the activity of amylase in plant, which plays an important part in the process of seed germination. In general, if chromium levels in drinking water is more than 5 mg/l then it is considered to be chronic poison [81].

In a study the wastewater from a tannery wastewater treatment plant (TWTP) as the research object was taken, the influence of TWTP was evaluated from three aspects: root elongation, shoot length and germination rate on *P. australis*. Generally speaking, with the increase of the proportion of wastewater, the inhibition of seed germination is also enhanced. However, when the wastewater is treated, its toxicity is reduced. The germination rate of *P. australis* seeds was higher, and the germination rate of seeds from different substrates was similar, which further indicated that *P. australis* was a strong plant with tolerance to different wastewater and growth conditions.

In another study three-time 54 plant species, 24 water samples, and 18 soil samples were obtained from the leather industry's upstream, treatment plant, and downstream. The results were analyzed using R software. The bioaccumulation of chromium was higher in the lower vegetable samples than in the upper vegetable samples. The chromium content in root vegetables was the highest, and that in fruit vegetables was the lowest. The content of Cr in vegetable, water, and soil of different upstream and downstream were significantly different ($p < 0.01$, $p < 0.05$, $p < 0.001$). The bioaccumulation order of Cr was root diet > leaf diet > fruit diet. (A study of irrigated farmland in Wukro) [82]. Toxic trace metal poisoning can cause neurological and cardiovascular disorders, as well as renal failure. The most prevalent illnesses affecting big populations include cholera, hepatitis, dysentery & typhoid. Wastes may have an indirect impact on human health by contaminating drinking water, the food chain, or seafood.

The toxic effects of heavy metals on crops such as water chestnut, cabbage, tomatoes, peppers & rice have been observed. Continuous metal exposure of plants affects physiological processes such as water relationship, photosynthesis & mineral nutrition. The main toxic effects were chlorosis, yellowing of leaves, immature defoliation, stunted growth, and decline of flower, fruit, and green yield. Metabolic changes induced by metal exposure have also been described as direct effects on enzymes or another metabolism. This may be due to the interaction between metals and plant nutrients resulting in plant nutrients and nutritional disorders [83]. Table 2.3 elaborate various diseases caused due to chemicals released from tannery industry.

Table 2.3: List of various diseases caused by heavy metals released from tannery industry [98].

Metals	**Health hazards**
Cadmium	Inhibits functioning of enzymes, affects gastrointestinal tract, lungs, and bones, causes renal problems
Mercury	Headache, intestinal problem, blood malfunctioning
Chromium	Carcinogenic, leads to kidney disorders, ulcer, nervous disorder
Lead	Anemia, abdominal pain, damage to nerves, convulsion, hypertension
Arsenic	Liver damage, ulcers, kidney problems, dermatological disorders
Copper	Mental stress, coma, uremia
Zinc	Kidney problems, pain in legs, vomiting
Nickel	Decreases body weight, damages heart and liver, causes skin irritation
Fluoride	Fluorosis
Aluminum	Weakens nervous system

Total chromium (Cr), total Cr (III), and total Cr (VI) were measured in Kombolcha tannery effluent and samples from the surrounding area (soil and lettuce plant). By using flame atomic absorption spectroscopy and UV/ Vis spectrophotometry, the concentrations of total Cr (VI) and total Cr (III) in wastewater, soil, and lettuce were measured and compared to total Cr (VI) (VI). The total Cr, Cr (III), and Cr (VI) concentrations in the collected samples were highest near the discharge site and lowest downstream of the ruby tannery effluent (400m from the junction of leather industry wastewater). The lowest concentration of Cr (VI) in lettuce, on the other hand, was discovered. All samples, with the exception of soil samples, showed total chromium levels that exceeded the WHO/FAO permitted limit. Except at a distance of 400 meters from the junction, the Cr (III) concentration in all wastewater samples above the permissible threshold, while the Cr (VI) concentration in the wastewater also exceeded the acceptable level. So, appropriate treatment procedures for wastewater should be used to guarantee that it is properly treated and does not harm the environment [84].

Under laboratory circumstances, the effects of tannery effluent on seed germination of six vegetable crops were investigated. The major goal of this research was to perform a thorough investigation of the germination of vegetable seeds treated with various types of sewage in East Shoa, Ethiopia. Measured amount of tannery effluent was mixed with 0,25,50,75 and 100% distilled water, to treat vegetable seeds of onion, carrot, swiss chard, beetroot, cabbage, tomato. Complete randomized design was used to carry out seed germination experiments, each experiment was repeated 3 times, with each time 5 sewage concentration. The effects of sewage and distilled water (control) were compared on all vegetable seedlings cultivated at varying sewage concentrations. Germination rate, shoot length (cm), root length (cm), seedling length (cm), fresh weight per plant (mg), dried weight per plant (mg), and relative toxicity (percent) were the sewage treatment parameters. Except for onion, carrot, and tomato, which were sensitive to 75 percent and 100 percent sewage mixtures, swiss Chard, beets, and kale were resistant to all sewage concentrations, the results showed that low concentration wastewater had no effect on seed germination and other morphological parameters. Vegetable seeds were sorted in the following order based on their resistance to tannery wastewater: cabbage > beetroot > tomato > carrot > onion > swiss chard. All process parameters decrease in proportion to the rise in effluent concentration. pH, temperature, EC, BOD, COD, TDS, cadmium, chromium, copper, iron, lead, and zinc were all measured in the water. Except for EC, COD, and Cr, the majority of the findings are below the optimum value [85].

Tannery effluents were collected and studied in Kano State's Sharada industrial region, under laboratory conditions, the physical and chemical parameters and heavy metal contents of tannery wastewater were analyzed, and the effects on seedling growth & seed germination of spinach, maize & lettuce were studied. Using completely random design method five concentrations of wastewater were made, 20%, 40%, 60%, 80%, 100%. When the concentration of wastewater was higher (80% and 100%), the average germination percentage, plant toxicity, seedling length, root length, dry weight & fresh weight were significantly decreased. The average toxicity of sewage concentration to plants was as follows: maize 82% > lettuce 75% > spinach 29%, germination rate of spinach 31% > lettuce 25% > maize 22%. The results showed that the sensitivity of maize seed to tannery wastewater was higher than that of other seeds, which indicated that tannery wastewater had adverse effects on maize seed germination and seedling growth. The physical and chemical parameters of irrigation water and the average value of heavy metals were within the standard allowable value of WHO, but the average value of chromium was 1.32 ± 0.35 mg/l. While some concentrations of heavy metals are within the allowable limits, the specific problem associated with heavy metals in the environment is their accumulation through the food chain, with long-term effects. At high concentrations, tannery effluent can impair seed types. The results show that morphological examination of tannery effluent promotes seed germination at lower concentrations while inhibiting seed germination at higher concentrations [86].

Chapter 3

Materials & Methods

This research study was conducted in greenhouse laboratory of Atta-Ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Pakistan, and the experiment was started in December 2020. Scheme of work to investigate the harmful impacts of tanneries wastewater on lettuce plant is given in figure 3.1.

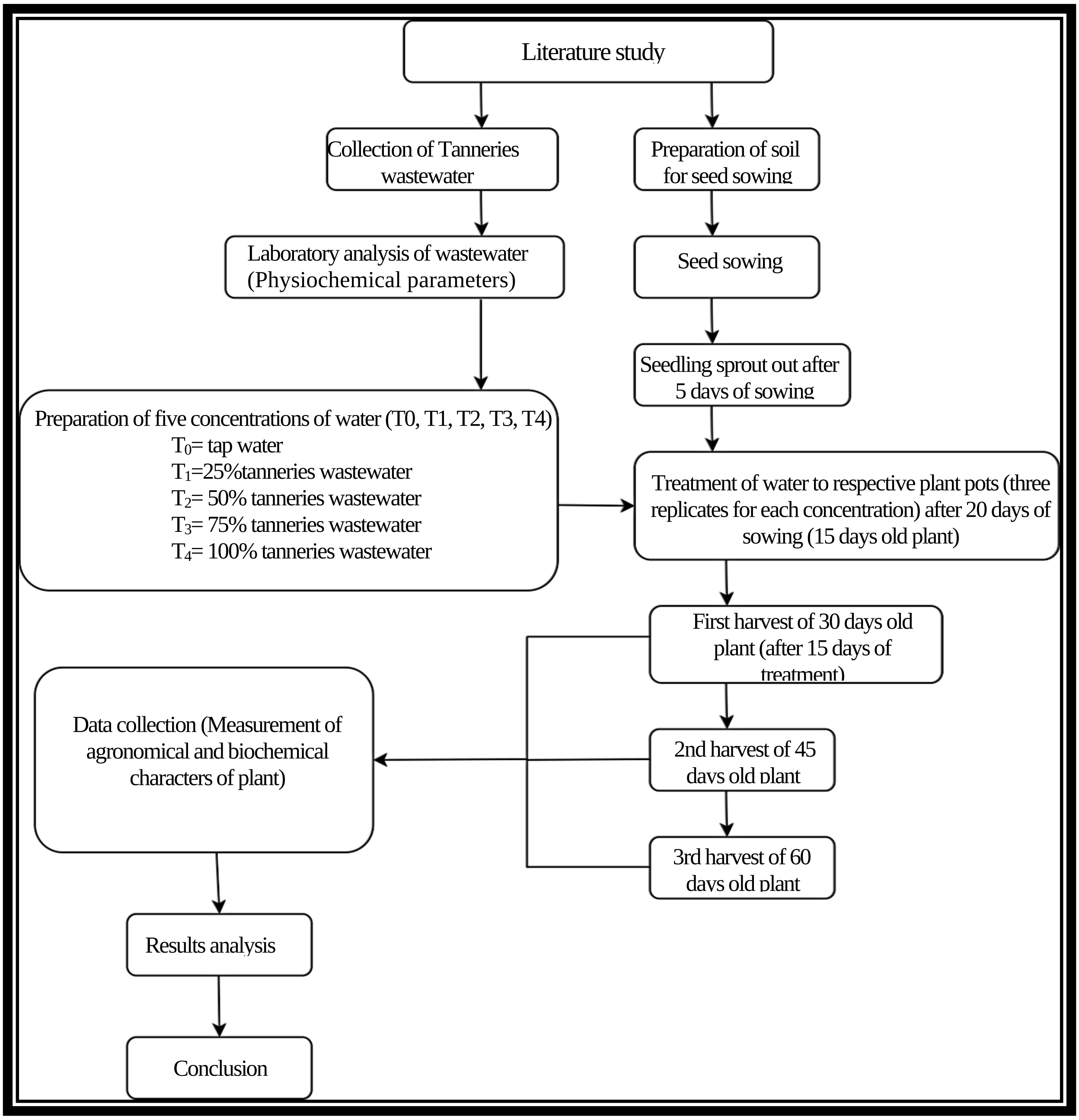

Figure 3.1: Different stages of scheme of work to determine impacts of tannery wastewater on *Lactuca sativa*

3.1 Selection of Lettuce plant

Lactuca sativa, also known as lettuce, is a cool-season, leafy vegetable that belongs to the cicoreae tribe of the compositae family. Figure 3.2 depict the typical image of *Lactuca sativa* variety of family compositae. Lettuce is a well-known green vegetable that may be used in a variety of ways, from salad to medicinal preparations. Since the notion of green goods has taken hold across the world, lettuce has grown in popularity not just as a food item, but also in a variety of other forms. There are a lot of characteristics that make lettuce ideal for research. It has a brief life cycle, is entirely self-fertile, and has a high natural self-pollination rate.it is possible to carry out a large number of crosses on one plant, and individual plants require only a moderate amount of space [87]. Seeds of Lettuce (*Lactuca sativa*) were obtained from NUST nursery. Various biological attributes and cultural conditions of selected plant are given in table 3.1.

Figure 3.2: Image for *Lactuca sativa* of family compositae

Table 3.1: Various attributes and cultural conditions of selected plant [87]

Attributes	
Genus	Lactuca
Species	Sativa
Family	Asteraceae
Life Cycle	Annual
Recommended Propagation Strategy	seed
Region of Origin	Mediterranean
Edibility	Leaves can be used raw or cooked in salads, sandwiches, and other dishes.
Whole Plant Traits	
Plant Type	Annual, edible, vegetable
Woody Plant Leaf Characteristics	Deciduous
Habit/Form	Erect
Growth Rate	Rapid
Cultural Conditions	
Light	Full sun (6 or more hours of direct sunlight a day) partial shade
Soil Texture	High organic matter
Soil pH	Neutral (6.0-8.0)
Soil Drainage	Good drainage/moist
Available Space to Plant	Less than 12 inches

3.2 Collection of Tanneries Wastewater

Tanneries effluent was collected from Tanneries Effluent Treatment Plant, Kasur. Discharge of tanneries effluents in Kasur tannery treatment plant is given in figure 3.3. The effluents were kept in open container for its use in experiment. One liter of the effluent was refrigerated at 4C^o for chemical analysis.

Figure 3.3: Discharge of tanneries wastewater at Kasur tannery treatment plant

3.3 Soil Treatment

Sandy loam soil was taken from NUST Nursery and was used for filling in pots after mixing of animal manure and garden manure in 3:1 ratio. Each pot was filled with 1kg of soil in 3:1. Soil was measured on weighing balance as shown in figure 3.4

Figure 3.4: Measurement of weight of one pot of soil

500 ml of tap water was given to each pot on first day. In the second day pots were given 250ml tap water and left for seven days and were observed daily to assess moisture level. After a week the soil had retained suitable moisture for sowing.

3.4 Wastewater Concentrations for Irrigation

Following five concentrations were used for pot irrigation during the experiment.

T_0: control, tube well water was used.

T_1: **25%,** 25 ml of effluent + with 75ml of tube well water.

T_2: 50%, 50 ml of effluent + with 50 ml of tube well water.

T_3: 75%, 75 ml of effluent + with 25 ml of tube well water.

T_4: **100%,** Effluent water was directly used.

Figure 3.5 is a typical demonstration of wastewater concentrations from T_1 to T_4.

Figure 3.5: Four different effluent concentrations for irrigation

3.5 Pot Dimensions

Plastic pots of size 15.24x15.24cm were used. Each pot has a hole at bottom for drainage.

3.6 Seed Sowing

Seeds were sown in January 2021, for each treatment three replicates were used i.e., total 15 replicates in total. In each pot about four seedling plants were cultivated and watered with normal tap water according to their need and pots were covered with polythene bags to keep then enough warm as shown in figure 3.6. Seedling sprout out after 5 days of sowing as can be seen in figure 3.7.

Figure 3.6: Covering of pots with polythene bag in order to give warmness for seedling sprout out

Figure 3.7: Sprouting of *Lactuca sativa* seedling

3.7 Effluent Treatment

First treatment of effluents was given to 15 days old seedlings about 20 days after planting and 250 ml of respective concentrations of effluents were added to each pot, while 250 ml of tap water was added to the control pots. Plant had enough growth at 15 day, and it was ready for treatment as can be seen in figure 3.8. During the growth season three such treatments were given periodically. Various agronomic characters were studied, and data recorded three times during the experimental study. First sampling was done after 15 days of treatment. Sampling interval between 2^{nd} and 3^{rd} harvest was of 15 days. Figure 3.9 depict the final harvest of all forms of treated plant from T_0 to T_1.

Figure 3.8: 15 days old plant of *Lactuca sativa*

Figure 3.9: Final harvest of *Lactuca sativa* plant

At each sampling stage following parameters were observed:

3.8 Plant Height

Height of the sampled plants was measured with the help of meter rod from the soil level to the tip of the plant.

3.9 Fresh Weight of Plants

The fresh weight of root, leaves and overall plant was measured with the help of weighing balance.

3.10 Dry Weight of Plants

The dry weight of root leaves and overall plant weight was measured by drying them in oven and then weight was measured in weighing balance.

3.11 Chlorophyll Determination

The chlorophyll content of Lettuce leaves was determined after 15, 30 and 50 days of planting. The leaf blade was taken, and its discs were subjected to grinding with liquid nitrogen to convert it into fine paste as shown in figure 3.10, 20 ml of 80 percent acetone was added to the homogeneous mixture, and it was stirred well, the homogenous paste was filtered in a 50 ml volumetric flask, filtered using Whatman No 1 filter paper. 5 ml of 80 percent acetone was added to the residue and repeat the extraction operation. 1 ml chlorophyll extract was transferred to a 10 ml volumetric flask, and the volume was adjusted using 80% acetone. On a spectrophotometer, the absorbance of the chlorophyll extract was measured at 645 nm and 663 nm and expressed in unit mg g^{-1} [88, 89].

Calculation

$$\text{Chl fresh weight (mg g}^{-1}) = (\text{A }645 \text{ x } 0.0202) + (\text{A}663 \text{ x } 0.00802) \text{ x } 10 \text{ x } 50$$

$\text{A}645 = \text{absorbance at } 645 \text{ nm}$

$\text{A}663 = \text{absorbance at } 663 \text{ nm}$

$0.0202 = \text{specific absorption co} - \text{efficient of Chlorophyll at } 645 \text{ nm.}$

$0.802 = \text{absorption co} - \text{efficient of Chlorophyll at } 663 \text{ nm.}$

$10 \text{ nd } 50 = \text{dilution volumes.}$

Figure 3.10: Grinding leaves for sample preparation for spectrophotometry

3.12 Protein Determination

The protein determination was carried out with Bio-Rad method (Bradford, 1976) [90].

3.12.1 Reagent

a. **Bio-Rad Solution** Dissolve 0.1 g brilliant blue in 50 ml of 95% ethanol. To this added 100 ml of phosphoric acid (85%) and mixed the contents. The volume of solution was adjusted and filtered through Whatman No. 1 filter paper. The solution was prepared fresh and stored in cold cabinet.

b. **Protein Standards** Dissolved 0.15 g bovine serum albumin (BSA) in 100 ml distilled water. Protein standard solution containing 10-100 µg protein ml^{-1} were made by appropriate dilution of stock solution.

3.12.2 Sample Preparation

Fresh leaf weighing 0.2 g was thoroughly crushed in a mortar. Finally added 10 ml of distilled water and the contents were mixed and then centrifuged at 5000 rpm for 15 minutes. Decanted the supernatant and 200 µl aliquot was used for estimation of protein.

3.12.3 Preparation of standard curve

A series of 10 grades ranging 10 to 100 µg per 100 µl were placed in 10 ml test tube. To each 100 µl protein grade added 900 µl distilled water, and 5 ml Bio-Rad reagent. After 15 min tubes were thoroughly shaken and absorbance was recorded at 595 nm on spectrophotometer. Blank containing distilled water and Bio-Rad was run in conjunction with the standard sample tubes and used for zero setting. Similar procedure was carried out for leaves. Total protein in the sample was calculated from the linear regression using the equation:

$$\mathbf{Y = a + bx}$$

Were,

Y = optical density

a = intercept

b = slope

x = µg protein in 100 µl sample

3.13 Determination of Trace Elements in Lettuce Plant

The plant samples were oven dried and converted into powder form to detect heavy metals by energy dispersive X-ray analysis in Soil survey of Pakistan Lahore [91]. Uptake of trace elements ($\mu g\ g^{-1}$) by shoot, root and leaves of lettuce plant. Samples were analyzed at maturity.

3.14 Available Ions Present in Soil

Available ions in the soil were measured by Atomic Absorption spectrophotometer [92] in Soil survey of Pakistan, Lahore. Soil was analyzed at the end of experiment. The soil was irrigated three times during the growth period with different concentrations of tanneries effluent.

3.15 Trace Elements in Soil

Trace elements in the soil were measured by Atomic Absorption spectrophotometer [92] in Soil survey of Pakistan, Lahore. Soil was analyzed at the end of experiment. The soil was irrigated three times during the growth period with different concentrations of tanneries effluent.

Chapter 4

Results

4.1 Chemical Analysis of Tanneries Effluents

Effluents taken from the Kasur tanneries effluent pretreatment plant, Kasur, were analyzed to investigate their quality. The results are presented in Table 4.1. The effluents were classified with regard to their quality from irrigation point of view using pH, EC, and SAR as basic criteria. Analysis of the effluent revealed an EC value 1.27 ds m^{-1}, pH 8.13 and SAR value 52.04 meq l^{-1}

Thus, it belongs to irrigation water quality class C_3-S_4, that is considered to be a poor-quality class for irrigation purpose. The effluents used in the experiment possessed very high amount of BOD and COD values 880 mg l^{-1} and 2695 mg l^{-1} respectively. The effluents contained much higher amounts of TDS (734 mg l^{-1}) and TSS (685 mg l^{-1}). The amount of various nutrients in (meq l^{-1}) was as: calcium + magnesium was 12, carbonate 9.2, bicarbonates 124, sodium 145, chloride 129 and sulphates were 1632 meq l^{-1} respectively. Among the analyzed trace elements, the amount of iron, manganese, zinc, chromium, lead, and chromium was 0.24, 0.07, 0.08, 0.02, 1.5 mg l^{-1} respectively.

Table 4.1: Physiochemical characteristics of tanneries effluent

Parameters	Effect Of Concentrations
Color	Light to dark grey
Temperature	23^{o}C
pH	8.13
Electrical Conductivity	1.27dsm^{-1}
Total Settleable Solids	685 mg l^{-1}
Total dissolve solid	734 meq l^{-1}
Biological Oxygen demand	880 mg l^{-1}
Chemical Oxygen demand	2695 mg l^{-1}
SAR	52.04 meq l^{-1}
Calcium+Magnesium	12 meq l^{-1}
Sulphates	1632 meq l^{-1}
Sodium	145 meq l^{-1}
Chlorides	129 meq l^{-1}
Carbonates	9.2 meq l^{-1}
Bicarbonates	l24 meq l^{-1}
Copper	0.2 mg l^{-1}
Iron	0.24 mg l^{-1}
Manganese	0.07 mg l^{-1}
Zinc	0.08 mg l^{-1}
Lead	0.02 mg l^{-1}
Nickel	Not detected
Chromium	1.5 mg l^{-1}

4.2 Soil Analysis

The results of the soil analysis that was treated with different concentrations of tanneries effluents are presented in Table 4.2. The pH of the soil treated with full strength tanneries effluent was higher (7.9) which was more as compared to the soil which received fresh tube well water (7.05). The EC of the non-treated soil was (58.3 dsm^{-1}), which increased marginally in the treated soil samples and in full treated sample it is (58.9 dsm^{-1})

The amount of available chloride (Cl^{-1}) and bicarbonate (HCO_3^{-1}) decreased with successive increase in the strength of effluents. Sulphate (SO_4^{-2}) concentration in the soil increased with continuous increase in tanneries effluents concentrations and in T_4, 4-folds increase in SO_4^{-2} was recorded. The value of Ca+Mg in control soil sample was 28.4 mg g^{-1} and across the four treatments it ranged toward 48.4 mg g^{-1} . Amount of organic matter (OM) in the non-treated soil was 8.2 %. Tanneries effluents treatments did not affect much on the soil OM and the amount of OM fluctuated between 8.2 to 7.7% across the treatments.

The amount of Na and sodium absorption ratio (SAR) was much higher in control and a considerable decrease in both values was found in soil treated with various concentrations of effluents. The value of Na in control was 37.3 mg g^{-1} while 30.2, 17.4, 8.4, and 7.3 mg g^{-1} Na was recorded in T_1, T_2, T_3 and T_4 soil respectively. The SAR value in control soil was 4.3 meq g^{-1} whereas in T_1, T_2, T_3 and T_4 soils the value of SAR was 5.8, 2.7, 2.1 and 1.4 respectively.

Residual sodium carbonate (RSC) in control soil sample (T_0) was 1.2 meq g^{-1}. A phenomenal increase in this value was registered with successive increase of effluents strength and the value of RSC in T_1, T_2, T_3 and T_4 was 15, 16.7, 25.7 meq g^{-1} and 31.9 meq g^{-1} respectively. Cation exchange capacity (CEC) increases between control to fully treated soil from, and it was 16.1 to 22.2 meq g^{-1} respectively.

4.3 Total Trace Elements Present in Soil

Results recorded for total trace elements present in the soil (Table 4.3) showed that amount of Cu in non-treated soil (T_0) was 83 µg $g^{-1}$and a slight increase in this value was recorded in T_1 (87). While in T_2, T_3 and T_4 soil samples the concentration of Cu increase and its concentrations were 91, 95 and 112 µg g^{-1} respectively. The amount of iron (Fe) in the non-treated soil sample was 482.1 µg g^{-1}. Treatment with effluents caused slight increase of this element in the soil. The increase in Fe concentration of the treated soil across four treatments ranged from 489.0 to 631.4 µg g^{-1}. In case of zinc (Zn) increase of this trace element was recorded across the treated soil samples when compared with the control. Addition of different dilutions of tanneries effluents resulted in increase in Zn concentration when compared to control soil (121-176 µg g^{-1}). Mn shows continuous increase in effluent concentration with increase in treatment that ranged from 180-678 µg g^{-1}. Increase in chromium (Cr) concentration of the treated soil was much more pronounced than the Mn and increase was recorded from T_1 to T_4. Maximum increase in Cr concentration was found in T_4 28-47 µg g^{-1}. The amount of cobalt in the non-treated soil was 8 µg $g^{-1}$and this value almost increases slowly in T_1, T_2, T_3, and T_4 respectively. Amount of T_4 was recorded as 14 µg g^{-1}. The lead (Pb) contents in the non-treated soil were 84 µg g^{-1} and it did increase with the effluent treatment and in T_4 the value was 112 µg g^{-1}. The value of nickel increased with treatment from 24 to 38 µg g^{-1} respectively.

Table 4.2: Available ions present in soil used for lettuce plant growth. Soil was analyzed at the end of the experiment. The soil was irrigated 3 times during the growth period with different concentrations of tanneries effluents.

Treatment	pH	EC	Cl^{-1}	CO3	HCO3	$SO4^{-2}$	Ca + Mg	CaCO3	Na	RSC	CEC	O.M %	SAR
		dsm^{-1}	$mg\ g^{-1}$							$meq\ g^{-1}$			
T_0	7.05	58.3	32	18	25	7.2	28.4	4.2	37.3	1.2	16.1	8.2	4.3
T_1	7.13	60.2	35	23	21	13.4	27.1	3.3	30.2	15	18.3	5.7	5.8
T_2	7.15	59	24	12	18	15.1	34.8	4.4	17.4	16.7	15.8	8.3	2.7
T_3	7.2	60.1	29	14	17.3	24	46.3	4.8	8.4	25.7	17.5	8.0	2.1
T_4	7.9	58.9	21	0.8	12.9	26.6	48.4	7.2	7.3	31.9	22.2	7.7	1.4

Key

T_0 = Tap water

T_1 = 25% effluent

T_2 = 50% effluent

T_3 = 75% effluent

T_4 = 100% effluent

Table 4.3: Amount of trace elements (ug g^{-1}) present in soil samples along with different concentrations of tannery effluent

S. No	Cu	Fe	Zn	Mn	Cr	Co	Pb	Ni
T_0	83	482.1	121	180	28	08	84	24
T_1	87	489.0	134	360	36	09	82	28
T_2	91	495.3	154	461	38	13	97	27
T_3	95	624.5	189	630	22	15	102	33
T_4	112	631.4	176	678	47	14	112	38

4.4 Fresh weight of Lettuce plant, root, and leaves

Tanneries effluent treatment considerably affected fresh weight of root, leaves and lettuce plant.

4.4.1 Fresh Weight of Lettuce Plant

Fresh weight of lettuce plant decreases with increase in effluent treatment except for T_1 where there is increase in fresh weight as compared to T_0 among all intervals of days. At T_1 4.9 %, 22 % and 8% increase was observed in fresh weight of lettuce with respect to 15, 30, 45 DAT. After T_1 reduction in fresh weight was observed with increase in effluent treatment. At 100% effluent treatment (T_4) the reduction in fresh weight was 47%, 41% and 49% respectively as compared to control. Statistical analysis of the data (Table 4.4) showed that tanneries effluents treatment significantly affected ($P < 0.0001$) the fresh weight of lettuce plant. Impacts of tanneries effluent on fresh weight of plant from first harvest to final harvest can be seen in Figure 4.1

4.4.2 Fresh Weight of Root

Fresh weight of root increase in 25 % effluent treatment and the increase was 25%, 27 % and 3% respectively as compared to control. Fresh weight of root decreases with increase in effluent. The reduction in fresh weight of root in 100% effluent treatment was 31%, 36% and 42 % respectively with DAT intervals. Statistical analysis of the data (Table 4.5) showed that tanneries effluents treatment significantly affected ($P< 0.001$) the fresh weight of root of lettuce plant. Change in fresh weight of root as a result of tanneries effluent, with respect to intervals of days can be seen in Figure 4.2.

4.4.3 Fresh Weight of Leaves

The results for fresh weight of leaves are similar to fresh weight of plant and root. In T_1 increase in fresh weight is observed whereas further on the fresh weight decrease with increase in effluent treatment. Increase in T_1 was 4.3%, 4.7% and 7%. Final decrease in T_4 was 31%, 31% and 47% as compared to control. Statistical analysis of the data (Table 4.6) showed that tanneries effluents treatment affected ($P < 0.05$) the fresh weight of lettuce plant. Reduction in fresh weight of leaves with respect to days after treatment can be seen in Figure 4.3.

Table 4.4: Impacts of tanneries effluent on fresh weight of plant from first harvest to final harvest

Treatments	Days after Treatment		
	15	30	45
T_0	4.69	5.17	6.2
T_1	4.92	6.33	6.7
T_2	4.20	5.78	5.43
T_3	3.67	4.11	4.90
T_4	2.47	3.02	3.16

Table 4.5: Impacts of tanneries effluent on fresh weight of root of plant from first harvest to final harvest

Treatments	Days after Treatment		
	15	30	45
T_0	0.453	0.489	0.466
T_1	0.567	0.595	0.480
T_2	0.423	0.412	0.382
T_3	0.387	0.340	0.280
T_4	0.312	0.311	0.268

Table 4.6: Impacts of tanneries effluent on fresh weight of leaves from first harvest to final harvest

Treatments	**Days after Treatment**		
	15	**30**	**45**
T_0	1.85	2.73	2.96
T_1	1.93	2.86	3.17
T_2	1.56	2.23	2.33
T_3	1.34	2.13	1.87
T_4	1.26	1.87	1.56

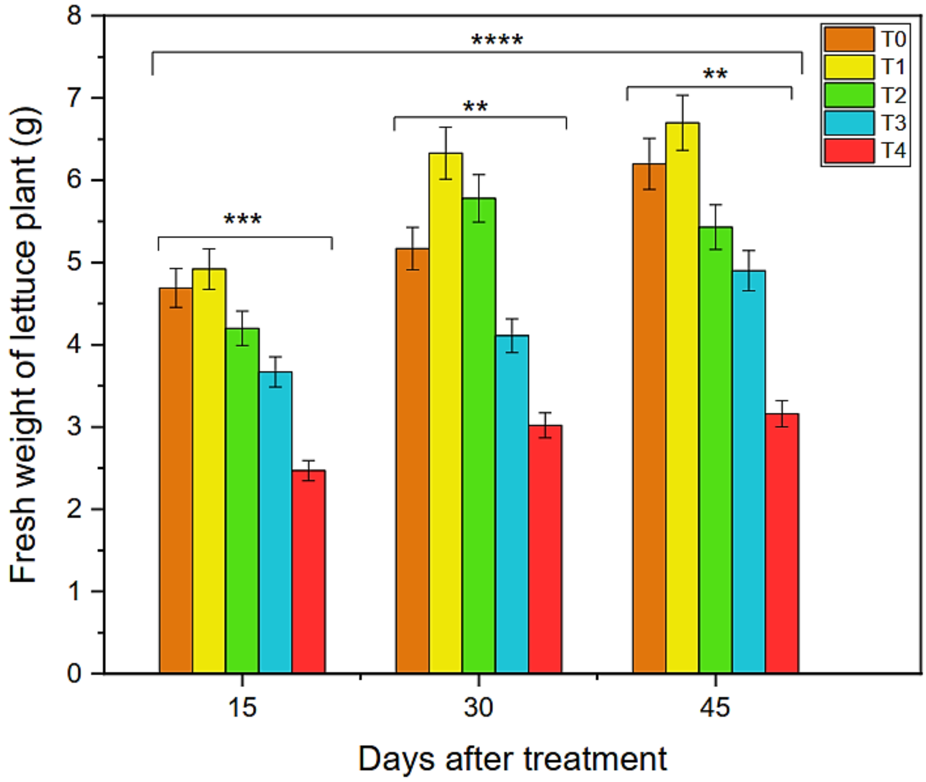

Figure 4.1: Impacts of tanneries effluent on fresh weight of plant from first harvest to final harvest

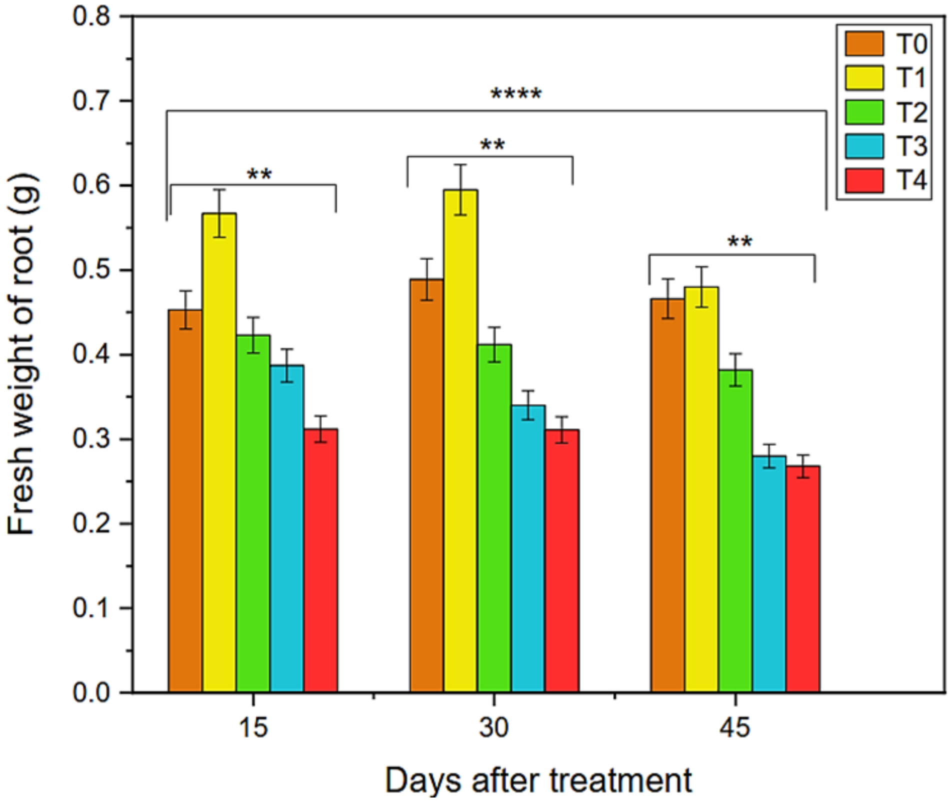

Figure 4.2: Impacts of tanneries effluent on fresh weight of root from first harvest to final harvest.

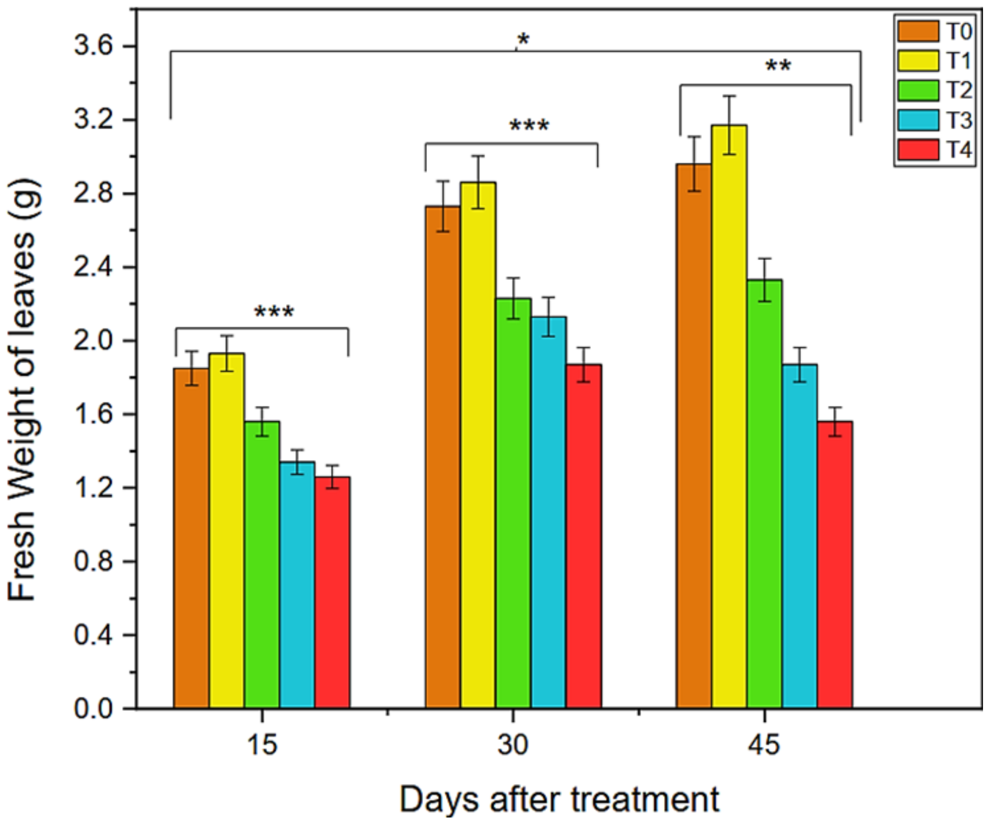

Figure 4.3: Impacts of tanneries effluent on fresh weight of leaves from first harvest to final harvest.

4.5 Dry Weight of Lettuce Plant, Root, and Leaves

Data recorded for dry weight at 15 DAT showed slight increase in dry weight of component parts of plant exposed to lower concentrations of tanneries effluents. While later in the season, tanneries effluent treatment had resulted in considerable reduction in dry weight in all three component parts of the plants.

4.5.1 Effect of Tanneries Effluent on Dry Weight of Lettuce Plant

In dry weight of lettuce plant, increase in weight was observed in T_1 with 22%, 9%, and 21% respectively (15, 30, 45 DAT) as compared to T_0. The reduction in T_2 at 15 DAT 11% as compared to T_0. At 30 DAT the reduction was 36% as compared to control. However, at 45 day the results were close to T_0, and slight reduction was observed (1.5%) as compared to control. Reduction in dry weight in T_4 was 56%, 59% and 72 % respectively. Statistical analysis of the data (Table 4.7) showed that tanneries effluents treatment significantly affected ($P < 0.0001$) the dry weight of lettuce plant. Impacts of tanneries effluent on dry weight of plant can be seen in Figure 4.4.

4.5.2 Dry Weight of Root

Results of table 4.8 depict that after T_1 reduction was observed in dry weight of root with increase in effluent treatment. At 15 DAT decrease in dry weight of root was 16%, 29% and 41 % with respect to T_2, T_3 and T_4 as compared to control. At 30 DAT the reduction was 24%, 46% and 65% as compared to control with respect to T_2, T_3 and T_4. At 45 DAT the reduction in T_2 was 32 %, T_3 was 42% and in T_4 it was 56%. In all DAT (15, 30, 45) T_1 show increase in dry weight of root. Statistical analysis of the data (Table 4.8) showed that tanneries effluents treatment significantly affected ($P < 0.0001$) the dry weight of root. Reduction in dry weight of root as a result of application of tanneries effluent can be seen in Figure 4.5.

4.5.3 Dry Weight of Leaves

Increase in dry weight of leaves was observed in T_1. The increase at 15 DAT was 18%, 30 DAT was 6%, and 45-day increase was 4.9 % in T_1. As the effluent treatment increase the reduction was observed in dry weight of lettuce leaves. At T_4 decrease was 36%, 50% and 68 % respectively with 15,30,45 DAT. Figure 4.6 clearly depict the reduction in dry weight of leaves. Statistical analysis of data of Table 4.9 showed that tanneries effluent treatment effected dry weight of leaves ($P<0.05$).

Table 4.7: Impacts of tanneries effluent on dry weight of lettuce plant from first harvest to final harvest

Treatments	**Days after Treatment**		
	15	**30**	**45**
T_0	1.8	2.2	1.9
T_1	2.2	2.4	2.3
T_2	1.6	1.4	1.87
T_3	0.98	1.2	0.76
T_4	0.78	0.89	0.52

Table 4.8: Impacts of tanneries effluent on dry weight of root from first harvest to final harvest

Treatments	**Days after Treatment**		
	15	**30**	**45**
T_0	0.072	0.089	0.075
T_1	0.086	0.095	0.083
T_2	0.06	0.067	0.051
T_3	0.051	0.048	0.043
T_4	0.042	0.031	0.033

Table 4.9: Impacts of tanneries effluent on dry weight of leaves from first harvest to final harvest

Treatments	**Days after Treatment**		
	15	**30**	**45**
T_0	0.198	0.397	0.487
T_1	0.235	0.424	0.511
T_2	0.195	0.323	0.312
T_3	0.135	0.257	0.187
T_4	0.126	0.196	0.151

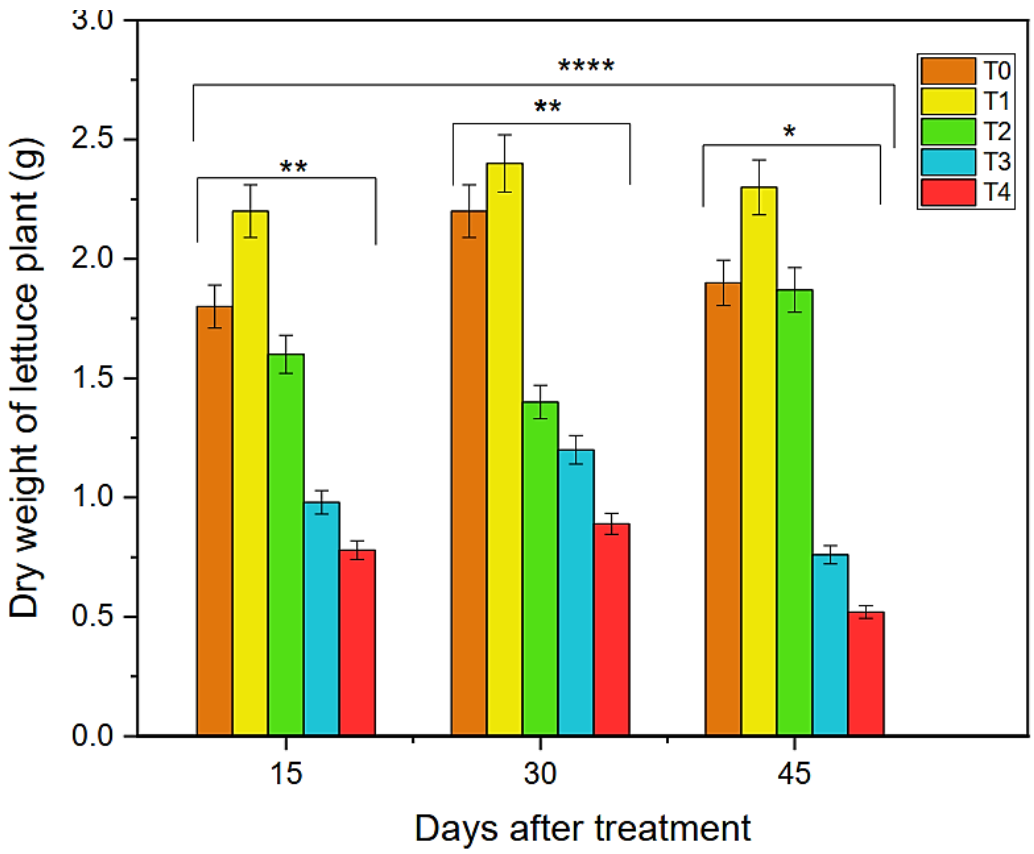

Figure 4.4: Impacts of tanneries effluent on dry weight of lettuce plant from first harvest to final harvest.

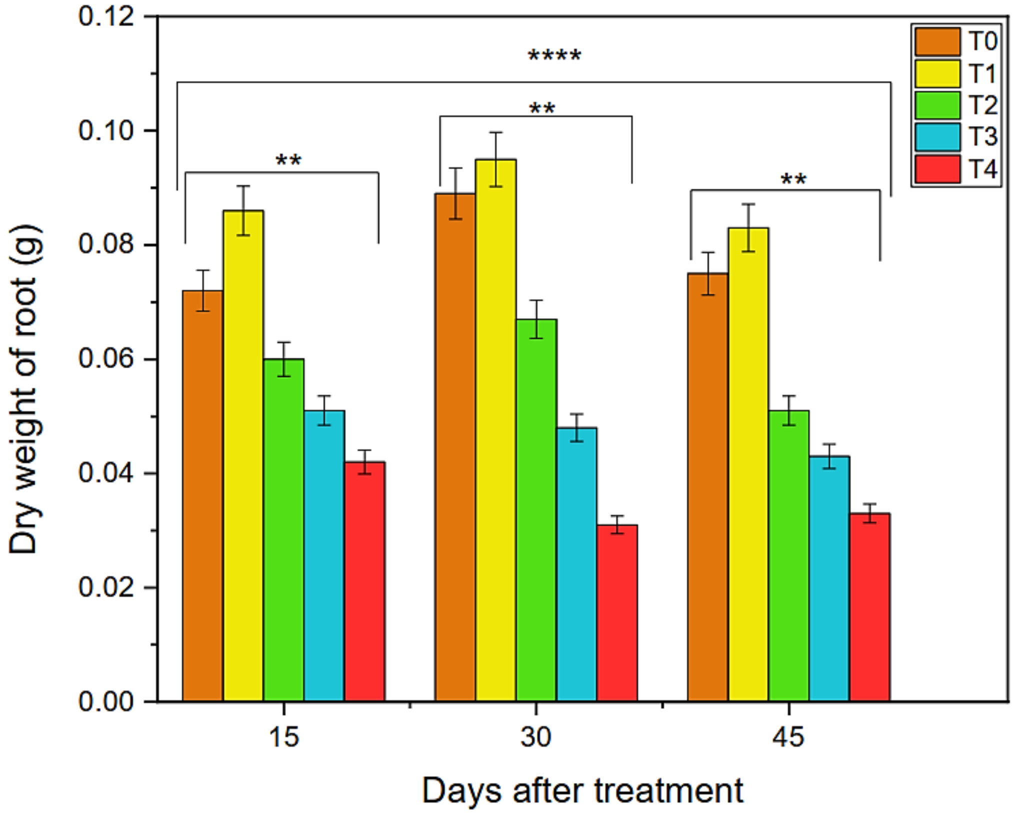

Figure 4.5: Impacts of tanneries effluent on dry weight of lettuce leaves from first harvest to final harvest.

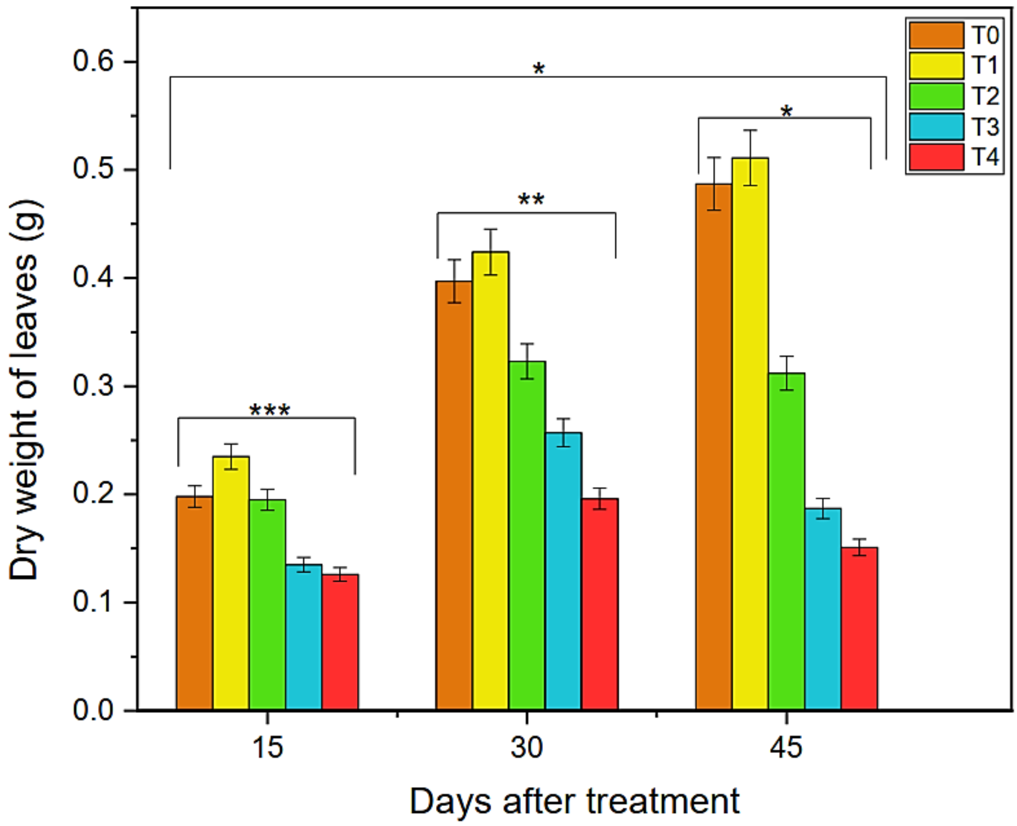

Figure 4.6: Impacts of tanneries effluent on dry weight of leaves from first harvest to final harvest.

4.6 Effect of Tanneries Wastewater on Plant Height

Analysis of plant height revealed that with increase in effluent treatment the relative growth rate of plant height as compared to T_0 and T_1 decreases. In T_0 plant grown to maximum height. Results of growth rate in T_1 were almost similar to T_0. In T_2, T_3 and T_4 plant do no show growth as much as in T_0 and T_1. Statistical analysis of data of Table 4.10 depicts that tanneries effluent significantly affected the plant heigh ($P < 0.0001$). Impact of tanneries effluent on plant height is demonstrated in figure 4.7.

Table 4.10: Impacts of tanneries effluent on height of lettuce plant from first harvest to final harvest

Treatments	**Days after Treatment**		
	15	**30**	**45**
T_0	41	47	50
T_1	38	45	48
T_2	34	37	42
T_3	28	32	37
T_4	12	15	19

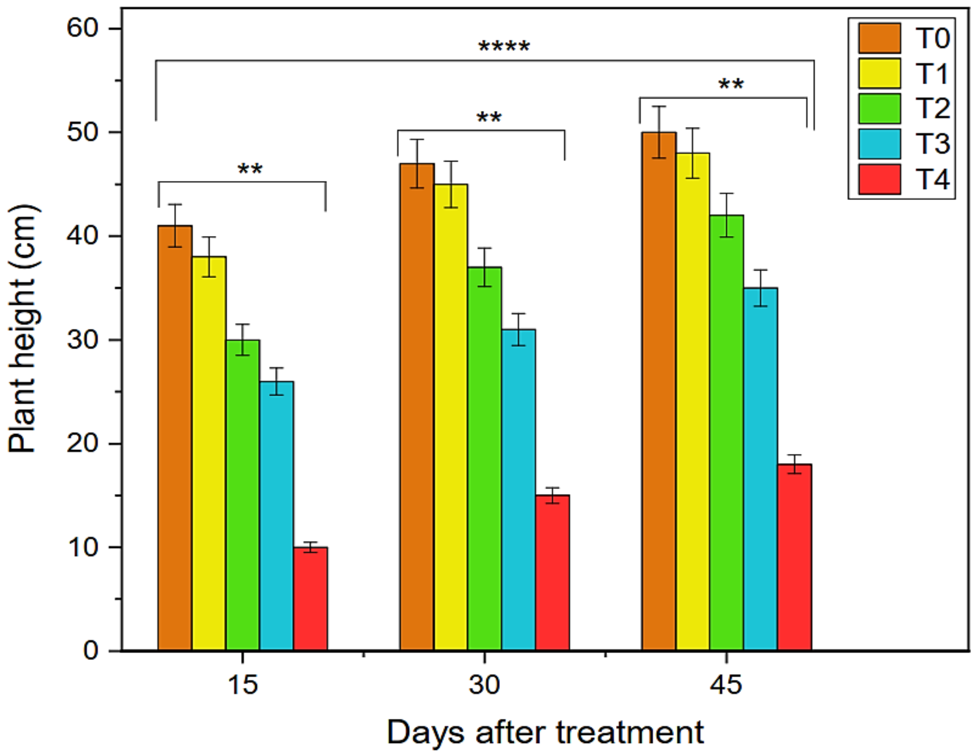

Figure 4.7: Impacts of tanneries wastewater on height of plant along all three harvests.

Analysis of lettuce roots, shoots and leaves for uptake of various trace elements was carried out at maturity and the results are presented in Table 4.11

In copper maximum uptake was observed in lettuce shoot followed by lettuce root followed by lettuce leaves. The copper uptake varies from 0.47 to 0.18.ug $g^{-1.}$ In lettuce leaves uptake of Cu increases with increase in effluent treatment. Uptake of trace elements by roots is depicted in Figure 4.8. In shoot the uptake of Cu also increases, it is almost same at T_2 and T_3. The uptake of trace elements in shoot is elaborated in Figure 4.9. In lettuce root the uptake of copper decreases with increase in effluent treatment from 0.39 to 0.22 ug $g^{-1.}$

In iron maximum uptake was observed in root followed by leaves and then shoot. In lettuce leaves uptake of iron decrease in T_1 and then goes on increase, maximum increase was observed in T_3 and T_4. Same results were observed in shoot and leaves also. Overall maximum uptake was observed in T_3 and T_4. The range varies from 16.5 to 1.2ug g^{-1} .

In Mn maximum uptake was observed in leaves followed by root and then shoot. Lettuce leaves, root and shoot show continuous uptake of Mn with increase in effluent treatment. The range of Mn uptake varies from 0.39 ug g^{-1} being the highest in leaves and 0.06 being the lowest in shoot. In Zn maximum uptake was observed in lettuce leaves followed by shoot and then root. Continuous decrease in Zn uptake was observed with increase in effluent treatment. The maximum uptake observed was 0.45 ug g^{-1} and minimum uptake was 0.18 u g^{-1}

The uptake of cobalt was null in T_0, T_1, T_2 in lettuce leaves, in shoot and root cobalt was null in T_1 and T_2. Minimum quantity of Co was taken up by plant in T_3 and T_4.

In lead continuous uptake was observed in lettuce leaves from 0.12 ug g^{-1} to 0.26 ug g^{-1}. Whereas in shoot and root continuous decreasing trend was observed with increase in effluent treatment. The maximum range of uptake of lead was 0.26 ug g^{-1} in leaves and lowest range was 0.08 ug g^{-1} in shoot.

Ni uptake in lettuce leaves was observed in T_3, the amount being 0.01 ug g^{-1} and in T_4 it was 0.06 ug g^{-1}. In T_1, T_2 and T_3 nickel was not observed in lettuce leaves. Figure 4.10 represent the uptake of trace elements by leaves. In shoot and root nickel uptake decrease with increase in effluent treatment. The highest value observed for nickel uptake was 0.09 ug g^{-1} and lowest was 0.01 ug g^{-1}.

The uptake of Cr shows increasing trend with increase in effluent treatment. The maximum uptake was observed in lettuce root followed by shoot and then leave. In leaves very low amount of Cr was observed being zero in $T_{0.}$

Table 4.11: Impact of tannery effluents on uptake of trace elements (µg g^{-1}) by shoot, root and leaves

Lettuce Leaves	**Cu**	**Fe**	**Mn**	**Zn**	**Co**	**Pb**	**Ni**	**Cr**
T_0	0.18	3.5	0.2	0.45	0	0.12	0	0
T_1	0.19	3.2	0.14	0.43	0	0.14	0	0.03
T_2	0.21	3.7	0.18	0.39	0	0.17	0	0.04
T_3	0.37	4.6	0.23	0.36	0.02	0.22	0.01	0.04
T_4	0.41	5.9	0.39	0.33	0.04	0.26	0.06	0.07
Lettuce Shoot								
T_0	0.18	1.3	0.06	0.36	0	0.24	0.09	0.04
T_1	0.28	1.2	0.09	0.34	0	0.23	0.08	0.06
T_2	0.42	1.5	0.14	0.33	0.01	0.19	0.07	0.09
T_3	0.42	1.8	0.16	0.31	0.02	0.14	0.06	0.13
T_4	0.47	1.9	0.2	0.29	0.02	0.08	0.03	0.19
Lettuce Root								
T_0	0.39	7.5	0.12	0.18	0	0.22	0.09	0.09
T_1	0.38	5.9	0.15	0.115	0	0.21	0.09	0.09
T_2	0.35	9.3	0.19	0.36	0.01	0.19	0.08	0.12
T_3	0.25	14.2	0.24	0.27	0.03	0.18	0.07	0.21
T_4	0.22	16.5	0.28	0.21	0.04	0.16	0.04	0.25

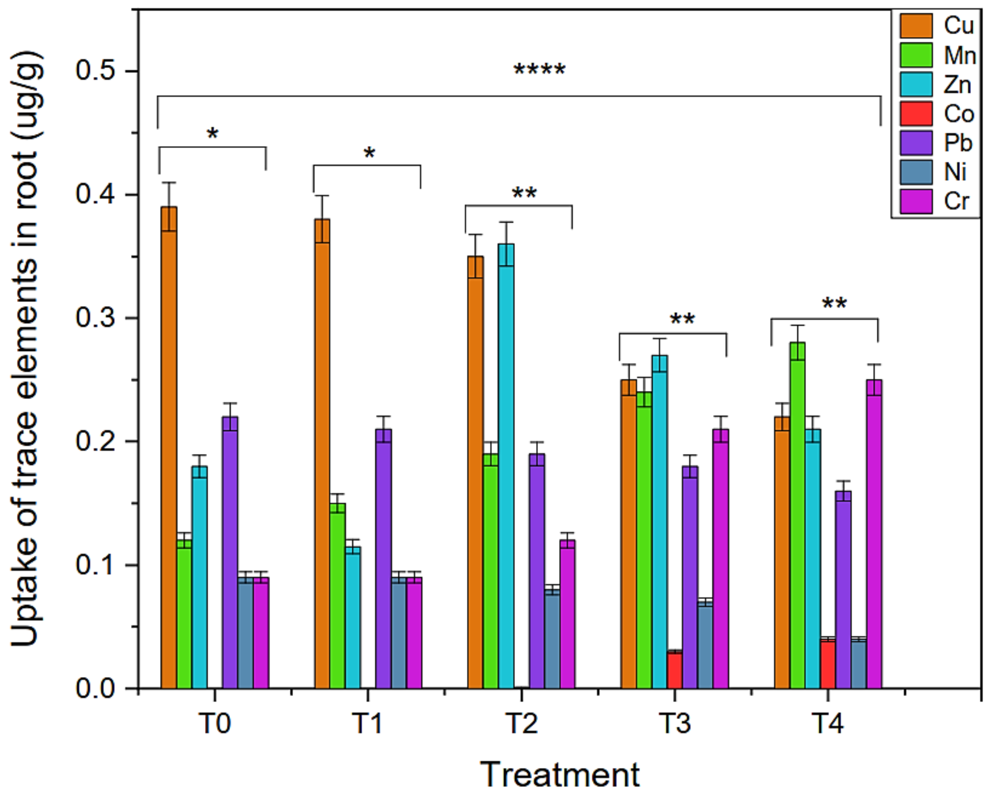

Figure 4.8: Uptake of trace elements by roots along with different concentrations of effluent.

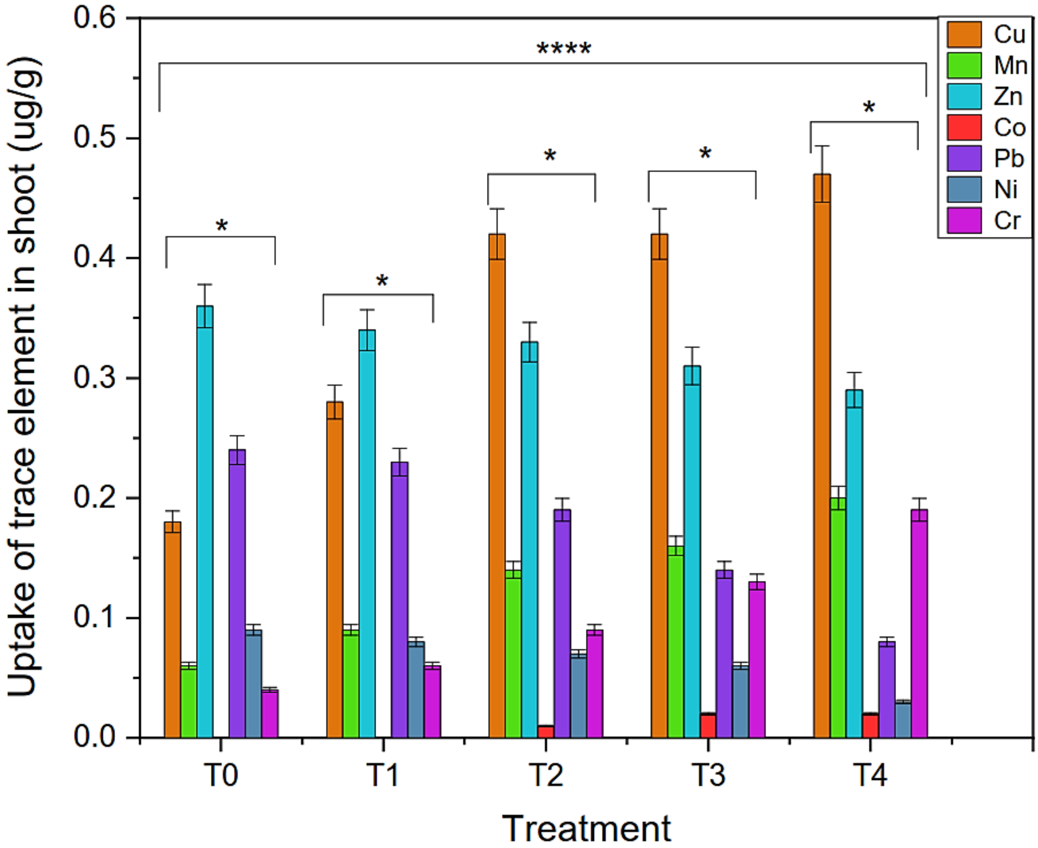

Figure 4.9: Uptake of trace elements by shoot along with different concentrations of effluents.

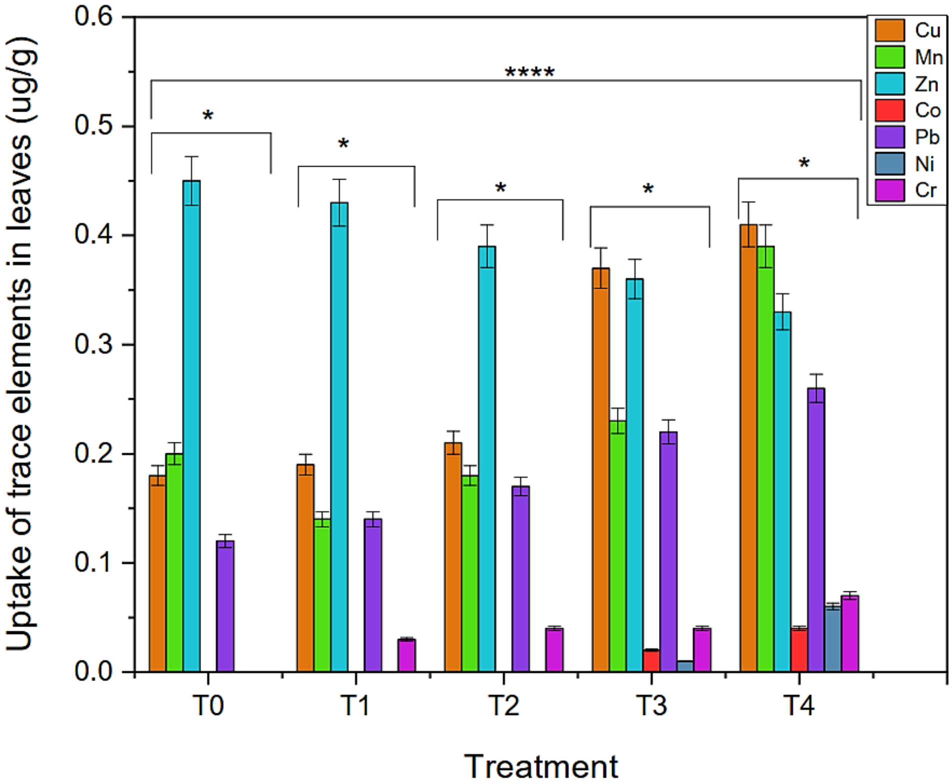

Figure 4.10: Uptake of trace elements by leaves along with different concentrations of effluent.

4.7 Effect of Tanneries Effluent on Soluble Protein Content in Leaves

Soluble protein content in lettuce leaves decrease with increase in effluent treatment except for T_1 where increase was observed in all DAT (15,30,45). At 15 DAT protein content increases in T_1 and T_2. Maximum increase was in T_2. Increase in T_1 and T_2 was 25 % and 40% respectively as represented in Figure 4.11. In T_3 the soluble protein content was higher than control but lower than T_2 and T_3. Reduction in T_4 was 14%. At 30 days after treatment maximum increase was observed in T_1(9%). Reduction in protein content in T_4 was 59%. At 45 DAT increase in T_1 was 0.8%, further on the protein content decreases with 66% decrease in T_4. Overall protein content increase with increase in days interval. Statistical analysis of the data presented in Table 4.12 showed that tanneries effluent treatment had significant effect on protein content in leaves.

Table 4.12: Impacts of tanneries effluent on soluble protein content in lettuce leaves along with different concentrations of effluents

Treatments	Days after Treatment		
	15	**30**	**45**
T_0	7.453	36.618	39.670
T_1	9.347	42.120	40.001
T_2	10.45	26.2	31.280
T_3	9.456	18.78	19.119
T_4	6.341	15.53	13.23

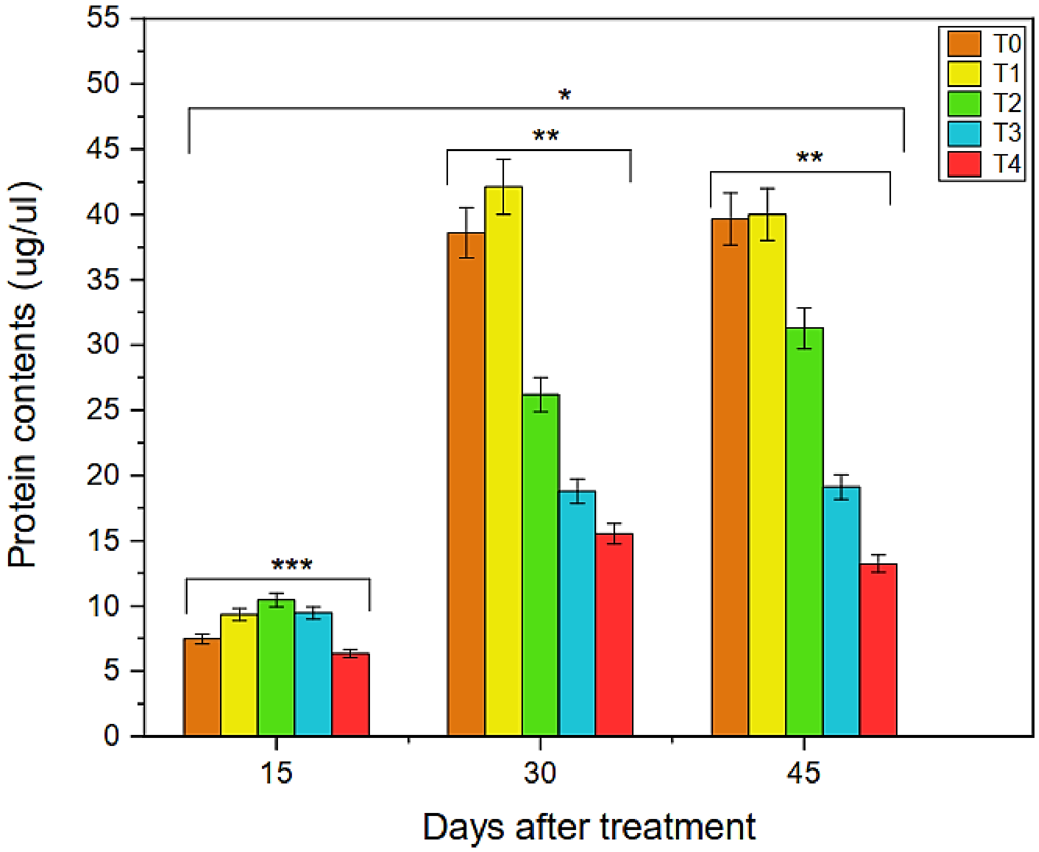

Figure 4.11: Impacts of tanneries effluent on protein content in leaves along with different concentrations of effluents.

4.8 Effect of Tanneries Effluent on Total Chlorophyll a+b (mg/g)

Chlorophyll content of data of Table 4.13 suggest that in all intervals of days chlorophyll content increases up to T_1 and then subsequently decrease along with increase in effluent treatment. Maximum increase was observed at T_1 of 15 days after treatment which was 15%. Increase in T_1 of 30 and 45 DAT was 6% and 8% respectively as compared to control. The reduction in chlorophyll content was 49%, 77% and 83% respectively with 15,30 and 45 DAT at T_4. The results of the data suggest that reduction in chlorophyll content decrease with increase in days as well as with increase in effluent treatment as shown in Figure 4.12. Effluent treatment and days interval has a very significant effect ($p<0.0001$) chlorophyll content.

Table 4.13: Impacts of tanneries effluent on total chlorophyll content a+b (mg/g) along with different concentrations of effluent

Treatments	**Days after Treatment**		
	15	**30**	**45**
T_0	1.934	2.29	2.10
T_1	2.239	2.43	2.27
T_2	1.912	1.89	1.68
T_3	1.072	1.41	1.53
T_4	0.985	0.52	0.34

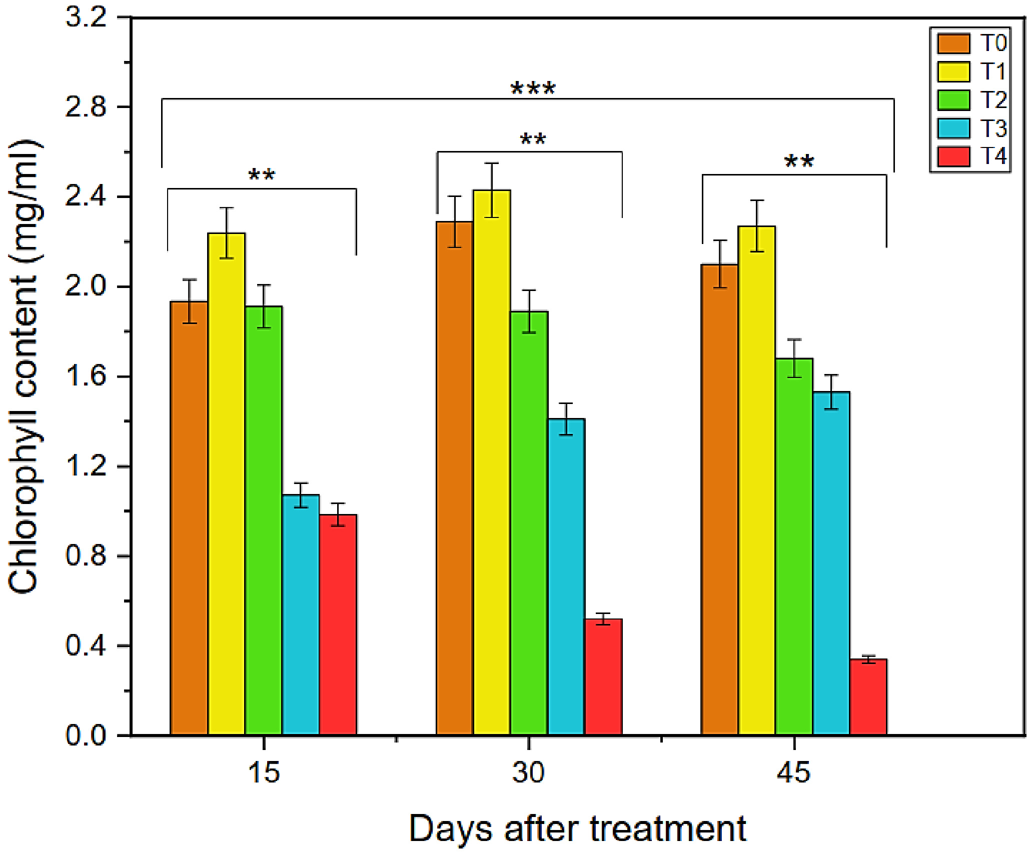

Figure 4.12: Impacts of tanneries effluent on total chlorophyll content a+b mg/g along with different concentrations of effluent

Chapter 5

Discussion

Effluents collected from settling tank of Kasur tanneries effluents pretreatment plant were analyzed for various parameters of water quality. The effluents were found slightly basic with high EC and very high SAR. Effluents contained much higher amounts of TDS, TSS, and heavy metals. Very high values of BOD and COD revealed that the effluents used in the experiment possess high organic and inorganic pollution load. This type of water is regarded as very poor-quality water because its use is generally unsatisfactory for irrigation purpose. It is saline due to its high EC and possess sodicity hazard because of very high SAR value. Similar results were obtained by (Khan *et al.,* 2013) [95].

Poor crop performance is caused by extremely alkaline water with high EC, BOD, COD, and SAR values. Because the quantity of iron, manganese, zinc, and chromium in tanneries wastewater is relatively high in number, it is not suitable for irrigation due to the high mineral and heavy high metal content. In the Kasur district of Pakistan, tannery effluents utilized for irrigation proven to be hazardous to the growth and production of a crop. Results are similar to the findings of (Qureshi,2015) [43].

More alarming is the situation when Cr (VI) enters the food chain. A study conducted on chicken who were fed on solid tannery waste showed that significant number of heavy metals enters into the liver of chicken (Mazumder *et al.*,2013) [93]. Similar findings were obtained by a researcher in research (Bari *etal.,* 2015) [69] where heavy metal buildup in chickens was discovered as a result of giving skin cut waste-based diet, by ingesting the infected chicken, these heavy metals are transferred to humans and pose a danger to human health. In another study high accumulation of chromium was observed in plant grown with tannery wastewater. The accumulation and distribution of chromium was detected, and it was observed that the concentration of chromium was higher in the roots and shoots of plants. Cr concentrations in the soil were high, which may be taken up by plants and food crops in the region, and eventually incorporated into the food chain (Bharani *etal.,* 2015) [94]. The salinity of the soil is also rising as a result of dissolved salts in effluents, resulting in low agricultural yields in such areas (Khan *et al.,* 2013) [95]. In addition to all of these problems, the chromium produced during the chrome tanning process is a serious problem due to its long-term persistence and possible toxicity. It has varying levels of influence on plant and human life after being absorbed by crop plants growing in polluted soils and transmitted to humans. Environmental cancer is one of the rare illnesses whose causes have been understood for more than a century and are attributed to chromium (Shahid *etal.,* 2017) [96].

Irrigation with tannery water may promote sodicity and salinity, deteriorating soil and jeopardizing future crop output. Salts and chromium in tannery effluent may influence soil processes and crop productivity. EC and OM of the soil increase which is irrigated with tanneries wastewater. The amount of Na and SAR value of the effluents were quite high whereas Na and SAR Value decrease in the treated soil. This could be due to high Ca + Mg contents of the soil source used in the experiment which was probably replaced by Na and hence might be the reason for significant increase of available Ca + Mg in the treated soil and residual sodium carbonates. Similar findings were observed (Qureshi,2015) [43]. The increasing content of trace elements Cu, Fe, Zn, Mn, Cr, Co, Pb, Ni in soil is linked with tanneries wastewater treatment. Copper, iron, zinc, and manganese are considered essential trace elements and are transported from soil to various plant parts. The source of elements could be the soil or tanneries effluents used in the experiment since these elements were found both in soil and effluents. Co and Ni are required by plants in a very low concentration and were transported to various plant parts from soil source since these elements were not found in the effluents. Cr and Pb are both known toxic elements to plants even at lower concentrations. Although Pb concentration was very low in the effluents, however its amount was quite high in the soil used for the experiment. The possible reason for its increase could be repeated irrigation with wastewater. Most likely the contaminated soil was the source of Pb transported to various plant parts. The amount of chromium increases with increase in concentration of effluent, as the concentrations of Cr increased by effluents treatment, Cr was transported to all parts of plant. Results of heavy metal uptake by plant are comparable to the work done of by (Balkhair *etal.,* 2016) [97].

Wastewater adversely affected the plant height of Lettuce grown in effluent treated water, the relative growth as compared to control seem to decrease in all concentrations of effluent, the findings related effect of wastewater on plant growth are similar to the to the findings of (Hussain *et al*., 2010) [44]. Fresh weight and dry weigh of Lettuce leaves, root, lettuce plant and total weight of plant decreases with increase of effluent as compared to control. Similar results were observed by (Zareen *etal.,* 2013) [40].

Chlorophyll content of leaf significantly decreased with increasing effluent concentration from T_2 to T_4. However, chlorophyll content increase in T_1 as compared to control in all intervals (15, 30, 45 DAT). Protein content in lettuce leave increase in T_1 as compared to control and then subsequent decreases T_2-T_4 in all intervals. Supplemental zinc and iron can enhance the plant's superoxide detoxification mechanism by producing hydrogen peroxide. The growth of these metals under adverse conditions will produce reactive oxygen species, destroy the photosynthetic mechanism. Protein content in lettuce leave increase in T_1 as compared to control and then subsequent decrease from T_2-T_4 in all intervals. (Srivastava *etal.,*2014) [68].

Chromium, Iron, Manganese, Nickel, Lead and Mercury are the toxic heavy metals that are detected in leaves, root and shoot of lettuce plant. The uptake of these elements increases with increase in effluent concentration. The source of these elements could be soil and effluent because from effluent these metals become part of soil after treatment. Chromium, Lead and Mercury are toxic to plant even at lower concentration. The amount of chromium was high in effluent and uptake of Cr increase with increase in effluent concentration. The findings suggested a possible route for human exposure to heavy metal poisoning through indirect consumption of vegetables produced on heavy metal-affected soil watered by polluted water sources (Balkhair *etal.,* 2016) [97].

The overall results of the investigation reveal that low concentration of tanneries effluents about 25% was stimulatory during the early period of growth. However, as the plant matures the tanneries effluents was found to have adverse effect on overall growth of the plant and increase in concentration (50-100%) of tanneries effluents further exacerbated the situation.

Chapter 6

Summary

A pot experiment was conducted to investigate the impact of tannery effluent on agronomical and biochemical aspects of Lettuce. The plants were subjected to four different tannery effluent concentrations i.e., 25%,50%, 75% and 100% and one control group based on tap water was used. The treatment was conducted for 45 days and three harvest were taken at 15, 30 and 45 DAT respectively. In all harvest agronomical and chemical properties of lettuce were analyzed.

For agronomical analysis plant height, fresh and dry weight of leave, root, and lettuce plant were evaluated. In chemical analysis chlorophyl content, protein content and uptake of element in root, shoot and leaves were evaluated.

Statistical analysis revealed that agronomical and chemical parameters were significantly affected by tanneries wastewater. Young plants were sensitive to various effluent concentration and considerable decrease in agronomical parameters was observed with increase in effluent concentration. Effluent in lower dilution 25% has stimulatory impact on plant whereas 50-100% effluent cause reduction in all agronomical properties.

Chlorophyll content and protein content increase in T_1 (25%) effluent and then decrease with increase in effluent concentration. Tanneries wastewater significantly affected the chlorophyll content ($p \leq 0.0001$). Protein content was maximum at 30^{th} day in all concentrations and the effect of effluent was significant ($p \leq 0.05$). Overall Uptake of trace element seem to increase with increase in effluent concentration, and the effects were highly significant ($p \leq 0.0001$). Tanneries wastewater significantly affected the height of plant ($p \leq 0.0001$), had highly significant affect ($p \leq 0.0001$) on dry & fresh weight of whole plant & root, the overall impact on dry & fresh weight of leaves was significant ($p \leq 0.05$).

Chemical analysis of effluent revealed that it was highly polluted. The number of chlorides, sulphates, carbonates, bicarbonates, and sodium was also very high. The heavy metals were also measured in significant amount in tannery effluent. The amount of chromium was found highest among the studied microelements. The physiochemical parameters of effluent were above the permissible limit by WHO.

Chapter 7

Conclusion & Recommendations

5.1 Conclusion

Pakistan is the world's second largest Muslim country. In Pakistan Eid-ul-Azha is celebrated with great zeal. Around 8 million animals are slaughtered in Pakistan each year at the occasion of Eid-ul-Azha and the raw hides produced are sent to various tannery units. The results of this study conducted on tannery wastewater purposed that that tannery effluent contains a large number of heavy metals in it along with organic pollutants. The analysis of physiochemical parameters of wastewater depict that it belongs to a poor-quality class of water for irrigation purpose. The number of chlorides, sulphates, carbonates, bicarbonates, and sodium was also very high. The heavy metals were also measured in significant amount in tannery effluent. The amount of chromium was found highest among the studied microelements, when plant is irrigated with this wastewater, the biochemical property of plant falls to decline with increase in effluent concentration. Decrease in chlorophyll and protein content at final harvest with full concentration of effluent was 83% and 66 % respectively. 50-100% concentration of this wastewater cause reduction in all agronomic properties of plant. With full concentration of effluent, the decrease in fresh and dry weight of plant was 49% and 72 % respectively. Analysis of uptake of trace elements in plants revealed that maximum uptake was in shoot followed by leaf and then root. Overall uptake of heavy metals seems to increase with increase in effluent concentration. Based on the results of this study it is obvious that use of tanneries effluent for irrigation purpose is highly detrimental for health, the heavy metals in it are not only carcinogenic but they also destroy the normal structure and texture of soil.

5.2 Recommendations

On the basis of results of this study following are some recommendations that may reduce harmful impacts of tanneries wastewater on environment:

I. Tanneries wastewater must be reused and recycled after proper treatment

II. Wastewater technologies must be employed in tannery units and wastewater must be treated before discharge to open ponds

III. National sanitation policy must be implicated in tannery units

IV. National air clean act and water conservation act must be implemented in tannery units to save the atmosphere

V. Government should make a law that must prohibit the discharge of industrial waste to open lands

VI. Micro-organisms should be used to treat the wastewater in tannery units

VII. Proper dress code, gloves and masks must be provided to those workers that have direct contact with heavy metals

References

[1] R. Chandra, A. Abdulsalam, N. Salim, and J. T. Puthur, “Distribution of Bio accumulated Cadmium and chromium in two Vigna species and the associated histological variations,” J. Stress Physiol. Biochem., 2010.

[2] S. Hema, T. Subramani, and L. Elango, “GIS study on Vulnerability assessment of water quality in a part of cauvery river,” Int. J. Environ. Sci., 2010.

[3] A. Rehman and M. S. Anjum, “Cadmium uptake by yeast from industrial effluents and its potential use in wastewater clenup operations,” Water, Air Soil Pollut., 2010.

[4] F. Çiner, “Treatability of Tannery Wastewater by Electrocoagulation Process,” Period. Eng. Nat. Sci., 2017.

[5] M. R. Azom, K. Mahmud, S. M. Yahya, A. Sontu, and S. B. Himon, “Environmental Impact Assessment of Tanneries: A Case Study of Hazaribag in Bangladesh,” Int. J. Environ. Sci. Dev., 2012

[6] S. Banuraman and T. P. Meikandaan., “Treatability Study of Tannery Effluent by Enhanced Primary Treatment,” Int. J. Mod. Eng. Res., 2013.

[7] Z. M. Ghafoor A, “Determinants of leather exports from Pakistan: a time series analysis. Transactions on Edu., 2014.

[8] G. J. Hashmi, G. Dastageer, M. S. Sajid, Z. Ali, M. F. Malik, and I. Liaqat, “Leather Industry and Environment: Pakistan Scenario,” Int. J. Appl. Biol. Forensics, 2017.

[9] M. Ummar, “Impact of tanneries on quality of ground water in Kasur district.” MS thesis, Islamia University of Bahawalpur., 2015.

[10] S. Kassim, “wastewater treatment practices by tanneries: case of skyfat tannery and leather industries of uganda tanneries”, jinja, uganda., 2018.

[11] Mongol, “Pakistan Leather Industry,” 2017, [Online]. Available: http://mongolleather.pk/pakistan-leather-industry/.

[12] M. A. Mr.Aamir Siddiqui and Ms.Fakhia Nadeem, “Sectoral Competitiveness and Value Chain Analysis,” Trade Dev. Auth. Pakistan, 2016.

[13] “Annual International Trade Statistics by Country (HS02),” Trend Economy. 2020, [Online]. Available: https://trendeconomy.com/data/h2/Pakistan?time_period=2020,2019,2018,2017,2016,2015,2014,2013,2012,2011,2010,2009.

[14] “Export of leather and leather products from pakistan during july-june’2018-2019 vs july-june’2019-2020,” Pakistan Tanners Association. 2020,

[Online]. Available: http://pakistantanners.org/industrial_statistics.html.

[15] A. Farooqi, “Mechanism of Arsenic and Fluoride 3 Release: A Case Study from Lahore and Kasur, Punjab Pakistan,” springer India, 2015.

[16] M. A. Islam, K. Fatima, and M. A. Ali, “Impact of tanneries waste disposal on environment in some selected areas of dhaka city corporation,” J. Environ. Sci. Nat. Resour., 2014.

[17] “Leather tanneries in Kasur and their effect on water,” The Financial Daily. 2019, [Online]. Available: https://thefinancialdaily.com/leather-tanneries-in-kasur-and-their-effect-on-water/.

[18] L. Wei, “Online determination of trace amounts of tannic acid coloured tannery wastewater by automatic reference flow analysis,” J. Autom. methods Manag. Chem., 2010.

[19] A. A. Belay, “Impacts of chromium from tannery effluent and evaluation of alternative treatment options,” J. Environ. Prot. (Irvine, Calif)., 2010.

[20] S. Naaz and S. N. Pandey, “Effects of industrial wastewater on heavy metal accumulation growth and biochemical responses of lettuce (*Lactuca sativa*),” J. Environ. Biol., 2010.

[21] R. Subramanian and K. Saravanan, “Histopathologiczl changes in the hepatopancreas of the Estuarine clam Meretrix casta exposed to Cadmium,” J. Ecotoxicol. Environ. Monit., 2010.

[22] M. Kavitha, “Effect of Lead acetate on Biochemistry of selected tissue of rats,” J. Ecotoxicol. Environ. Monit., 2010.

[23] N. Dongre, N. A. Suryakar, A. J. Patil, and B. D. Rathi, “Biochemical effects of occupated lead exposure to workers of North Karnataka (India),” J. Environ. Heal. Res., 2010.

[24] A. Rajendran, “Applicability of toxic liquid in the removal of chromium from tannery effluents: A green chemical approach.,” African J. Pure Appl. Chem., 2010.

[25] M. Ummar, “impact of tanneries on quality of ground water in kasur district.” MS thesis, Islamia university of Bahawalpur., 2015.

[26] Ajwah, “Kasur Tanneries and Their Impact on the Environment,” ENVPK. 2021, [Online]. Available: https://www.envpk.com/kasur-tanneries-and-their-impact-on-the-environment/.

[27] M. Abbas, M. A. ur Rahman, and A. Safdar, “Detection of Heavy Metals Concentration due to leather Tanning Industry and Prevalent Disease Pattern in Kasur, Pakistan,” Environ. Urban. Asia, 2015.

[28] E. El-Bestawy, F. Al-Fassi, and R. Amer, “Biological Treatment of Leather-Tanning Industrial Wastewater Using Free Living Bacteria,” Biol. Treat. Leather-Tanning Ind. Wastewater Using Free Living Bact., 2013.

[29] J. Harris and A. McCartor, “The World’s Worst Toxic Pollution Problems,” Top Ten Toxic Twenty., 2011.

[30] Ş. Sungur and A. Özkan, “Characterization of Wastewaters Obtained from Hatay Tanneries.,” Nat. Eng. Sci., 2017.

[31] et al. Jahan, M., “Characterization of tannery wastewater and its treatment by aquatic macrophytes and algae,” Bangladesh J. Sci. Ind. Res., 2015.

[32] M. Sathish, B. Madhan, K. J. Sreeram, J. R. Rao, and B. U. Nair, “Alternative carrier medium for sustainable leather manufacturing - A review and perspective.,” J. Clean. Prod., 2016.

[33] S. Dixit, A. Yadav, P. D. Dwivedi, and M. & Das, “Toxic hazards of leather industry and technologies to combat threat: a review.,” J. Clean. Prod., 2014.

[34] E. Zuriaga-Agustí, Galiana-Aleixandre, A. M. V., BesPia, J. A. Mendoza-Roca, V. Risueno-Puchades, and V. & Segarra, “Pollution reduction in an ecofriendly chrome-free tanning and evaluation of the biodegradation by composting of the tanned leather wastes.,” J. Clean. Prod., 2015.

[35] M. Hutton and M. Shafahi, “Water Pollution Caused by Leather Industry: A Review”, ASME2019 13th International Conference on Energy Sustainability, 2019.

[36] “Pollutants in tannery Effluent,” Leather Panel. 2018,

[Online]. Available: https://leatherpanel.org/content/pollutants-tannery-effluent.

[37] M. Chowdhury, M. G. Mostafa, T. K. Biswas, A. M. Saha, &, and A. Kumar, “Characterization of the Effluents from Leather Processing Industries,” Environ. Process., 2015.

[38] O. M. Nyabaro, Pollutants levels in wastewater from tannery effluent: A case study of Nakuru tanneries, Kenya. 2013.

[39] Sarahaznam and P. Date, “12 Chemicals Used in the Tanning Process,” AZChemistry.Com. 2017.

[40] A. Zereen, A. Wahid, “Effect of Tannery Wastewater on the Growth and Yield of Sunflower (Helianthus Annuus L.).,” Bangladesh J. Bot., 2013.

[41] N. Kamlesh, “Long term effects of tannery effluents toxicity on crops and modulation of toxicity through zinc, iron, and potassium.,” Environ. Life Sci, 2009.

[42] K. H. Sheikh and M. Irshad, “Wastewater Effluents from a Tannery: Their Effects on Soil and Vegetation in Pakistan,” Cambridge Univ. Press, 2009.

[43] M. A. Qureshi, “Effect of Heavy Metals from Tannery Effluent on the Soil and Groundwater using Multivariate Analysis in District Sheikhupura, Pakistan,” Res. J. Chem. Environ., 2015.

[44] F. Hussain *et al*., “Effect of tannery effluents on seed germination and growth of two sunflower cultivars,” African J. Biotechnol., 2010.

[45] G. J. Hashmi, G. Dastageer, M. S. Sajid, Z. Ali, M. F. Malik, and I. Liaqat, “Leather Industry and Environment: Pakistan Scenario,” Int. J. Appl. Biol. Forensics, 2017.

[46] G. Gnanasekara, *etal*., “Ambient air pollution from the leather tanneries in Vellore district in reference to Asthma,” J. Chem. Pharm., 2010.

[47] A. H. M, S. ul A. M, and A. Jor., “Gaseous air pollutant and its environmental effect emitted from the tanning industry at Hazaribagh, Bangladesh,” AJER, 2015.

[48] K. Ramish and V. Thirumangai, “Impacts of tanneries on quality of ground water in Pallavaram, Chennai metropolitan city.,” IJERA, 2014.

[49] M. Moneeza Abbas, and A. Safdar, “Detection of Heavy Metals Concentration Due to Leather Tanning Industry and Prevalent Disease Pattern in Kasur, Pakistan,” Environ. Urban. ASIA, 2012.

[50] S. Anwar and M. Anwar, “Patterns of Groundwater Pollution along Industrial Zones of Lahore, Kasur and Kala Shah Kaku: A Comparative Analysis,” J. Res. Soc. Pakistan, 2020.

[51] L. Shakir et al., “Ecotoxicological risks associated with tannery effluent wastewater,” Env. Toxicol Pharmacol, 2012.

[52] S. Dixit, A. Yadav, P. D. Dwivedi, &, and M. Das, “Toxic hazards of leather industry and technologies to combat threat: A review.,” A Rev. J. Clean. Prod., 2015.

[53] M. Dotaniya, H. Das, &, and V. Meena, “Assessment of chromium eficacy on germination, root elongation, and coleoptile growth of wheat (Triticum aestivum L.) at different growth periods.,” Environ. Monit. Assess., 2014.

[54] R. D. Tripathi et al., “Roles for root iron plaque in sequestration and uptake of heavy metals and metalloids in aquatic and wetland plants,” Metallomics, 2014.

[55] S. Cheng et al., “Temporal variations in physiological responses of Kandelia obovata seedlings exposed to multiple heavy metals.,” Mar. Pollut. Bull., 2017.

[56] M. Maleki, M. Ghorbanpour, &, and K. Kariman, “Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress,” Plant Gene, 2017.

[57] R. Gopal, &, and Y. Sharma, “Effects of chromium (VI) on groundnut (Arachis hypogeae L.) metabolism.,” J. Plant Physiol. Pathol., 2014.

[58] P. Johnson et al., “Plant extract as environmental-friendly green catalyst for the reduction of hexavalent chromium in tannery effluent.,” Environ. Technol., 2018.

[59] T. B. Asfaw, T. M. Tadesse, &, and A. M. Ewnetie, “Determination of total chromium and chromium species in Kombolcha tannery wastewater, surrounding soil, and lettuce plant samples, South Wollo, Ethiopia.,” Adv. Chem., 2017.

[60] V. Sinha, K. Pakshirajan, &, and R. Chaturvedi, “Chromium tolerance, bioaccumulation and localization in plants: An overview.,” J. Environ. Manage., 2018.

[61] A. Habib, N. Riaz, I. Ahmad, and T. Shafiq, “Ground water contamination by effluent from tanneries from Kasur (Pakistan),” J.Chem.Soc.pk., 2008.

[62] A. Zereen, A. Wahid, Z. U. D. Khan, and A. A. Sardar, “Effect of tannery wastewater on the growth and yield of sunflower (Helianthus annuus L.),” Bangladesh J. Bot., 2013.

[63] A. Shakiri and M. Farid, “The impact of an industrial complex on freshly deposited sediments, Chener Rahdar river case study, Shiraz, Iran,” Environ. Monit. Assess., 2010.

[64] Y. Ashutosh, *etal*., “Organic pollutants and pathogenic bacteria in tannery wastewater and their removal strategies.,” 2016.

[65] S. Barkat and N. Hafiz, “Preparation of nano zinc oxide and its application in leather as a retanning and antibacterial agent.,” Can. J. Sci. Indus, 2011.

[66] N. C. Das and S. kishore Munsh, “Influence of tannery wastes and the surrounding environment of tannery industries on microbial growth and proliferation,” Stamford J. Microbiol., 2017.

[67] V. Kannan, M. Vijayasanthi, and M. Chinnasamy, “Bioremediation of chromium in tannery effluent by filamentous Cyanobacteria Anabaena flos-aquae West,” Int. J. Environ. Sci., 2012.

[68] S. Srivastava, K. Mishra, and P. K. Tandon, “Ameliorating Effects of Iron and Zinc on Vigna mungo L. Treated with Tannery Effluent,” J. Toxicol., 2014.

[69] N. K. Mohammad Latiful Bari, *etal.*, “Potential Human Health Risks of Tannery Waste-contaminated Poultry Feed,” J. Heal. Pollut., 2015.

[70] R.Chandra, *etal*., “Bacterial diversity, organic pollutants and their metabolites in two aeration lagoons of common effluent treatment plant (CETP) during the degradation and detoxification of tannery wastewater,” Bioresour. Technol., 2011.

[71] S. Biswas, A. Bhattacharya, and P. Basak, “Effect of Tannery Effluent on Germination and Early Seedling Growth of Oryza sativa Var. IET-4786,” Bioresour. Util. Manag., 2017.

[72] E. Souza, &, and L. Silva, “Energy recovery from tannery sludge wastewaters through photocatalytic hydrogen production.,” J. Environ. Chem. Eng., 2016.

[73] J. Hu *et al.,* “Ecological utilization of leather tannery waste with cicrular economy model.,” J. Clean. Prod., 2011.

[74] T. Ahmed and Z. U. Chowdhury, “Enviromental Burden of Tanneries in Bangladesh.,” 36th Annu. Conf. Int. Assoc. Impact Assessment. Japan, 2016.

[75] P. Saikia, *etal*., “Development of a flexible composite from leather industry waste and evaluation of their physico-chemical properties.,” Clean Technol. Environ. Policy, 2017.

[76] K. Bien, J. Celary, and P. Wystalska, “The problems in acheiving sustainable development in the tannery industry in regard to sewage sludge management.,” J. Ecol. Eng., 2017.

[77] G. Liknaw, T. Tekalign, and K. Guya, “Impacts of Tannery Effluent on Environments and Human Health,” J. Environ. Earth Sci., vol. 7, no. 3, pp. 88–97, 2017, [Online]. Available: www.iiste.org.

[78] K. Saima, H. Irfana “Effect of Wastewater Irrigation on Heavy Metal Accumulation, Growth and Yield of Vegetables” IJPE., 2017.

[79] Dr. A. M. Ghouri, “Environmental Pollution: Its Effects on Life and Its Remedies,” J. Arts, Sci. Commer., 2011.

[80] R. Rahmaty, “Effects of vesicular arbuscular mycorrhiza Glomus intraradices on photosynthetic pigments, antioxidant enzymes, lipid peroxidation, and chromium accumulation in maize plants treated with chromium.,” Turkish J. Biol., 2011.

[81] B.A. Adelekan and K. D. Abegunde, “Heavy metals contamination of soil and groundwater at automobile mechanic villages in Ibadan, Nigeria.,” Int. J. Phys. Sci., 2011.

[82] A. K. Weldemariam, “Assessment of Lowest Chromium Bioaccumulation Vegetables Irrigated by Sheba Leather Industry Contaminated Water In Wukro, Tigray - Ethiopia,” Curr. World Environ., 2020.

[83] P. B. Shakila, “Effect of tannery effluent on water and soil profile, plant growth and human health.” 2010.

[84] T. M. Tadesse and A. M. Ewnetie, “Determination of Total Chromium and Chromium Species in Kombolcha Tannery Wastewater, Surrounding Soil, and Lettuce Plant Samples, South Wollo, Ethiopia,” Adv. Chem., 2017.

[85] A. Asfaw, M. Sime, and F. Itanna, “Determining the Effect of Tannery Effluent on Seeds Germination of Some Vegetables in Ejersa Area of East Shoa, Ethiopia,” Int. J. Sci. Res. Publ., 2012.

[86] S. Ibrahim and S. A. Nafi’u, “In vitro Effects of tannery effluents on seed germination and growth performance of maize (zea mays), spinach (spinacia caudatus) and lettuce (Lactuca sativa),” Int. J. Appl. Biol. Res., 2017.

[87] R. Das and C. Bhattacharjee, “Lettuce,” Nutr. Compos. Antioxid. Prop. Fruits Veg., 2020.

[88] S. Su, Y. Zhou, J. G. Qin, W. Yao, and Z. Ma, “Optimization of the Method for Chlorophyll Extraction in Aquatic Plants,” J. Freshw. Ecol., 2010.

[89] C. Parry, B. Bugbee, “In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship,” Plant, Cell Environ., 2013.

[90] “Bradford Protein Assay,” Bio-Rad Laboratories.

[Online]. Available: https://www.bio-rad.com/featured/en/bradford-protein-assay.html.

[91] M. Scimeca, S. Bischetti, H. K. Lamsira, R. Bonfiglio, and E. Bonanno, “Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis,” Eur. J. Histochem., 2018.

[92] A. Maury, L. Kesharwani, and M. K. Mishra, “Analysis of Heavy Metal in Soil through Atomic Absorption Spectroscopy for Forensic Consideration,” Int. J. Res. Appl. Sci. Eng. Technol., 2018.

[93] L. T. Mazumder, S. Hasan, and M. L. Rahman, “Hexavalent Chromium in Tannery Solid Waste Based Poultry Feed in Bangladesh and Its Transfer to Food Chain,” J. Environ. Sci. Toxicol. Food Technol., 2013.

[94] V. Bharani, S. Sathyaraj, A. Sinha, D. Sugankumar, “A Survey on the Accumulation of Chromium in Plants near Various Tannery Industries of Vellore District, T.N.,” Res. J. Pharm. Technol., 2015.

[95] S. A. Khan, M. Ibrahim, Y. Jamil, and M. S. Islam, “Spectrochemical Analysis of Soil around Leather Tanning Industry Using Laser Induced Breakdown Spectroscopy,” J. Chem., 2013.

[96] M. Shahid and S. Shamshad, “Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system, ‘Chromium speciation, bioavailability, uptake, toxicity, and detoxification in soil-plant system: A review,’ Chemosphere, 2017.nt system: A,” Chemosphere, 2017.

[97] K. S.Balkhair and M. Aqeel Ashraf, “Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia,” Saudi J. Biol. Sci., 2016.

[98] S. A. Mashi and M. M. Alhassan, “Effects of wastewater discharge on heavy metals pollution in Fadama soils in Kano City, Nigeria,” Biomed Env. Sci, 2007.